2022
NIH-FDA
Immunology Interest Group Workshop

Dear Fellow Immunologists,

The NIH/FDA Immunology Interest Group (IIG) Steering Committee welcomes you to the 2022 NIH/FDA IIG Workshop. This will be our first hybrid meeting and first in-person event since the onset of the COVID-19 pandemic. We continue to evolve the meeting to meet the circumstances, and the steering committee is grateful for the support of the NIH/FDA immunology community. This Workshop, the IIG weekly seminar series, and all of the IIG activities are made possible by the generous financial support of NIAID (DIR, VRC, and CHI); NCI; FDA/CBER, FDA/OCE, and FDA/CDER; NHLBI; NIAMS; NHGRI; NINDS; NIDCR; NEI; NIA; NIDCD; NIEHS; NIAAA; NIDDK; and NICHD. Please be sure to thank your Scientific Directors for their support!

Intramural NIH scientists and affiliated FDA researchers represent the largest concentration of professional immunologists in the world. Our mission is to carry out research into disease mechanisms and to develop the scientific basis for therapeutically beneficial treatments and cures, as well as to identify effective measures to prevent disease. This Workshop is a critical part of this mission. It is a rare opportunity for immunologists of all levels, from multiple sub-disciplines and across multiple institutes and agencies, to come together to exchange ideas and to discover common ground across fields of inquiry. Moreover, this Workshop is a vital step in training the next generation of biomedical researchers who will continue to advance human health.

As always, the goal of this meeting to provide an opportunity for discussion and discovery. Because we can newly appreciate the joys of face-to-face interactions, please take every opportunity to ask questions, talk with one another, and share your work. We hope you will give and receive constructive feedback and succeed in creating new networks of collaboration that will hasten discovery. We look forward to many exciting and stimulating scientific connections at this Workshop.

We are especially grateful to our two gurus, Dr. Erika Pearce and Dr. Yasmine Belkaid, for their active participation in the 2022 Workshop. We also thank the attending journal editors --- Laurie Dempsey (*Nature Immunology*), Ifor Williams (*Science Immunology*), Shachi Bhatt (*The Journal of Experimental Medicine*), and Julian Vastl (*Current Protocols in Immunology)* --- especially for their interest in the findings and ongoing discussions in immunology.

Finally, we thank all of you for once again sharing your time, knowledge, and data during these two days of scientific presentations and insightful discussions.

Welcome and enjoy!

Sincerely,

The IIG Steering Committee

Please visit our website at https://www.niaid.nih.gov/research/immunology-interest-group

The 2021-2022 IIG Steering Committee members:

Nihal Altan-Bonnet (NHLBI)
Catharine Bosio (NIAID)
Gregory Constantine (NIAID)
Karen Elkins (CBER/FDA)
Michel Enamorado (NIAID)
Rosandra Kaplan (NCI)
Laurie Krug (NCI)
Daniel Lagasse (CBER/FDA)
P'ng Loke (NIAID)
Vivien Maltez (NIAID)
Robert Maul (NIA)
Ronit Mazor (CBER/FDA)
Stefan Muljo (NIAID)
Christine Nelson (NIAID)
Alexandra O'Sick (NIAID)
Maria Parkhurst (NCI)
Roxane Tussiwand (NIDCR)
Neha Wali (NCI)
Chuan Wu (NCI)
Howard Young (NCI)

The 2022 – 2023 IIG Steering Committee members:

Gregoire Altan-Bonnet (NCI)
Gregory Constantine (NIAID)
Julie Fox (NIAID)
Amy Hsu (NIAID)
Sabina Kaczanowska (NCI)
Rosandra Kaplan (NCI)
Daniel Lagasse (CBER/FDA)
Ha Na Lee (CDER/FDA)
P'ng Loke (NIAID)
Robert Maul (NIA)
Christian Mayer (NCI)
Stefan Muljo (NIAID)
Alexandra O'Sick (NIAID)
Oyebola Oyesola (NIAID)
Michael Sack (NHLBI)
Susannah Shissler (NCI)
Roxane Tussiwand (NIDCR)
Masashi Watanabe (NCI)
Alexandria Wells (NIAID)
Chuan Wu (NCI)
Li Yang (NCI)
Howard Young (NCI)
Amy Zhang (NEI)

Thanks to our 2022 Gurus!

Dr. Yasmine Belkaid
Chief, Metaorganism Immunity Section
Chief, Laboratory of Host Immunity and Microbiome
NIAID/NIH

Dr. Belkaid is a Distinguished Investigator at the National Institute of Allergy and Infectious Diseases at the National Institute of Health (Bethesda). She obtained her Masters in Biochemistry at the University of Science and Technology Houari Boumediene in Algiers, Algeria, and her Ph.D. from the Pasteur Institute in France. Following a postdoctoral fellowship at NIAID on immune regulation during infection, she started her research program at the Children's Hospital Research Foundation in Cincinnati. In 2005, she came back to NIAID and was appointed a senior investigator in 2008. Her laboratory explores fundamental mechanisms that regulate tissue homeostasis and host immune responses. The work has uncovered key roles for the microbiota and dietary factors in the control of immunity and protection to pathogens. Dr. Belkaid is the Chief of the Laboratory of Hist Immunity and Microbiome, the director of the trans NIH Center for Human immunology, and the founder and Director of the NIAID Microbiome program. Dr. Belkaid is a member of the National Academy of Sciences, the American Academy of Arts and Sciences, and the National Academy of Medicine. She is the recipient of numerous awards, including the Lurie Prize in Biomedical Sciences, the Emil von Behring Prize, the Sanofi-Institut Pasteur Award, and the Robert Koch Award.

Dr. Erika Pearce
Bloomberg Distinguished Professor
Department of Biochemistry and Molecular Biology
Bloomberg School of Public Health
Johns Hopkins University

Dr. Pearce obtained her Ph.D. in Cell and Molecular Biology in 2005 at the University of Pennsylvania in Philadelphia, where she studied the regulation of T cell responses during infection. During her postdoctoral studies, also at the University of Pennsylvania, she began her research into how cellular metabolic processes govern immune responses to infection and cancer. She launched her independent career in 2009, holding faculty positions at the Trudeau Institute in New York and then Washington University School of Medicine in St. Louis. She moved her research group to Europe in 2015 to become a Director at the Max Planck Institute for Immunobiology and Epigenetics in Freiburg, Germany. In 2018, she was awarded the Gottfreid Wilhelm Leibniz Prize from the DFG for her work on immunometabolism. In 2021, she became a Bloomberg Distinguished Professor at the Johns Hopkins University in Baltimore. Her work continues to investigate the connection between metabolism and cell function.

On-line poster displays and virtual attendance information

Please note the location within the Natcher Conference Center for the session of interest, which will either be the main auditorium or the balcony and select the corresponding Zoom link with associated information. These links are the same for both meeting days. Links are reprinted within the body of the program as well for convenience.

You are invited to a Zoom webinar.
When: Dec 8 and 9, 2022 08:00 AM Eastern Time (US and Canada)
Topic: IIG 2022 Workshop Auditorium

Please click the link below to join the webinar in the auditorium:
https://nih.zoomgov.com/j/1606510188?pwd=MXNNd3ZMMm92UDZrYWx5TC9BbldGQT09
Passcode: 438851
Or One tap mobile:
US: +16692545252,,1606510188#,,,,*438851# or +16468287666,,1606510188#,,,,*438851#
Or Telephone: Dial (for higher quality, dial a number based on your current location):
US: +1 669 254 5252, or +1 646 828 7666, or +1 551 285 1373, or +1 669 216 1590
Webinar ID: 160 651 0188
Passcode: 438851
International numbers available: https://nih.zoomgov.com/u/aeCJxIe3jc

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You are invited to a Zoom webinar.
When: Dec 8 and 9, 2022 08:00 AM Eastern Time (US and Canada)
Topic: IIG 2022 Workshop Balcony

Please click the link below to join the webinar in the balcony:
https://nih.zoomgov.com/j/1614602721?pwd=Z2FPYVBSUlhQVjBwTEZ4clRQcEZPZz09
Passcode: 744538
Or One tap mobile :
US: +16692545252,,1614602721#,,,,*744538# or +16468287666,,1614602721#,,,,*744538#
Or Telephone: Dial (for higher quality, dial a number based on your current location):
US: +1 669 254 5252, or +1 646 828 7666, or +1 669 216 1590, or +1 551 285 1373
Webinar ID: 161 460 2721
Passcode: 744538
International numbers available: https://nih.zoomgov.com/u/aemu9FFaVC

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Access all on-line posters from Monday, December 5 through Friday, December 9 by going to:

https://events.cancer.gov/user/login
Enter username: iigworkshop22
Enter password: 22IIGworkshop

Both username and password are case sensitive.

AGENDA
Thursday, December 8, 2022
NIH/FDA Immunology Interest Group Workshop 2022 – DAY 1

Plenary Session
Main Auditorium and
https://nih.zoomgov.com/j/1606510188?pwd=MXNNd3ZMMm92UDZrYWx5TC9BbldGQT09

09:00 – 09:10 **Welcome and Introduction – Karen Elkins, CBER/FDA**

09:10 – 09:15 **Guru Introduction – Roxane Tussiwand, NIDCR**

09:15 – 10:15 **Dr. Yasmine Belkaid**

Chief, Metaorganism Immunity Section
Chief, Laboratory of Host Immunity and Microbiome

"Multi-kingdom control of immunity"

10:15 – 10:30 **Break**

10:30 – 12:00 **Concurrent Sessions 1 and 2, with six short talks**

Session 1: Vaccination, Viral Immunity, and COVID-19

Auditorium and
https://nih.zoomgov.com/j/1606510188?pwd=MXNNd3ZMMm92UDZrYWx5TC9BbldGQT09

***Co-chairs*: Christine Nelson (NIAID) and Julie Fox (NIAID)**

10:30 – 10:45 **Qin (Sandy) Xu, NIAID (#101)**

Robust, prolonged adaptive immune responses to SARS-CoV-2 in tonsils and adenoids of convalescent children

10:45 – 11:00 **Paul Baker, NIAID (#8)**

Immunological history of the lung governs innate resistance to SARS-CoV-2

11:00 – 11:15 **Siddharth Krishnamurthy, NIAID (#48)**

Nutritional and Microbiota Synergy Control Enteric Coronavirus Infection

11:15 – 11:30 **Guoli (Scarlett) Shi, NCI-Frederick (#114)**

Rapalogs downmodulate intrinsic immunity and promote cell entry of SARS-CoV-2

11:30 – 11:45 **Leda Lotspeich-Cole, FDA-CBER (#61)**

Sustained antigen delivery improves germinal center reaction and increases antibody responses in neonatal mice

11:45 – 12:00 **Jiangyuan Li, NIA (#55)**

Predicting the $CD8^+$ TCRs recognizing a dominant influenza virus (IAV) epitope

Session 2: Innate Immunity and Inflammation

Balconies and
https://nih.zoomgov.com/j/1614602721?pwd=Z2FPYVBSUlhQVjBwTEZ4clRQcEZPZz09

***Co-chairs*: Robert Maul (NIA) and Sabina Kaczanowska (NCI)**

10:30 – 10:45 **Clinton Bradfield, NIAID (#14)**

Biphasic JNK Signaling Reveals Discrete MAP3K Complexes Licensing Inflammasome Formation and Pyroptosis

10:45 – 11:00 **Thierry Gauthier, NINDCR (#31)**

TGF-beta promotes glycolysis through PFKL in activated macrophages and exacerbates sepsis by disrupting blood coagulation

11:00 – 11:15 **Tomi McGuire, NCI-Bethesda (#73)**

Loss of tissue resident macrophages protects against tumor, but hampers immune initiation and tissue repair

11:15 – 11:30 **Bo-Ran Choi, NINDS (#18)**

Monocyte-derived IL-6 Programs Microglia to Rebuild Damaged Brain Vasculature

11:30 – 11:45 **Kalpana Manthiram, NIAID (#70)**

Trisomy 8-associated Autoinflammatory Disease (TRIAD) is Characterized by Dysregulated Myeloid Cells

11:45 – 12:00 **Yi Ding, NCI-Bethesda (#23)**

Two developmental pathways generate functionally distinct populations of natural killer cells

12:00 – 12:30 **Lunch, *Atrium* – Eurest pre-order and**
12:30 – 2:15 ***Atrium and Lobby***

NIH/FDA Immunology Interest Group - Day 1 Poster Session (#1-74, A-Me)

2:15 – 3:45 Concurrent Sessions 3 and 4, with six short talks

Session 3: Lymphocyte and Cytokine Biology

Auditorium and
https://nih.zoomgov.com/j/1606510188?pwd=MXNNd3ZMMm92UDZrYWx5TC9BbldGQT09

***Co-chairs*: Laurie Krug (NCI), Alexandra Wells (NIAID), and Ronit Mazor (CBER/FDA)**

2:15 – 2:30 **Nurcin Liman, NCI-Bethesda (#56)**

Death Receptor 3 is a co-stimulatory molecule for iNKT cells that potentiates their agonistic activation and triggers systemic inflammation

2:30 – 2:45 **Zenia Kaul, NIAID (#46)**

IL-2 rescues impaired T cell function upon loss of Itk via metabolic reprogramming

2:45 – 3:00 **Erin West, NHLBI (#137)**

CD4 T cell intrinsic arginase 1 controls the kinetics of Th1 induction and contraction

3:00 – 3:15 **Farhat Parween, NIAID (#93)**

Chemokine localization determines non-redundant roles for their receptors in extravasation of human pathogenic type 17 Th cells

3:15 – 3:30 **Jiro Sakai, CBER/FDA (#108)**

STAT5-regulated autocrine IL-6 signaling dictates IL-10-dependent regulatory functions of neonatal B10 cells

3:30 – 3:45 **Stormy Ruiz, NIA (#107)**

Does Transcriptional Regulation Target AID to Immunoglobulin Loci?

Session 4: Infection, Host Defense, and Microbiome

Balconies, and
https://nih.zoomgov.com/j/1614602721?pwd=Z2FPYVBSUlhQVjBwTEZ4clRQcEZPZz09

***Co-chairs*: Amy Hsu (NIAID) and Amy Zhang (NEI)**

2:15 – 2:30 **Victor Band, NIAID (#10)**

Sulfides in the gut mediate protection against gastrointestinal infection via alterations to local immunity and the microbiome

2:30 – 2:45 **Inta Gribonika, NIAID (#34)**

Host colonization with cutaneous commensals induces humoral immunity via the formation of dermal tertiary lymphoid organs

2:45 – 3:00 **Miranda Oakley, FDA-CBER (#84)**

CD47 regulates parasite burden and promotes pathogenesis in murine malaria models

3:00 – 3:15 **Alexandria Wells, NIAID (#136)**

Adaptive immunity against ancient retroelements controls the tissue threshold of activation

3:15 – 3:30 **Warakorn Kulalert, NIAID (#50)**

The neuroimmune CGRP/RAMP1 axis functionally tunes adaptive immunity to the microbiota

3:30 – 3:45 **Mattia Bonsignori, NIAID (#12)**

A Zika virus-specific IgM elicited in pregnancy exhibits ultrapotent neutralization

3:45 – 4:00 **Break**

04:00 – 05:00 **Pulling Back the Curtain on Clinical Trials**

Auditorium and
https://nih.zoomgov.com/j/1606510188?pwd=MXNNd3ZMMm92UDZrYWx5TC9BbldGQT09

This session will provide an introduction to clinical trials for immunologists to get a sense of how science is translated into the clinic. The material will include an overview of clinical trial design and considerations, as well as experts sharing their experiences on developing, writing, and running clinical trials.

***Moderators*:** **Rosandra Kaplan**, Senior Investigator
Pediatric Oncology Branch, CCR/NCI

Jacqui Klicka-Skeels, Advanced Clinical Fellow
Pediatric Oncology Branch, CCR/NCI

***Panelists*:** **Christopher Kanakry**, Lasker Clinical Research Scholar
Center for Immuno-Oncology, CCR/NCI

Jason Redman, Assistant Research Physician
Center for Immuno-Oncology, CCR/NCI

James Kochenderfer, Senior Investigator
Surgery Branch, CCR/NCI

Haneen Shalabi, Assistant Research Physician
Pediatric Oncology Branch, CCR/NCI

Social Hour: All invited for an informal (pay-as-you-go) gathering at ~ 5:30
Rock Bottom Brewery, main level bar and patio
7900 Norfolk Ave, Bethesda, MD 20814

End DAY 1

AGENDA
Friday, December 9, 2022

NIH/FDA Immunology Interest Group Workshop 2022 – DAY 2

09:00 – 10:30 Concurrent Sessions 5 and 6, with six short talks

Session 5: Immunoregulation, Metabolism, and Cancer

Auditorium and
https://nih.zoomgov.com/j/1606510188?pwd=MXNNd3ZMMm92UDZrYWx5TC9BbldGQT09

***Co-chairs*: Michael Sack (NHLBI), Maria Parkhurst (NCI), and Li Yang (NCI)**

9:00 – 9:15 **Dan Corral, NIAID (#20)**

Mammary intra-epithelial lymphocytes regulate mammary gland development and lactogenesis

9:15 – 9:30 **Qiaoya Lin, NCI (#57)**

IFN-gamma targets tumor vascular endothelial cells causing impaired perfusion and tumor growth suppression in adoptive T cell therapy

9:30 – 9:45 **Amelie Lopes, NCI (#60)**

Interrogating the role of the immune microenvironment in brain metastases response to immunotherapy using new preclinical melanoma models

9:45 – 10:00 **Abir Panda, NIAID (#91)**

Inhibition of NK and myeloid cell inhibitory receptor interactions by anti-MHC-I augments innate and adaptive immunity in both mouse and man

10:00 – 10:15 **Kannan Natarajan, NIAID (#82)**

Mechanistic aspects of tapasin mediated antigen presentation revealed by structure of a tapasin/MHC-I complex

10:15 – 10:30 **Vivien Maltez, NIAID (#67)**

Hijacking suppression: Anti-CD40 converts regulatory T cells into Type I effectors

Session 6: Dysregulation: Autoimmunity, Allergy, and Immunodeficiencies

Balconies and
https://nih.zoomgov.com/j/1614602721?pwd=Z2FPYVBSUlhQVjBwTEZ4clRQcEZPZz09

***Co-chairs*: Gregory Constantine (NIAID) and Christian Mayer (NCI)**

9:00 – 9:15 **Dominic Golec, NIAID (#33)**

PI3K-delta shapes Th2 lineage restriction through coordination of IL-2 signaling, Foxo1 inactivation and epigenetic remodeling

9:15 – 9:30 **Joshua Taylor, NIA (#123)**

Unmasking arterial-resident autoreactive B cells involved in atherosclerosis and peripheral artery disease progression

9:30 – 9:45 **Angela Thornton, NIAID (#126)**

Acquired lipodystrophy is mediated by a Treg specific deletion of Helios

9:45 – 10:00 **Amy Zhang, NEI (#142)**

Human gut commensals support development of spontaneous ocular autoimmunity in genetically predisposed mice

10:00 – 10:15 **Blake Warner, NIDCR (#135)**

Single Cell and Spatial Transcriptomics Identifies Altered Cellular Neighborhoods in the Salivary Glands of Sjogren's Disease Patients

10:15 – 10:30 **Page Murray, NIAID (#80)**

Prostaglandin E2 signaling via EP4 on macrophages protects against acute colitis by preserving intestinal barrier function

10:30 – 10:45 **Break**

10:45 – 12:00 **From Bench to Bedside: Behind the Scenes with Technology Transfer**

Auditorium and
https://nih.zoomgov.com/j/1606510188?pwd=MXNNd3ZMMm92UDZrYWx5TC9BbldGQT09

This session will delve into what goes on behind the scenes when moving a technology out of the lab, from “bench to bedside.” The technology transfer (TT) process involves many aspects of science, business, and law. including research, clinical trial and other agreements, patents, market assessments, industry partnerships, licenses, and more. The session will include an introduction to TT, how to pursue a career in the field, and insights from a panel of TT professionals. Panelists will share their career paths and highlight TT successes from their own offices, to illustrate the impact of TT and the professionals who practice it.

Moderator: **Laura Prestia**, Communications & Strategic Initiatives Manager
Technology Transfer Center (TTC), NCI/NIH

Panelists: **Andrew Burke**, Senior Technology Transfer Manager, TTC, NCI/NIH

Amy Petrik, Senior Technology Transfer and Patent Specialist
Technology Transfer and Intellectual Property Office (TTIPO), NIAID/NIH

Nisha Narayan, Intellectual Property and Partnerships Lead
CBER/FDA

12:00 – 12:30 **LUNCH, *Atrium***
12:30 – 2:30 ***Atrium and Lobby***

NIH/FDA Immunology Interest Group - Day 2 Poster Session (#75 – 144, 147, Mo – Z)

Plenary Session
Main Auditorium and
https://nih.zoomgov.com/j/1606510188?pwd=MXNNd3ZMMm92UDZrYWx5TC9BbldGQT09

2:30 – 2:35 **Guru Introduction – Naomi Taylor, NCI**

2:35 – 3:35 **Dr. Erika Pearce**

Bloomberg Distinguished Professor
Johns Hopkins University

"Delineating Cell Intrinsic Metabolic Pathways That Regulate T Cell Function"

3:35 – 04:00 **Recognition of IIG Steering Committees and Hand-off**

Conclusion

P'ng Loke (NIAID) and Stefan Muljo (NIAID)

END Day 2

Agenda Outline by Time Blocks

Natcher Conference Center
NIH Bethesda campus, Building 45

<table>
<tr><th></th><th colspan="2">Thursday, December 8</th><th colspan="2">Friday, December 9</th></tr>
<tr><td>Morning</td><td colspan="2">Welcome
Auditorium
9 – 9:15 AM

Plenary Session
9:15 – 10:15 AM
Guru address
Yasmine Belkaid, NIAID/NIH
“Multi-kingdom control of immunity”

Break 10:15 – 10:30 AM</td><td>Immunoregulation, Metabolism, and Cancer
Auditorium
9:00 – 10:30 AM

Break 10:30 – 10:45 AM</td><td>Dysregulation: Autoimmunity, Allergy, & Immunodeficiencies
Balconies
9:00 – 10:30 AM

Break 10:30 – 10:45 AM</td></tr>
<tr><td></td><td>Vaccination, Viral Immunity, & COVID-19
Auditorium
10:30 – 12:00 PM</td><td>Innate Immunity and Inflammation
Balconies
10:30 – 12:00 PM</td><td colspan="2">Professional Development Session
Auditorium
10:45 – 12 PM
From Bench to Bedside: Behind the Scenes with Technology Transfer</td></tr>
<tr><td>Mid-day</td><td colspan="2">Poster Session #1 and lunch

Lunch 12 – 12:30 PM

Posters 12:30 – 2:15 PM</td><td colspan="2">Poster Session #2 and lunch

Lunch 12 – 12:30 PM

Posters 12:30 – 2:30 PM</td></tr>
<tr><td>Afternoon</td><td>Lymphocyte and Cytokine Biology
Auditorium
2:15 – 3:45 PM

Break 3:45 – 4:00</td><td>Infection, Host Defense, & Microbiome
Balconies
2:15 – 3:45 PM

Break 3:45 – 4:00</td><td colspan="2" rowspan="2">Plenary Session
Auditorium
2:30 – 3:30 PM
Guru address
Erika Pearce, Johns Hopkins University
“Delineating Cell Intrinsic Metabolic Pathways That Regulate T Cell Function”

3:30 – 4:00
Steering Committee Hand-off
Closing</td></tr>
<tr><td></td><td colspan="2">Professional Development Session
Auditorium
4:00 – 5:00
Pulling Back the Curtain on Clinical Trials</td></tr>
<tr><td>Early evening</td><td colspan="2">Social hour – Rock Bottom Brewery, ~ 5:30</td><td colspan="2"></td></tr>
</table>

IIG Posters, Day 1, Thursday

Poster number	Attendance	Last Name	First Name	Organization	Abstract Title
1	In Person	Abed	Mehdi	NIDCR	The Role of cGAS-STING Pathway Activation in Sjögren's Disease: Driver of IFN production and Putative Therapeutic Target
2	In Person	Achar	Sooraj	NCI-Bethesda	T cells are bad at math: Dual activation of CAR and TCR can antagonize CAR-T function
3	In Person	Angeles	Benjamin	NCI-Bethesda	Generation of novel anti-tumor chimeric antigen receptors incorporating T cell signaling proteins
4	In Person	Ansaldo Gine	Eduardo	NIAID	Homeostatic intestinal immunity is dominated by microbiota-independent cytotoxic Th1 cells
5	In Person	Aqdas	Mohd	NIA	Double knock-in reporter mice: a novel tool to study age-associated NF-κB dynamics in primary microglia
6	In Person	Araya	Romina	NCI-Bethesda	Macrophage remodeling by chronic inflammation protects against metastasis
7	In Person	Azodi	Nazli	FDA-CBER	Application of Metabolomic Analysis towards the Discovery of Biomarkers of Immunogenicity and Efficacy of Parasitic Vaccines
8	In Person	Baker	Paul	NIAID	Immunological history of the lung governs innate resistance to SARS-CoV-2
9	In Person	Balsamo	Joseph	FDA-CDER	Comparative assessment of assays to detect innate immune response modulating impurities
10	In Person	Band	Victor	NIAID	Sulfides in the Gut Mediate Protection Against Gastrointestinal Infection via Alterations to Local Immunity and the Microbiome
11	In Person	Bohrer	Andrea	NIAID	PGE2 signaling via EP2 limits a host protective CD8+ T cell response in Mycobacterium tuberculosis
12	In Person	Bonsignori	Mattia	NIAID	A Zika virus-specific IgM elicited in pregnancy exhibits ultrapotent neutralization.
13	In Person	Boyd	Lisa	NIAID	Structural definition of MHC-I epitopes recognized by commonly used monoclonal antibodies.
14	In Person	Bradfield	Clinton	NIAID	Biphasic JNK Signaling Reveals Discrete MAP3K Complexes Licensing Inflammasome Formation and Pyroptosis
15	In Person	Cachau	Raul	NIAID	CD8: nature's template for Chimeric Antigen Receptors. Information transduction across the membrane, a working hypothesis.
16	In Person	Cannons	Jennifer	NIAID	PI3Kdelta coordinates transcriptional, epigenetic and metabolic changes to promote effector differentiation at the expense of memory and exhaustion CD8 T cells differentiation.
17	In Person	Chi	Liang	NIAID	Androgen signaling shapes the sexual differences of skin immunity by regulating ILC2 and dendritic cells.
18	In Person	Choi	Bo-Ran	NINDS	Monocyte-derived IL-6 Programs Microglia to Rebuild Damaged Brain Vasculature
19	In Person	Claudio-Etienne	Estefania	NIAID	PI3K□ gain of function mutations in innate cells predispose to allergy
20	In Person	Corral	Dan	NIAID	Mammary Intra-Epithelial Lymphocytes Regulate Mammary Gland Development and Lactogenesis.
21	In Person	Cui	Kairong	NHLBI	Restraint of IFN-γ expression through a distal silencer CNS–28 for tissue homeostasis
22	In Person	Davis	Katelin	NIAID	Modeling trained immunity and its proposed role in food allergies
23	In Person	Ding	Yi	NCI-Bethesda	Two developmental pathways generate functionally distinct populations of natural killer cells
24	In Person	Donko	Agnes	NIAID	Analysis of novel RAC2 variants associated with neutrophil dysfunction, lymphopenia, and primary immunodeficiency
25	In Person	Enamorado	Michel	NIAID	IMMUNITY TO THE MICROBIOTA PROMOTES SENSORY NEURON REGENERATION
26	In Person	Epping	Madeline	NIAID	TNIP1: A novel primary immunodeficiency candidate gene tied to NF-kB signaling
27	In Person	Fang	Difeng	NIAID	Cytokine-mediated NK Cell Contraction Prevents Immunopathology during Toxoplasma gondii Infection
28	In Person	Farley	Taylor	NIAID	Fates of Commensal Specific, Innate-like CD8+T cells in the Gut
29	In Person	Fukutomi	Keisuke	NIDDK	Pathogenesis of Pregnancy-Related Flares of Liver Disease in Women with Chronic Hepatitis B Virus Infection
30	In Person	Gasilina	Anjelika	NHLBI	Microtubule-binding Myosin-X (Myo10) is required for efficient dendritic cell migration in complex environments
31	In Person	Gauthier	Thierry	NIDCR	TGF-beta promotes glycolysis through PFKL in activated macrophages and exacerbates sepsis by disrupting blood coagulation
32	In Person	Ghabdan Zanlu	Nagela	NINDS	Venous plexus-associated lymphoid hubs support meningeal humoral immunity
33	In Person	Golec	Dominic	NIAID	PI3K-delta shapes Th2 lineage restriction through coordination of IL-2 signaling, Foxo1 inactivation and epigenetic remodeling
34	In Person	Gribonika	Inta	NIAID	Host colonization with cutaneous commensals induces humoral immunity via the formation of dermal tertiary lymphoid organs
35	In Person	Harrison	Mitra	NIAID	Assessment of Key Elements Required for Efficient Induction of an HIV-1-Specific Neutralizing Antibody Response
36	In Person	Hemani	Humza	NIA	Longitudinal somatic mutation analysis of individual human CD8+ T cells by a UMI-based single cell mutation detection (USCMD) method
37	In Person	Hirofumi	Shibata	NIAID	CRISPR screening for cytotoxic granule degranulation pathway in CD8 T cell
38	In Person	Ikeuchi	Tomoko	NIDCR	Fibroblasts specific response in the oral inflammatory disease periodontitis
39	In Person	Ireland	Derek	FDA	Neonatal mouse model of SARS-CoV-2 and variants of concern to evaluate therapeutics.
40	In Person	James	Alyssa	NIAID	Damaging OSMR variants differentiate severe non-allergic pruritus from atopic dermatitis in humans
41	In Person	Javaid	Ayesha	NIDCR	Quantitation and Characterization of the Immune Cell Response within COVID-associated Pernio Lesions
42	In Person	Jiang	Jiansheng	NIAID	Structures of Antibodies and Nanobodies in Complex with Spike/RBD: The Vital Role of CDR Loops in Capturing Epitopes
43	In Person	Jones	Madalyn	NIAID	CD8+Helios+ T cells: A unique functional T cell subpopulation?
44	In Person	Kaczanowska	Sabina	NCI-Bethesda	Myeloid-based approaches for cancer immunotherapy
45	In Person	Kang	Byunghyun	NIAID	Segmented filamentous bacteria (SFB) drives increased T cell-dependent polyclonal IgA and IgG2b responses in Peyer's patches by enhancing cDC-T cell interactions and the conversion of Th17 to Tfh cells.
46	In Person	KAUL	ZENIA	NIAID	IL-2 rescues impaired T cell function upon loss of Itk via metabolic reprogramming
47	In Person	Kim	Yong-Hee	NIAID	Treg cell depletion in adult mice results in activation of antigen-presenting cells prior to fatal autoimmune disease
48	In Person	Krishnamurthy	Siddharth	NIAID	Nutritional and Microbiota Synergy Control Enteric Coronavirus Infection
49	In Person	Krug	Laurie	NCI-Bethesda	Vaccination with a Replication-Dead Gammaherpesvirus Protects against Wild-Type Virus Replication, Reactivation, and Disease in Mice
50	In Person	Kulalert	Warakorn	NIAID	The neuroimmune CGRP/RAMP1 axis functionally tunes adaptive immunity to the microbiota
52	In Person	Lam	Khiem	NCI-Bethesda	Tumor-intrinsic factors dictate beneficial effect of microbiota-targeted therapies
53	In Person	Lee	Ha-Na	FDA-CDER	Differential effects of therapeutic antibodies targeting the Ebola glycoprotein on rVSVΔG-EBOV-GP-induced acute and chronic ocular diseases
54	In Person	Lee	Sang	NIAID	MHCII- dermis resident macrophages orchestrate localized ILC2-eosinophil circuitries to maintain M2-like properties in cutaneous leishmaniasis
55	In Person	Li	Jiangyuan	NIA	Predicting the CD8+ TCRs recognizing a dominant influenza virus (IAV) epitope
56	In Person	Liman	Nurcin	NCI-Bethesda	Death Receptor 3 is a co-stimulatory molecule for iNKT cells that potentiates their agonistic activation and triggers systemic inflammation
57	In Person	Lin	Qiaoya	NCI-Bethesda	IFN-gamma targets tumor vascular endothelial cells causing impaired perfusion and tumor growth suppression in adoptive T cell therapy
58	In Person	LIN	BIN	NIAID	NEMO Exon 5 Skipping led to a systemic autoinflammatory syndrome via excessive cell death
59	In Person	liu	lunhua	FDA-CBER	Age specific differences in murine alveolar endothelial cells to pathological conditions
60	In Person	Lopes	Amelie	NCI-Bethesda	Interrogating the role of the immune microenvironment in brain metastases response to immunotherapy using new preclinical melanoma models
61	In Person	Lotspeich-Cole	Leda	FDA-CBER	Sustained antigen delivery improves germinal center reaction and increases antibody responses in neonatal mice
62	In Person	Lubkin	Ashira	NIAID	Candida albicans pathogenesis in the context of mucosal type II interferonopathy
63	In Person	Maeng	Hoyoung	NCI-Bethesda	Evidences of immune response against epitope-enhanced peptide when vaccinated with peptides or peptide-pulsed DCs targeting TARP in patients with biochemically recurrent prostate cancer.
65	In Person	Majumdar	Shamik	NIAID	Ackr1-deficient mice are protected from lethal SARS-CoV-2 challenge ---- POSTER WILL BE PRESENTED ON FRIDAY
66	In Person	Mak	Nelly	NCI-Frederick	Evolutionary divergence in the IFITM genes of bat and avian species compromises antiviral function: implications for reservoirs of zoonotic viruses
67	In Person	Maltez	Vivien	NIAID	Hijacking Suppression: Anti-CD40 Converts Regulatory T Cells Into Type I Effectors
68	In Person	Manangeeswar	Mohanraj	FDA-CDER	BSL2-compliant lethal mouse model of SARS-COV-2 and variants of concern to evaluate therapeutics targeting the Spike protein
69	In Person	Mansoori	Mohammad	NIAID	Tregs suppress antigen-specific CD8+ T cells in vivo by depleting pMHC-I complexes from Dendritic Cells
70	In Person	Manthiram	Kalpana	NIAID	Trisomy 8-associated Autoinflammatory Disease (TRIAD) is Characterized by Dysregulated Myeloid Cells
71	In Person	Maul	Robert	NIA	Variable gene splicing promotes AID activity during somatic hypermutation
72	In Person	Mayer	Christian	NCI-Bethesda	Distinct sites and mechanisms of apoptosis prior to B lymphocyte activation limit autoimmune disease
73	In Person	McGuire	Tomi	NCI-Bethesda	Loss of tissue resident macrophages protects against tumor, but hampers immune initiation and tissue repair
74	In Person	Mendoza	Mirian	FDA-CDER	Developing a new immunocompetent mouse model for Dengue virus infection

IIG Posters, Day 2, Friday, and Virtual only posters

Poster number	Attendance	Last name	First name	Organization	Abstract title	
75	In Person	Mody	Drashty	NIDCR	Characterization of ZG16b protein as a potential biomarker for salivary gland damage from onset of chronic graft vs. host disease	
76	In Person	Moon	Sookjin	NIEHS	Membrane protein Flotillin-2 regulates T cell activation and division by increasing T cell receptor signaling threshold	
77	In Person	Morales-Sanch	Abigail	NCI-Bethesda	Reversion of thymus involution rescues old mice from fatal Toxoplasma gondii infection	
79	In Person	Murphy	Caitlin	NEI	Exposure to Commensal Microbiota Promotes Ocular Autoimmunity in Retina-Specific T Cell Receptor Transgenic Mice	
80	In Person	Murray	Page	NIAID	Prostaglandin E2 signaling via EP4 on macrophages protects against acute colitis by preserving intestinal barrier function	
81	In Person	Nagai	Motoyoshi	NIAID	The importance of dietary factors in regulating oral tolerance	
82	In Person	Natarajan	Kannan	NIAID	Mechanistic Aspects Of Tapasin Mediated Antigen Presentation Revealed By Structure Of A Tapasin/MHC-I Complex.	
83	In Person	Nelson	Christine	NIAID	IL-10 suppresses T cell expansion while promoting tissue-resident memory cell formation during SARS-CoV-2 infection in rhesus macaques	
84	In Person	Oakley	Miranda	FDA-CBER	CD47 regulates parasite burden and promotes pathogenesis in murine malaria models	
85	In Person	Oh	Yeuran	NIA	Modeling NF-κB regulation of the IL12b locus	
86	In Person	Oh	Jihoon	NIDDK	Wild-derived microbiota modulate the host immune system and mitigates virus-induced hepatitis.	
87	In Person	Okada	Reona	NCI-Bethesda	BET bromodomain inhibitor as priming agent for immune checkpoint blockade in neuroblastoma	
88	In Person	Oliveira Silva S	Camila	NIAID	Heterogeneity of immune response during schistosomiasis in inbred mouse strains	
89	In Person	Oyebola	Oyesola	NIAID	Previous helminth infection enhances murine host resistance to SARS-CoV-2 through pulmonary macrophage dependent T cell activation.	
90	In Person	Palmieri	Erika	NCI-Frederick	Distinct environments change metabolically upon inflammation	
91	In Person	Panda	Abir	NIAID	Inhibition of NK and myeloid cell inhibitory receptor interactions by anti-MHC-I augments innate and adaptive immunity in both mouse and man.	
92	In Person	Parvathaneni	Swetha	FDA-CBER	Unique features of IL-6 mediated germinal center responses to vaccines in neonatal mice	
93	In Person	Parween	Farhat	NIAID	Chemokine localization determines non-redundant roles for their receptors in extravasation of human pathogenic type 17 Th cells	
94	In Person	Patel	Shil	NCI-Bethesda	Investigation of a putative Bim cis-regulatory sequence in agonist and negative selection	
95	In Person	Patino Molano	Liliana	NIDCR	Differential methylation in Foxp3 locus between CD4 Tregs and CD8 Tregs	
96	In Person	Peluf	Victoria	NIAID	Temporal and tissue-coordinated requirements for the IL-12 response in Th1-mediated control of systemic infection	
97	In Person	Peng	Dingkang	NIAID	Foxp3 M370I mutation allows the activation of T effector program in Tregs	
98	In Person	Pessenda	Gabriela	NIAID	Kupffer Cells Heterogeneity Contributes to Visceral Leishmaniasis Resistance	
99	In Person	Pichler	Anrea	NIAID	PI3K: a key driver of effector differentiation under conditions of T cell exhaustion	
100	In Person	Pontejo	Sergio	NIAID	Mast cells enhance MCMV infection of macrophages: critical effects of heparin and macrophage scavenger receptors	
101	In Person	Qin	Xu	NIAID	Robust, prolonged adaptive immune responses to SARS-CoV-2 in tonsils and adenoids of convalescent children	
102	In Person	Rahman	Shah Md Tou	NIA	Decoding ligand-specificity using simultaneous monitoring of RelA and c-Rel signals in primary mouse macrophages	
103	In Person	Rao	Indira	NIAID	Sympathetic regulation of skin microbiota-induced innate-like T cells	
104	In Person	Rappaport	Jessica	NCI-Bethesda	Characterization of metastatic burden of new immunocompetent preclinical melanoma brain metastasis models to optimize immunotherapy approaches	
105	In Person	Rivera	Claudia A.	NIAID	Endogenous retroviruses modulation of intestinal immune homeostasis and oral tolerance development	
106	In Person	Roberts	Lydia	NIAID	Utilization of multiomics to identify breakdowns in pulmonary vaccine efficacy	
107	In Person	Ruiz	Stormy	NIA	Does Transcriptional Regulation Target AID to Immunoglobulin Loci?	
108	In Person	Sakai	Jiro	FDA-CBER	STAT5-regulated autocrine IL-6 signaling dictates IL-10-dependent regulatory functions of neonatal B10 cells	
109	In Person	Salazar Cavazc	Emanuel	NCI-Bethesda	LEVERAGING THE STOCHASTICITY OF IMMUNE RESPONSES AGAINST TUMORS TO IDENTIFY THE SPARK T CELLS THAT INITIATE SUCCESSFUL CANCER IMMUNOTHERAPIES	
110	In Person	Schrock	Dillon	NHLBI	LFA-1 ligation and tropomyosin promote the formation of the pSMAC actomyosin arc network in mouse CD8+ T cells	
111	In Person	Segrist	Elisha	NIAID	Role of endogenous retroviruses in the control of immunity in the Female Reproductive Tract	
112	In Person	Sepahpour	Telly	FDA-CBER	Role of IRF-7 mediated Type I Interferon response in the protective immunity induced by LmCen-/- parasites against Visceral Leishmaniasis	
113	In Person	Sharma	Rahul	NHLBI	BLOC1S1: An Unexpected Regulator of Th2 Cell-driven Inflammatory Responses	
114	In Person	Shi	Guoli	NCI-Frederick	Rapalogs downmodulate intrinsic immunity and promote cell entry of SARS-CoV-2	
115	In Person	Shissler	Susannah	NCI-Bethesda	Investigation of adult thymic epithelial cell progenitors using Foxn1 lineage tracing	
116	In Person	Silva	Lakmali Muna	NIDCR	Fibrin is a critical regulator of neutrophil effector functions at the oral mucosa	
117	In Person	Songkiatisak	Preeyaporn	NIA	Studying the role of NF-κB in Alzheimer's Disease in vitro using hiPSC-derived brain models	
118	In Person	Stassenko	Elizabeth	NCI-Bethesda	SLP-76 interaction with PLC-γ1 fine-tunes TCR signal strength for appropriate thymocyte selection and peripheral T-cell activation	
119	In Person	Steffke	Emily	NCI-Bethesda	ChAdOx1 and MVA Vaccines for the Treatment of a P1A-Expressing SB28 Glioblastoma Model in C57BL/6 Mice	
120	In Person	Sultana	Sabrina	NIAID	Determining the function of the long non-coding RNA H19 and microRNA mir675 in fetal hematopoiesis	
121	In Person	Sung	Mia	NIA	Double Knock-in Reporter Mice Reveal NF-kappaB Trajectories in Signaling, Immune Cell Development, and Aging	
122	In Person	Swanbery	Nathan	NCI-Bethesda	Enforced expression of Myc and T58A-mutant Myc in lineage-traced thymic epithelial cells	
123	In Person	Taylor	Joshua	NIA	Unmasking arterial-resident autoreactive B cells involved in atherosclerosis and peripheral artery disease progression	
124	In Person	Teijeiro	Ana	NIAID	Breast cancer remodels the bone marrow immune microenvironment to favor metastasis	
125	In Person	Thacker	Seth	FDA	Protein aggregate morphology and presence of IIRMI can impact immunogenicity.	
126	In Person	Thornton	Angela	NIAID	Acquired Lipodystrophy is Mediated by a Treg Specific Deletion of Helios	
127	In Person	Ticas Rodas	Carlos	NIA	Follicular B cells from old mice are hyper-responsive and inhibit antigen-specific antibody responses.	
128	In Person	Tolnay	Mate	FDA-CDER	Lymphocytes sense antibodies through human Fc receptor-like proteins: emerging roles in mucosal immunity	
129	In Person	Torres Juarez	Flor	NIAID	Metabolipidomic profiling of omega-3 and omega-6-derived bioactive lipid mediators in lungs of Mtb infected mice and nonhuman primate granulomas	
130	In Person	Trichka	Josephine	NCI-Bethesda	The ESCRT protein CHMP5 controls skeletal muscle homeostasis and coordination of myeloid cell-mediated tissue repair	
131	In Person	Valentina	Ottaviani	NIDCR	Dissecting the role of TGF-b in the skin	
132	In Person	Valtierra Alvara	Monica	NIAID	Obese Mice Have Attenuated Inflammatory Responses Following Infection with Bordetella pertussis	
133	In Person	Wahlsten	Madison	NCI-Bethesda	Deep learning a model of cytotoxic T cell activation in the tumor microenvironment	
134	In Person	Wang	Yihui	FDA-CBER	The Comparison of the Duration of Immunity Induced by Pertussis Vaccines and Infection in a Baboon Model	
135	In Person	Warner	Blake	NIDCR	Single Cell and Spatial Transcriptomics Identifies Altered Cellular Neighborhoods in the Salivary Glands of Sjogren's Disease Patients	
136	In Person	Wells	Alexandria	NIAID	Adaptive immunity against ancient retroelements controls the tissue threshold of activation	
137	In Person	West	Erin	NHLBI	CD4 T cell intrinsic arginase 1 controls the kinetics of Th1 induction and contraction	
138	In Person	Williamson	Kim	Uniformed Servi	Plasmablast Ig repertoire dynamics through repeat Plasmodium falciparum challenges	
139	In Person	Yamada	Eiko	NIDCR	Exploring cGAS-STING Pathway in Sjögren's Disease: Driver of IFN production and Potential Therapeutic Target	
140	In Person	Yang	Neil	NIAID	The functional role of Helios in Foxp3- T conventional cells	
141	In Person	Yoon	Sung Hwan	NIAID	A Proteomic Investigation of Antibiotic Resistance and Susceptibility in Mycobacterium abscessus and Mycobacterium massiliense in response to Clarithromycin.	
142	In Person	Zhang	Amy	NEI	Human Gut Commensals Support Development of Spontaneous Ocular Autoimmunity in Genetically Predisposed Mice	
143	In Person	Zhang	Hongwei	NIAID	Leukocyte Trafficking in Severe COVID-19	
144	In Person	Zhu	Xiaoliang	NIAID	Optimal CXCR5 Expression during Tfh Maturation Involves Bhlhe40-Pou2af1 Axis Downstream of Bcl6-Blimp1	
145	Virtual	Ahmad	Javeed	NIAID	Bivalent molecules from structure-guided design effectively neutralize SARS-CoV-2 and variants	
146	Virtual	Basu	Rahul	NIAID	A focused genetic screen uncovers genes which contribute to increased La Crosse Virus susceptibility in children	
147	In Person	Berkson	Julia	FDA-CBER	Immunological and Microbial Responses to Bacteriophage Therapy Targeting Vancomycin-Resistant Enterococcus colonization	
148	Virtual	Bettencourt	Ian	NCI-Frederick	The peritoneal tissue resident macrophage niche is dynamic and is supported by both stromal and circulating immune cells.	
149	Virtual	Bing	Sojin	FDA-CBER	Differential T Cell Immune Responses to Deamidated Adeno-associated Virus Vector	
150	Virtual	Costa-da-Silva	Ana Caroline	NIDCR	What are Exhausted Effector T CD8 cells, revealed by Single-cell RNAseq, doing in human mucosal chronic GVHD?	
151	Virtual	Fisher	Megan	NIAID	TCR Signal Strength Indirectly Regulates Complex N-Glycosylation of Recently Activated CD4+ T Cells Via a Soluble Factor	
152	Virtual	Hilligan	Kerry	NIAID	Pre-existing interferon gamma responses condition the lung to mediate early control of SARS-CoV-2 infection	
153	Virtual	Islam	Zohirul	NIAID	The role of Matrin 3 (MATR3) in innate immune response	
154	Virtual	Jessop	Forrest	NIAID	Prolyl Hydroxylase Inhibition and HIF-dependent Metabolic Reprogramming Protects Against Lethal SARS-CoV-2 Infection in Mice	
155	Virtual	Kim	Tae Sung	NIDCR	Neutrophil extracellular traps and extracellular histones mediate IL-17 inflammation and bone destruction in periodontitis	
156	Virtual	Krishnan	Anagha	NCI-Bethesda	Bottoms Up!: Inferring Relevant Immune Phenotypes from Bulk Cytokine Kinetics via Semi-Supervised Regression	
157	Virtual	Lopez-Munoz	Alberto D	NIAID	Cell Surface Nucleocapsid Protein: An Evolutionary Conserved Immunomodulatory Strategy of Betacoronaviruses?	
158	Virtual	O'Connell	Michael	NIAID	Enzymatically inactive tryptases function as partial agonists of LPS-mediated TLR4 activation	
159	Virtual	Sharma	Rubina	NIDCR	Cytotoxic role of γδ T-cells in oral Chronic Graft-Versus-Host Disease (cGVHD)	
160	Virtual	Singh	Satya	NIAID	Human CCR6+ Th memory cells form opposing extended gradients of Th17 and multilineage character with position-dependent mechanisms of plasticity	
161	Virtual	Xuan	Xie	NIAID	Eos is a critical transcription factor for T regulatory cell (Treg) function	

ABSTRACTS

Poster #1

Abed, Mehdi
NIDCR

The Role of cGAS-STING Pathway Activation in Sjögren's Disease: Driver of IFN production and Putative Therapeutic Target

Mehdi Abed, Eiko Yamada, DDS, Ph.D., Shyh-Ing Jang, Ph.D., Paola Perez, Ph.D., Thomas Pranzatelli, Kalie Dominick, Sarthak Gupta, M.D., and Blake M. Warner, DDS, Ph.D., MPH

NIDCR, Salivary Disorders Unit, Building 10, Room 1A01

Background: Sjogren's Disease (SjD) is a systemic autoimmune disease characterized by dry mouth and eyes, profound fatigue, and other systemic symptoms. A variety of biological pathways converge leading to a loss of tolerance and the development of autoimmunity in SjD. Altered interferon signaling is a hallmark of autoimmunity; understanding the drivers of altered innate immune signaling is an important investigative direction. Cytosolic DNA is recognized by the cGAS-STING pathway and is known to be activated in other autoimmune diseases including systemic lupus erythematous (SLE). Given the shared biology between SjD and lupus, we hypothesize that a subset of SjD patients exhibited cGAS-STING-driven inflammation and can be targeted for therapeutic intervention.

Methods: We used cellular bioassays and primary cell culture in vitro models from SjD and healthy control samples. Specifically, we performed STING agonism and antagonism studies on SjD and health controls primary salivary gland epithelial cells (pSGEC) and peripheral blood mononuclear cells (PBMC) using flow cytometry analysis, western blotting, and qRT-PCR.

Results: pSGEC exhibit a robust responsiveness to cGAMP, an agonist of STING, and increased TBK1, STING, and IRF3 phosporylation. Analysis of STING target gene expression exhibited a 30- and 15-fold increase in IFNB1 and MX1 expression, respectively. When pre-treated with Antagonist #5 followed by stimulation with cGAMP, we observed a reduction of pIRF3 translocation into the nucleus of pSGEC. Furthermore, qRT-PCR data showed Antagonist #5 affects STING signaling and resulted in approximately a two-fold reduction in the expression of IFNB1, CXCL10, ISG15, and MX1 in pSGEC in both SjD and HV pSGEC. These results confirm the involvement of the cGAS-STING in SjD pathogenesis and provide potential treatment for the disease. Preliminary data from our lab confirms that cGAS-STING pathway is activated in a subset of high IFN signature SjD patients.

Poster #2

Achar, Sooraj
NCI-Bethesda

T cells are bad at math: Dual activation of CAR and TCR can antagonize CAR-T function

Sooraj Achar, Taisuke Kondo, Francois Bourassa, Justyn DuSold, Guillaume Gaud, Madison Wahlsten, Paul Love, Paul Francois, Naomi Taylor, Gregoire Altan-Bonnet

Sooraj Achar, Madison Wahlsten, Gregoire Altan-Bonnet: Immunodynamics Group, Laboratory of Integrative Cancer Immunology, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA; Taisuke Kondo, Justyn DuSold, Naomi Taylor: Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA; Francois Bourassa, Paul Francois: Department of Physics, McGill University, Montréal, Québec, Canada; Guillaume Gaud, Paul Love: Section on Hematopoiesis and Lymphocyte Biology, Eunice Kennedy Shriver, National Institute of Child Health and Human Development, Bethesda, MD, USA.

Chimeric antigen receptor (CAR) T cells, are created by extracting T cells from a cancer patient, engineering them to express a CAR targeting a tumor specific molecule, then reintroducing them back into the patient. A patient’s T cells contain their own endogenous T cell receptors (TCRs) however, which could potentially interact with the exogenous CAR inserted into the cell. In this study, we examine how TCR and CAR signals interact. We show that weak TCR stimulation can reduce (antagonize) or increase overall CAR-T response, both in vitro and in vivo, across multiple tumor models, in both mouse and human T cells. We further show that the behavior of these TCR/CAR interactions can be manipulated by changing various characteristics of the TCR, CAR, and associated ligands. While this behavior is complex, we show that it can be described by a single mathematical model based on intracellular negative feedback. We then use the insights from our study to construct a novel type of CAR-T cell we term an “antanCAR-T cell”. An antanCAR-T cell is a CAR-T cell that also expresses a TCR that has been selected to respond weakly to a particular self antigen and strongly to the tumor neoantigen version of the self antigen. When an antanCAR-T cell encounters a non-tumor cell presenting a self antigen and a CAR target, the weak TCR signal it receives from the self ligand antagonizes its off target CAR response. When the antanCAR-T cell encounters a tumor cell however, the combined strong TCR signal and CAR signal it receives allows it to kill tumor cells more effectively than if it had received a CAR signal alone. This unique dual "brake/accelerator" role of the TCR dramatically increases the specificity of antanCAR-T cells, making them a potentially promising option to reduce off target CAR-T toxicity in patients.

Poster #3

Angeles, Benjamin
NCI-Bethesda

Generation of novel anti-tumor chimeric antigen receptors incorporating T cell signaling proteins

Benjamin Angeles (1), Lakshmi Balagopalan (1), Taylor Moreno (1), Jason Yi (1), Haiying Qin (2), Neriah Alvinez (1), Hide Yamane (1), Mariah Lee (1), Sandeep Pallikkuth (1), Andy Tran (1), Katherine McIntire (1), Naomi Taylor (2) and Lawrence E Samelson (1)

(1) Laboratory of Cellular and Molecular Biology, CCR, NCI; (2) Pediatric Oncology Branch, CCR, NCI

Chimeric antigen receptors (CARs) are molecules containing an antibody-derived extracellular domain combined intracellularly with signaling proteins from the T cell receptor (TCR). CAR-T cells bind specifically to tumor cells independently of MHCs to activate T cell signaling and mount an anti-tumor immune response. Although CAR-T cells have been a breakthrough clinically, there are still challenges to resolve such as their low efficacy in solid tumors and limited sensitivity to low target antigen density. Designing better CAR-T constructs may help overcome these obstacles. While several efforts have focused on identifying new cell surface target antigens, we have modified CAR intracellular domains, switching out the TCRz domain with downstream signaling proteins. This idea originated from a previous super-resolution microscopy study in which we revealed that adapter molecules form clusters distinct from the TCR complex and that full T cell activation appears to be dependent on passing a signaling threshold at the LAT phosphorylation step. With these findings and CAR sensitivity issues in mind, we developed Chimeric Adapter Proteins (CAPs) which contain signaling molecules downstream from the TCR that were hypothesized to bypass several proofreading steps required to cross the T cell activation threshold. Initial CAP designs that expressed adapter moieties enhanced basal cytokine production independent of antigen, a feature that correlates with increased cytotoxicity in vivo. In comparison, CAPs with only ZAP70 domains demonstrated antigen-specific cytokine production and cytotoxicity. In vivo murine models evaluated the ability of first (ZAP70) and second (ZAP70 and CD28 costimulatory domain) generation CAPs to clear CD19+ leukemia in an NSG xenograft model. Second generation CAPs illustrated high anti-tumor efficacy and T-cell expansion when compared to conventional 28z CAR-T cells used clinically. These findings are promising, and we are now interested in further understanding mechanisms of enhanced CAP performance and evaluating their efficacy in clearing solid tumors.

Poster #4

Ansaldo Gine, Eduardo
NIAID

Homeostatic intestinal immunity is dominated by microbiota-independent cytotoxic Th1 cells
Eduard Ansaldo 1, Taryn McFadden 1, Daniel Yong 1, Verena Link 1, Dan Corral 1, Yasmine Belkaid 1,2

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Barrier tissues such as the skin and gut form the first line of defense against pathogens and establish an immune dialogue with the resident microbiota. To that end, they harbor immune populations geared towards barrier function, tolerance, and repair. Of particular interest are the large numbers of T cells. Some are specific towards commensal microbes or recognize previously encountered pathogens. However, the origin, antigen specificity, and function of most barrier tissue-resident T cells remains elusive. We have identified a dominant CD4 T cell population in the small intestine lamina propria characterized by the expression of T-bet. Surprisingly, this population is present in both germ-free and conventionally reared (SPF) mice at homeostasis, and accounts for almost half of T cells at this site. Bulk and single-cell transcriptome analysis revealed that this population has cytotoxic potential. Furthermore, single-cell TCR sequencing showed parallel T cell repertoires in germ-free and SPF mice, suggesting similar ontogeny. To explore the ontogeny of this population, we have identified clonally expanded TCRs and are generating low frequency retrogenic mice. Additionally, we have generated several T cell hybridomas from small intestine Th1 cells. Interestingly, preliminary results suggest that small intestinal Th1 TCRs exhibit some degree of self-reactivity, which may determine their development and differentiation in a foreign antigen-independent manner. Collectively, these studies will shed light on the function of a dominant intestinal T cell population and reveal novel insights into homeostatic tissue immune networks.

Poster #5

Aqdas, Mohd
NIA

Double knock-in reporter mice: a novel tool to study age-associated NF-κB dynamics in primary microglia

Mohammad Aqdas1, Preeyaporn Songkiatisak1, Shah Md Toufiqur Rahman1, Kyu-Seon Oh1, Myong-Hee “Mia” Sung1

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Microglia are immune sentinels in the brain that are capable of orchestrating potent inflammatory responses in aging and neurodegenerative diseases like Alzheimer’s and Parkinson’s diseases. NF-κB signaling pathway is commonly recognized as a significant regulator of inflammation and aging. Mapping the spatiotemporal complexity of NF-κB signaling is crucial to understand its impact and function in vivo, but the lack of tools to directly monitor NF-κB protein components has hindered such efforts. Our lab has generated reporter mice with the endogenous RelA (p65) and c-Rel labeled with distinct fluorescent proteins and a double knock-in line with both labeled subunits. To understand how aging affects microglial function, we isolated primary microglia from young and old animals. We cultured them in vitro and validated by P2RY12+, CD11b+ and CD45int. We observed two different microglial populations, one motile, and the other forming clusters. Microglia from aged animals showed a higher prevalence of motile, free-roaming microglia. Live-cell imaging of NF-kB dynamics in primary microglia from young and old mice revealed a shift towards c-Rel in old brains. We began to examine microglial populations in brain slices from the mice ex-vivo. To functionally characterize these microglia populations, we stained for Ki67 as a proliferation marker and found that only the free-roaming microglia were proliferative. Furthermore, we are investigating the senescence pattern between the two populations to better understand the molecular mechanisms associated with age-dependent and inflammation-derived changes in young and old brains.

Poster #6

Araya, Romina
NCI-Bethesda

Macrophage remodeling by chronic inflammation protects against metastasis

Romina Araya1, Luisa Magalhaes1, Quanyi Chen1,2, April Huang1,3, Amelie Lopes1, Khiem Lam1, Gregoire Altan-Bonnet4, Romina Goldszmid1

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Macrophages are present in every tissue of the body, and they are also major components of the tumor microenvironment where they play dual roles: they can be either pro- or anti-tumorigenic and contribute to disease progression or response to therapies. Previous studies from our lab have shown that chronic inflammation, such as chronic infection with the intracellular pathogen T. gondii, remodels the monocyte-macrophage compartment in every tissue of the body. That prompted us to ask whether the persistent remodeling of the monocyte-macrophage compartment impacts tumor metastasis. We initially focused on the brain, the site of infection during T. gondii chronic infection, and used multiparametric flow cytometry and single-cell RNA sequencing to characterize immune populations. We found that chronically infected animals suffered the disappearance of homeostatic microglia followed by expansion of inflammatory macrophages and a microglia-like population with proliferative capacity and functions associated with an anti-tumor response. Interestingly, when we challenged these mice with brain metastatic melanoma, we observed a significantly lower brain metastasis burden than naïve mice. We next studied the systemic effect of macrophage remodeling focusing on the lung, a tissue with no parasite burden in this phase of infection. We found a loss of resident alveolar macrophages, which were replaced by inflammatory macrophages. Chronically infected animals challenged with B16 melanoma showed a marked reduction in lung metastasis burden compared to naïve controls. Interestingly, this was independent of NK cells and CD8 T cells, two immune populations known to play a protective role against B16 lung metastases. Moreover, a direct effect of IFNg on the tumor cells was ruled out using IFNg-receptor deficient B16 cells. Our findings demonstrate that chronic inflammation-induced remodeling of the macrophage compartment protects against metastasis development. Dissecting the molecular and cellular pathways associated with this phenomenon offers a unique opportunity to identify novel therapeutic targets.

Poster #7

Azodi, Nazli
FDA-CBER

Application of Metabolomic Analysis towards the Discovery of Biomarkers of Immunogenicity and Efficacy of Parasitic Vaccines

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FDA/CBER/OBRR; USDA; FDA/CBER/OBRR; Ohio State University; FDA/CBER/OBRR; FDA/CBER/OBRR

Leishmaniasis is a parasitic disease that is prevalent in approximately 88 countries, and yet no licensed human vaccine exists against it. Towards control of leishmaniasis, we have developed Leishmania major Centrin gene deletion mutant strains (LmCen-/-) as a live attenuated vaccine, which induces a strong Th1 response to provide IFN-γ-mediated protection to the host. However, the immune mechanisms of such protection remain to be understood. Metabolomic reprogramming of the host cells following Leishmania-infection has been shown to play a critical role in pathogenicity and shaping the immune response following infection. Here, we applied untargeted mass spectrometric analysis to study the metabolic changes induced by infection with LmCen-/- and compared with virulent L. major parasite infection to identify the immune mechanism of protection. Our data shows that immunization with LmCen-/- parasites, in contrast to virulent L. major infection, alters tryptophan metabolism to down-regulate Kynurenine-AhR signaling and promote a pro-inflammatory response.

Poster #8

Baker, Paul
NIAID

Immunological history of the lung governs innate resistance to SARS-CoV-2

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SARS-CoV-2 infection results in diverse outcomes ranging from asymptomatic infection to fatal disease. Co-morbidities, age, host genetics and various other factors can alter susceptibility to infection, but little is known about how the lung microenvironment at the time of exposure impacts outcome. Here we utilize multiple mouse models of respiratory infection and inflammation to understand the impact of recent pulmonary immune history on SARS-CoV-2 (SCV2) replication. Mice previously infected with Mycobacterium tuberculosis (Mtb) exhibit 1-3 log reduction in viral titers within one to three days following SCV2 infection. Previous acute pulmonary infection with Staphylococcus aureus or Influenza A virus (IAV) preceding SCV2 exposure similarly decreases viral titers 1-2 logs, and even ovalbumin/alum-induced asthma constrains early viral loads. Moreover, a single intranasal administration of toll-like receptor (TLR) agonists prior to infection suppresses early SCV2 viral replication. All these models induce quantitative and qualitative changes to lung resident myeloid cells, and upregulation of interferon (IFN) response markers on pulmonary epithelial cells. Protection mediated by TLR agonist pre-treatment is partially dependent on both type-I-IFN and TNF, suggesting that induction of multiple innate signaling pathways prior to infection can suppress subsequent SCV2 viral loads. Transcriptional analysis of TLR agonist pre-treated lungs also reveals activated macrophage gene expression profiles preceding SCV2 infection. This data suggests that diverse immunological stimuli can non-specifically, yet profoundly, impact SCV2 replication and suggest a potential tissue-resident macrophage (TRM)-epithelial crosstalk in this protective anti-viral state. Thus, SCV2 may benefit from immunologically quiescent environments, indicating that the outcome of SCV2 infection may be highly dependent on the individual's pulmonary immune status at the time of viral exposure.

Poster #9

Balsamo, Joseph
FDA-CDER

Comparative assessment of assays to detect innate immune response modulating impurities

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Therapeutic proteins, whether recombinant or naturally derived, are manufactured using complex expression/production systems. Even though manufacturing pipelines are designed to eliminate most impurities, the level and types of product and process related impurities in the final drug is dependent on the purification process and affected by manufacturing changes. It has been observed in the clinic that such impurities can trigger unintended immune responses that undercut safety and therapeutic efficacy of the active drug product. The development of in vitro assays to detect the presence of minute differences in innate immune response modulating impurities (IIRMI) has the potential to improve the risk assessment of manufacturing changes. However, understanding the parameters that make these assays sensitive to trace levels of impurities and predictive of product immunogenicity is incomplete. This is in part due to the heterogeneity of cellular platforms, assay readouts, and control strategies employed by different laboratories. In these studies, we use PBMC from healthy donors and/or monocytic cell lines challenged with products spiked with low levels of IIRMI (e.g., LPS, Zymosan) to provide a framework for assay comparison. Specifically, this work examines the response to IIRMI in insulin glargine, interferon beta, and adalimumab as determined by mRNA expression patterns, cytokine secretion, antigen uptake and cell surface markers on dendritic cells. Cells and supernatants are collected after twenty-four hours of stimulation to measure protein levels of innate cytokines (Luminex) and mRNA (Nanostring), as well as antigen uptake and cell surface expression of CD80/86/40 by flow cytometry. Side by side comparison of three products spiked with four different impurities will provide a foundation for selecting a platform to assess IIRMI in biologics. These studies should help understand the relative strengths and weaknesses of the different methods currently being used to assess IIRMI.

Poster #10

Band, Victor
NIAID

Sulfides in the Gut Mediate Protection Against Gastrointestinal Infection via Alterations to Local Immunity and the Microbiome

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Sulfides are gaseous, sulfur containing molecules with key roles in homeostasis throughout the body. Sulfides are produced endogenously by host tissues from dietary amino acids, but are also produced by the bacterial cells of the gut microbiome which results in the gut containing the highest concentrations of sulfide in the body. Local sulfides can be depleted by the compound bismuth subsalicylate (BSS), a common component of over-the-counter anti-diarrheal medication, which acts by sequestering sulfides in the gut. Administering BSS to mice by gavage, we observed that this treatment greatly reduced available sulfides within the gut and dramatically altered the composition of the gut microbiome. Key gut commensals such as Lactobacillus and segmented filamentous bacteria which are major mediators of gut immunity and resistance to pathogen colonization were profoundly depleted following treatment. Additionally, we observed significant downstream immune effects, specifically within the local immunity of the small intestine with depletion of gut sulfides resulting in > 50% reduction in the numbers of CD4 T cells in the lamina propria. These cells are key regulators of gut immunity and play a significant role in protection from gastrointestinal infection. Using a mouse model of Salmonella Typhimurium, mice treated with BSS were extremely susceptible to infection, with a 5 log increase in bacterial load in the colon at 24 hours post infection. Conversely, if we instead supplemented mice with sulfide in drinking water, we observed an enhanced protection to S. Typhimurium infection along with increased counts of small intestinal CD4 T cells. These data suggest that sulfides play a key role in gut homeostasis, and that usage of drugs that modify sulfide levels may have significant deleterious effects on gut health. Strategies to manage gut sulfide levels, including diet supplementation and microbiome engineering, could be a possible intervention to promote gut health.

Poster #11

Bohrer, Andrea
NIAID

PGE2 signaling via EP2 limits a host protective CD8+ T cell response in Mycobacterium tuberculosis

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Host defense against Mycobacterium tuberculosis (Mtb) is influenced by lipid mediators like Prostaglandin E2 (PGE2) that can be induced by Interleukin 1 (IL-1). PGE2 signals through four distinct receptors (EP1-EP4) that vary in expression and their downstream intracellular signals are known to influence inflammatory immune responses. Our group and others have shown that IL-1 receptor type I (IL-1R1)-deficient mice are severely susceptible to Mtb. Therefore, we hypothesized that IL1R1-dependent PGE2 signaling through its receptors would be protective against Mtb infection. In contrast to our hypothesis, we found that EP2-deficient animals had a higher pulmonary Mtb burden early but lived longer than wild-type controls. We were unable to find any differences in innate populations nor altered Mtb growth in in vitro macrophages cultures or in pulmonary myeloid cells of infected mice, each a common niche for Mtb. These results suggested the adaptive response may be modulated by EP2 signaling. CD4+ T cells are critical for host defense against Mtb; however, we found no changes in Mtb-specific CD4+ T cell responses. At the same time, we observed an increase of Mtb-specific CD8+ T cell frequency that could contribute to the increased survival observed in EP2-deficient mice. Indeed, adoptive transfer of EP2-deficient CD8+ T cells into T cell-deficient mice prolonged survival after Mtb infection compared to transfer of WT CD8+ T cells. These results suggest that EP2 signaling in CD8+ T cells limits their protective response to Mtb and might implicate EP2 as a potential target for host-directed therapy for tuberculosis.

Poster #12

Bonsignori, Mattia
NIAID

A Zika virus-specific IgM elicited in pregnancy exhibits ultrapotent neutralization

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Congenital Zika virus (ZIKV) infection results in neurodevelopmental deficits in up to 14% of infants born to ZIKV-infected mothers. Flavivirus infections are characterized by prolonged IgM responses. Neutralizing antibodies are a critical component of protective immunity and Zika virus (ZIKV)-specific serum IgM has been recently shown to contribute to ZIKV neutralization in vivo. However, prior observations did not include pregnant women and ZIKV neutralizing IgM monoclonal antibodies (mAb) have not been studied. Here, we demonstrate in a small cohort (n=10) of pregnant women that plasma IgM contributes to ZIKV immunity in pregnancy, mediating neutralization up to three months post symptoms. From a ZIKV-infected pregnant woman, we isolated a pentameric ZIKV-specific IgM (DH1017.IgM) that exhibited ultrapotent ZIKV neutralization dependent on the IgM isotype. DH1017.IgM did not cross react with Dengue virus serotypes 1-4 and, while DH1017.IgG mediated ADE in both K562 and THP-1 cells, DH1017.IgM did not. DH1017.IgM neutralized ZIKV >40-fold more potently and protected mice against viremia upon lethal ZIKV challenge more efficiently than when expressed as an IgG. Cryo-EM of the DH1017 Fab in complex with Zika virion indicated that DH1017.IgM targets a novel envelope dimer epitope within Domain II. The epitope arrangement on the virion is compatible with the concurrent engagement of all ten antigen-binding sites of DH1017.IgM. Electron micrographs of DH1017.IgM in complex with Zika virions supported a DH1017.IgM multivalent mode of antigen recognition on the same virion, a solution not available to IgG, as well as virion cross-linking. Our findings identify a unique role for antibodies of the IgM isotype in protection against ZIKV and posit DH1017.IgM as a safe and effective candidate immunotherapeutic, particularly during pregnancy.

Poster #13

Boyd, Lisa
NIAID

Structural definition of MHC-I epitopes recognized by commonly used monoclonal antibodies.

Lisa F. Boyd, Jiansheng Jiang, Kannan Natarajan, David H. Margulies

MBS, LISB, NIAID, NIH

Class I MHC molecules are integral to the recognition of self as well as to the immune response to infection and disease. Analyses of the reactivities of anti-MHC antibodies, first with sera from multiparous women and then with increasing precision using monoclonal antibodies (mAb) and recombinant MHC molecules, have been indispensable to our current understanding of MHC structure, evolution, and function. The regions of the MHC molecules recognized by a number of mAb have been previously mapped using various assays including cross-blocking studies, binding to chimeric MHC molecules (exon-shuffles), and site-directed mutagenesis. In order to map precisely the binding sites of selected anti-mouse and anti-human MHC-I mAbs we determined the crystal structures of MHC-I/Fab complexes. Fabs were prepared either by papain digestion of purified antibodies or by recombinant expression in transfected cells, following which MHC-I/Fab complexes were generated and purified by size exclusion chromatography. The mAb representing a pan anti-MHC as well as domain specific and peptide-dependent but not peptide-specific, reactivities were studied. Crystals of several MHC-I/Fab complexes were analyzed and high-resolution structures determined. By and large, the structural footprints of the mAb on MHC-I were consistent with previous studies, but topological orientation and definition of specific contacts explain further details of their reactivity. Such detailed mapping of MHC-I epitopic sites contributes to our understanding of the biological effects of these mAbs.

(Supported by the intramural research program of the NIAID, NIH)

Poster #14

Bradfield, Clinton
NIAID

Biphasic JNK Signaling Reveals Discrete MAP3K Complexes Licensing Inflammasome Formation and Pyroptosis

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Kinase signaling in the tiered activation of inflammasomes and associated pyroptosis is a prime therapeutic target for inflammatory diseases. While MAPKs subsume pivotal roles during inflammasome priming, specifically the MAP3K7:JNK1:NLRP3 licensing axis, their involvement in successive steps of inflammasome activation is poorly defined. Using live cell MAPK biosensors to focus on the inflammasome triggering event allowed us to identify a subsequent biphasic JNK activation. We find that post-trigger JNK signaling distinctly facilitates both mitochondrial reactive oxygen species generation needed to support core inflammasome formation, and gasdermin mediated cell permeation leading to release of active IL 1□ from human macrophages. We further identify and characterize a xanthine oxidase ROS activated MAP3K5:JNK2 substrate licensing complex as a novel regulator of GSDMD mobilization preceding pyroptosis. We find that inhibitors targeting this MAP3K5 complex alleviate morbidity in mouse models of colitis and dampen both augmented IL 1□ release and cell permeation in monocytes derived from patients with gain of function inflammasomopathies.

Poster #15

Cachau, Raul
NIAID

CD8: nature’s template for Chimeric Antigen Receptors. Information transduction across the membrane, a working hypothesis

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CD8 (cluster of differentiation 8) and CD28 (cluster of differentiation 28) are two frequently used templates for the design of chimeric antigen receptors (CAR), providing the sequences for transmembrane and hinge domains. However, the process of signal transduction by CD8 and CD28 is not well understood. We will discuss the state of our understanding and efforts to characterize CD8 by structure analysis methods and propose a hypothesis for the mechanism of information transduction across the membrane.

Poster #16

Cannons, Jennifer
NIAID

PI3Kdelta coordinates transcriptional, epigenetic and metabolic changes to promote effector differentiation at the expense of memory and exhaustion CD8 T cells differentiation

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Activated PI3K-delta syndrome (APDS) is a primary immunodeficiency caused by heterozygous activating mutations of Pik3cd, resulting in dysregulated immunity, recurrent respiratory infections and lymphoproliferation, yet underlying mechanisms behind these phenotypes remain unclear. Using patient samples and a mouse model (Pik3cdE1020K/+ mice), we evaluated CD8 T cell function both in vitro and in response to infectious agents, assessing cellular phenotypes, metabolism, gene expression and chromatin organization. Pik3cdE1020K/+ CD8 T cells exhibited accelerated differentiation to short-lived effectors, associated with increased mTORC1 and c-Myc pathways, as well as altered metabolic, transcriptional, and epigenetic circuits characterized by a pronounced IL-2/STAT5 signature associated with heightened IL-2 responses that prevented differentiation to memory-like cells in the presence of IL-15. Conversely, Pik3cdE1020K/+ CD8 T cells failed to sustain expression of proteins critical for maintenance of long-lived memory cells, including TCF1, and mounted inadequate central memory responses in vivo with enhanced generation of long-lived effector populations. In response to chronic infection using Clone 13 LCMV as a model pathogen, we also find that Pik3cdE1020K/+ mice fail to sustain TCF1+ progenitor CD8 T cells, leading to an imbalance of effector and exhausted cells associated with increased immunopathology. In addition, adoptive transfer of WT P14 into Pik3cdE1020K/+ mice revealed intrinsic and extrinsic factors contribute to CD8 T cell exhaustion and effector differentiation. Our data position PI3Kd as a central hub integrating multiple signaling nodes that promote an accelerated effector T cell program during both acute and chronic infections.

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Poster #17

Chi, Liang
NIAID

Androgen signaling shapes the sexual differences of skin immunity by regulating ILC2 and dendritic cells

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Male and females have different predisposition to autoimmune disorders and these differences can be at least in part explain by sexual dimorphism of the immune system. However, the difference in tissue immunity between gender and the mechanism underlying these differences remains largely unknown. This study aims to investigate sex differences in the skin immune system and decipher the underlying regulatory factors. We found that female mice have higher level of tissue resident lymphocyte and higher magnitude of adaptive immune responses in the context of microbiota association and intradermal infections. Correspondingly, we found that female skin harbor a significantly higher level of skin dendritic cells (DCs) and that in females, all DC subset express a higher level of activation. These sex differences were regulated by male sex hormones, as castration of males normalized the differences observed between males and females. Our data reveal that the androgen receptor is highly expressed in skin ILC2 and that females have a significantly higher level of ILC2 and more activated ILC2 than males. In addition, ILC2 from females have significantly higher level of ILC2-produced IL-13 and GM-CSF, both cytokines known to contribute to the accumulation and function of DC within the skin. Collectively our data propose that androgen signaling negatively regulates the level of skin ILC2 and DCs, thereby shaping sex-specific skin immunity within the skin. These findings uncover ILC2 as a central regulator of immune sexual dimorphism and may provide an explanation for the enhanced susceptibility to inflammatory disorder and enhanced control of pathogen observed in females.

Poster #18

Choi, Bo-Ran
NINDS

Monocyte-derived IL-6 Programs Microglia to Rebuild Damaged Brain Vasculature

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Cerebrovascular injury (CVI) is a common pathology caused by infections, injury, stroke, neurodegeneration, and autoimmune disease. Rapid resolution of a CVI requires a coordinated innate immune response. In this study, we sought mechanistic insights into how CNS infiltrating monocytes program resident microglia to mediate angiogenesis and cerebrovascular repair following intracerebral hemorrhage. In the penumbrae of human stroke brain lesions, we identified a subpopulation of microglia that express VEGFA. These cells, termed repair associated microglia (RAM), co-expressed membrane-bound IL-6Ra and were also observed in a rodent model of CVI. Cerebrovascular repair did not occur in IL-6 knockouts or in mice lacking microglial IL-6Ra expression, and single cell transcriptomic analyses revealed faulty RAM programming in the absence of IL-6 signaling. Infiltrating CCR2+ monocytes were the primary source of IL-6 following CVI and were required to endow microglia with proliferative and pro-angiogenic properties. Faulty RAM programming in the absence of IL-6 or inflammatory monocytes resulted in poor cerebrovascular repair, neuronal destruction, and sustained neurological deficits that were all restored via exogenous IL-6 administration. These data provide a molecular and cellular basis for how monocytes instruct microglia to repair damaged brain vasculature and promote functional recovery after injury.

Poster #19

Claudio-Etienne, Estefania
NIAID

PI3K□ gain of function mutations in innate cells predispose to allergy

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Activated phosphoinositide-3 kinase syndrome (APDS) is a disease characterized by gain-of-function mutations in PI3KCD or PI3KR1. Patients develop a broad range of symptoms that include frequent respiratory infections, lymphadenopathy, and autoimmunity. Additionally, we and others have found an increased prevalence of allergic disorders in this patient population, including eosinophilic gastrointestinal disease (EGID) and asthma. Furthermore, recent publications have demonstrated that PI3K□□inhibitors are able to ameliorate airway allergic inflammation in mouse models.

PI3Kinase proteins are composed of a catalytic subunit and a regulatory subunit and are involved in cellular mechanisms such as cell survival, proliferation, and cellular metabolism. Naïve CD4+ T cells from patients with APDS have been shown to have an increased propensity for T helper (Th2) skewing. The PI3K signaling pathway also acts downstream of cytokine receptors in innate immune cells. Thus, we hypothesized that altered PI3K signaling in response to IL-33 in innate lymphoid cells (ILC2s) and eosinophils may contribute to the allergic predisposition in APDS patients.

To address this question, we analyzed eosinophil precursors, mature eosinophils, and ILC2s in the peripheral blood of APDS patients and age/sex matched healthy volunteers. We found that patients exhibited greater accumulation of eosinophil precursors and ILC2s, and a trend for higher numbers of mature eosinophils, especially in patients prior to the initiation of treatment. Both patient and murine ILC2s harboring a knock-in allele of Pik3cdE1021K/WT commonly found in patients expressed higher amounts of IL-5 following stimulation with IL-33. Furthermore, Pik3cdE1021K/WT mice spontaneously developed increased frequencies of eosinophil precursors in bone marrow and mature eosinophils in spleen. These results suggest that dysregulation of type 2 responses by innate immune cells may also contribute to a predisposition for allergic disease in patients with APDS.

Poster #20

Corral, Dan
NIAID

Mammary Intra-Epithelial Lymphocytes Regulate Mammary Gland Development and Lactogenesis.

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Breastfeeding has long been associated with health benefits for the infant. Breast milk is composed of essential nutrients and bioactive molecules shaping the growth, and the immune development of the offspring. An optimal differentiation of the mammary gland (MG) epithelium into milk-secreting cells is critical to sustain lactation and its health benefits. Nevertheless, how the lactation process is regulated by the immune system remains unexplored. Intriguingly, we observed that a T cell deficiency changes the nutritional quality of milk as well as its yield. While a bias of type 1 to type 2 immunity is thought to be critical for normal pregnancy, our data reveal that MG is largely dominated by type-1 immunity. Notably mammary gland development is associated with the accumulation of T-bet+ lymphoid cells, including unconventional T lymphocytes, that are preferentially localized at the proximity and inside the mammary epithelium. Of note, these cells exhibit common features with gut CD8aa+ intra-epithelial lymphocytes and derive from the same thymic progenitor (IELp). Such process is associated with thymus involution during pregnancy and specific enrichment in thymic IELp that preferentially migrates into the mammary gland. Adoptive transfer of IELp into Rag2-/-□c-/- mice modulates the differentiation of mammary epithelial cells into milk-producing cells and their ability to express beta-casein, the main milk protein. While expressing high levels of T-bet, MG lymphocytes produce IL-4, a cytokine known to promote the lactogenesis. Thus, our data propose that innate-like lymphocytes are critical for the differentiation of the mammary epithelium into milk-secreting cells as well as in the control of the lactogenesis. Decoding the immune regulation of the MG during pregnancy will allow to understand the profound transformation of this compartment as a primary nutritional source for the offspring and may allow to understand how dysregulation of this process could affect the long-term development of newborns.

Poster #21

Cui, Kairong
NHLBI

Restraint of IFN-γ expression through a distal silencer CNS–28 for tissue homeostasis

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Interferon-□ (IFN-□) is a key cytokine in response to viral or intracellular bacterial infection in mammals. While a number of enhancers are described to promote IFN-□ responses, no silencers for the Ifng gene have been identified. By examining H3K4me1 histone modification in naïve CD4+ T cells within Ifng locus, we identify an unrecognized silencer (CNS–28) which is responsible for restraining Ifng expression. Mechanistic study further demonstrates that CNS–28 maintains Ifng silence by diminishing enhancer-promoter interactions within Ifng locus in a T-bet independent manner. Functionally, CNS–28 restrains Ifng transcription in Th1, Tc1 and NK cells during both innate and adaptive immune responses. Moreover, CNS–28 deficiency resulted in repressed type 2 responses due to elevated IFN-□ expression, shifting Th1 and Th2 paradigm. Thus, CNS–28 activity ensures immune cell quiescence by cooperating with other regulatory cis elements within the Ifng gene locus to minimize autoimmunity.

Poster #22

Davis, Katelin
NIAID

Modeling trained immunity and its proposed role in food allergies

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The rapidly growing prevalence of food allergies in Westernized countries outpaces a genetic explanation for this increase. A growing body of literature implicates environmental circumstances as contributing factors including limited microbial diversity, nutritional alterations, skin inflammation, and early life immune challenges. Furthermore, infants who go on to develop food allergies and fail to develop oral tolerance have elevated numbers of pro-inflammatory monocytes in circulation at birth and throughout early childhood. Together these factors suggest that early life immune training (TRIM) contributes to food allergy sensitization and prevention of food antigen tolerance. To better understand how previous immune challenges can alter subsequent food antigen sensitization and oral tolerance, we developed a model of TRIM in Balb/c mice using epicutaneous tape-stripping with or without ovalbumin antigen exposure. Mouse bone-marrow hematopoietic stem cell and multipotent progenitor populations were evaluated to demonstrate long term population alterations after epicutaneous antigen exposure suggestive of TRIM. To determine if bone-marrow progenitor cell alterations could result in changes in distant tissue-specific myeloid populations, the small intestine and splenic myeloid populations were evaluated and dendritic-cell antigen presentation and T-cell priming capabilities were assessed. Preliminary results suggest a previously undescribed skin-bone-marrow axis which may contribute to systemic myeloid cell alterations and altered susceptibility to food allergies.

Poster #23

Ding, Yi
NCI-Bethesda

Two developmental pathways generate functionally distinct populations of natural killer cells

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Natural killer (NK) cells function by eliminating virus-infected cells or tumor cells during early defenses. However, the early development of NK cells and lineage relationships between NK cells and helper innate lymphoid cells (ILCs) remain elusive. Common precursors for ILCs (ILCPs) can differentiate into both helper ILCs and NK cells. Here, we identified a NK lineage-restricted progenitor population, early NK progenitor (ENKP), which does not develop from ILCPs, thus ENKP may represent the ILCP-independent pathway of NK cell development. Competitive chimera experiment shows ENKPs generate NK cells more efficiently than ILCPs, suggesting that ENKP-dependent pathway is the major pathway for NK cell development. scRNA-seq shows ENKP-derived NK express Ly49 receptors and higher levels of cytotoxic genes whereas ILCP-derived NK cells have very low expression of Ly49 receptors and express higher levels of genes implicated in tissue residency such as CD69 and CD200R. Furthermore, Ly49H+ NK cells which response to MCMV infection mostly develop from ENKPs but not ILCPs. Our findings establish the existence of two pathways of NK cell development that generate functionally distinct NK cell subsets.

Poster #24

Donko, Agnes
NIAID

Analysis of novel RAC2 variants associated with neutrophil dysfunction, lymphopenia, and primary immunodeficiency

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RAC2 is a member of Rho-family GTPases, abundant in neutrophils serving roles in NADPH oxidase activation and cytoskeleton dynamics. RAC2 regulates neutrophil superoxide production by direct p67phox and cytochrome b558 interaction and actin cytoskeleton regulation affecting migration and membrane trafficking. Patients with dominant, activating mutations in RAC2 (E62K, N92T, Q61R) are associated with neutrophil dysfunction (excess ROS production, reduced cell migration), lymphopenia and immunodeficiency. We now characterize additional disease-related RAC2 variants with in-vitro analysis of RAC2 effector functions including NOX2-based superoxide production, regulation of PAK1-PDB binding, activation of AKT, protein stability, subcellular localization, and F-actin formation.

We identified both active and inactive variants associated with lymphopenia and immunodeficiency. Patients with dominant, active RAC2 variants with high protein stability (Q61R, Q61K) presented in the first days of life with severe combined immunodeficiency and absent lymphocytes. Activating variants with lower protein stability (I21S, E62K, N92K, N92S) presented as combined variable immunodeficiency with lymphopenia, detectable by newborn screening but clinically presenting later with recurrent sinopulmonary infections, reduced B-cells and hypogammaglobulinemia. A third class of mutations with unstable transcript or protein were phenotypic only in homozygosity (W56*, R68W). Severity of phenotype and initial clinical presentation was correlated with protein stability, with increased stability leading to neonatal presentation and decreased stability of dominant mutations having later clinical onset. Thus, heterologous expression and analysis of RAC2 variants is useful for deciphering the genetic and molecular mechanistic basis for the common underlying clinical phenotype related to lymphopenia and immunodeficiency.

Poster #25

Enamorado, Michel
NIAID

IMMUNITY TO THE MICROBIOTA PROMOTES SENSORY NEURON REGENERATION

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The microbiota plays a fundamental role in the induction and education of the immune system. In turn, the immune system operates to sustain and restore barrier tissue function in the context of threatening environmental exposures. All barrier tissues, including the skin, are densely innervated with sensory nerve fibers that are involved not only in the perception of touch and temperature, but also in the regulation of inflammation and protective immunity. These emerging observations underscore our understanding of the interconnection between the immune and nervous systems. Upon infection or injury, host survival requires protection and restoration of all tissue components, each requiring specific repair programs. Based on the alliance between the microbiota and its host, we hypothesized that the microbiota might play an important role in bridging biological systems to reinforce and restore barrier integrity. In this context, whether immunity to the microbiota can promote neuronal regeneration remains unclear. Here, we show that, upon injury, adaptive responses to the microbiota directly promote sensory neuron regeneration. At homeostasis, commensal-specific Th17 cells colocalize with sensory nerve fibers within the dermis and express a transcriptional program associated with neuronal repair. Following injury, commensal-specific Th17 cells promote axon growth and local nerve regeneration. Mechanistically, our data reveal that the cytokine interleukin 17 A (IL-17A) produced by commensal-specific T cells directly signal to sensory neurons via the IL-17 receptor A, the transcription of which is specifically upregulated in injured neurons. Collectively, our work reveals that microbiota-specific T cells can bridge biological systems by directly promoting neuronal repair, and identifies IL-17A as a major determinant of this fundamental process. Our findings that upregulation of the IL-17A/IL-17RA axis represents a conserved response in injured neurons open the door to novel therapeutic approaches to potentiate sensory recovery after injury, or limit neuropathies in the context of diabetes and chemotherapy.

Poster #26

Epping, Madeline
NIAID

TNIP1: A novel primary immunodeficiency candidate gene tied to NF-kB signaling

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Primary immunodeficiencies (PID) represent a group of over 450 rare inborn errors affecting the development and/or function of the immune system. Patients typically display an increased susceptibility to infection and many exhibit autoimmune conditions or malignancies. A growing number of PID patients are diagnosed in adulthood with sporadic, milder courses and increased clinical heterogeneity. Identification of causative genes for adult-onset PID may allow for better understanding of broader immune dysregulation and provide insight for possible therapeutic targets.
Whole genome-sequencing identified TNIP1 as a novel candidate gene associated with adult-onset PID. TNIP1 is a ubiquitin-sensing protein that regulates NFkB signaling by promoting proteasomal degradation of key pathway members and destabilization of the IKK-NEMO complex. An individual exhibiting adult-onset PID and pulmonary sarcoidosis with no known genetic basis was found to have a heterozygous nonsense variant in the critical ubiquitin-binding domain of TNIP1.
To evaluate t this variant, we generated a mouse model at the synonymous genetic location using CRISPR/Cas-9 genome-editing. Previous work indicated that homozygous deletion of TNIP1 is embryonic lethal, and we found that mice homozygous for the stop-gain variant represented only 3% of live births, suggesting that it is poorly-tolerated. Both heterozygous and homozygous variant mice revealed splenomegaly with increased splenocyte and lymph node cell counts. Homozygous mice exhibit a marked myeloid expansion and relative lymphopenia with a 10-fold reduction in T lymphocyte percentage and a 5-fold reduction in B cells. Homozygous mice displayed pronounced reductions in naïve cells, increased effector memory cells, and B and T cell activation, whereas heterozygous mice had slightly decreased T and NK cells with increased effector memory T cells and evidence of myelofibrosis.
Our results suggest that TNIP1 is a novel candidate gene for PID with a nonsense mutation in the ubiquitin-binding domain resulting in immune dysregulation in mice.

Poster #27

Fang, Difeng
NIAID

Cytokine-mediated NK Cell Contraction Prevents Immunopathology during Toxoplasma gondii Infection

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The rapid and robust activation of innate NK cells is critical for controlling parasite expansion at early-stage of Toxoplasmas gondii infection through the production of effector cytokine IFN-γ. However, the kinetics of NK cell population as well as the physiological and pathological consequence of aberrant NK cell activity during acute T. gondii infection are not well understood. In this study, we found that the NK cells located at the sites of primary infection expanded dramatically at early stage and contracted substantially coinciding with the onset of vigorous adaptive type 1 immune response upon T. gondii challenge. Pro-inflammatory cytokine IL-12-mediated upregulation of lineage-determining transcription factor T-bet conducted the self-destruction of terminally maturate NK cells, limited the persistence of IFN-γ-producing NK cell population, and thus prevented lethal immunopathology. Our study revealed an important mechanism through which cytokine-mediated feedback restrains the magnitude of innate immune response during T. gondii infection.

Poster #28

Farley, Taylor
NIAID

Fates of Commensal Specific, Innate-like CD8+T cells in the Gut

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Barrier tissues such as the skin and gut are host to a variety of bacteria, fungi, viruses and parasites collectively termed the microbiota. These co-inhabitants are vital for immune eduction, nutrient acquisition and protection from pathogens. To maintain host homeostasis, sufficient exposure to the microbiome is necessary, and diseases such as Inflammatory bowel disease (IBD), which encompases both Crohn's Disease and Ulcerative Colitis, have been well described as occurring admist breakdown in the normal communication between host and microbe. Fundamentally, IBD is a disease of aberrant immune activation to the microbiome and epithelial barrier disfunction. At a distal epithelial barrier, the skin, we have described the innate-like CD8+T cell response in mice after topical exposure to a commensal microbe. Under homeostatic conditions, topical association with particular commensals induce a non-inflammatory H2-M3 restricted CD8+T cell response characterized by IL-17A production that aids in barrier maintenance and epithelial repair. However, the residence of these H2-M3 restricted, commensal specific T cells in the gut, and potential contribution to barrier function in this tissue is unknown. We have now identified H2-M3 restricted CD8+T cells populating the lamina propria and intraepithelial compartments of both the colon and small intestine following novel commensal exposure. Further, during inflammatory conditions these cells drastically expand and produce proinflammatory Type 1 cytokines. Following these discoveries, we developed novel tetramer reagents to track and interrogate the function of H2-M3 restricted T cell responses to Helicobacter hepaticus, a common gut commensal with known roles in perpetuating colitis in immunocompromised animals. Utilizing these tools we hope to uncover how these non-classically restricted immune responses to the same commensal under homeostatic or inflammatory conditions differ, to identify potential targets to correct aberrant reactivity to the microbiota during colitis.

Poster #29

Fukutomi, Keisuke
NIDDK

Pathogenesis of Pregnancy-Related Flares of Liver Disease in Women with Chronic Hepatitis B Virus Infection

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Women with chronic hepatitis B are at risk of increased liver inflammation during and after pregnancy. To investigate the pathogenesis of these hepatic flares we studied immune responses and gut microbiota in 19 women with chronic hepatitis B (12 without and 7 with nucleotide analogue (NUC) treatment) and 3 uninfected controls during the first, second, and third trimesters and at two and six months postpartum. Increased serum alanine aminotransferase activity as a marker of flares in disease activity was defined as greater than the upper limit of normal (ULN) and greater than twice the baseline value in the respective patient.

While increased plasma levels of soluble CD163, inflammatory cytokines and chemokines generally occurred during the period of increased ALT activity, we also observed increased levels of IL-22 and IFN-gamma during pregnancy preceding a postpartum ALT increase. In contrast, spikes in IL-27, a cytokine that is produced by the trophoblast, occurred in individual patients who maintained normal ALT activity at all studied time points.

The fecal microbiota of HBV-infected patients with and without NUC treatment had reduced alpha-diversity (inverse Simpson index) compared to those of uninfected controls. The composition of the fecal microbiome clustered in a patient-specific manner (PCA analysis). To identify the factors that predict a postpartum ALT increase we examined patients who maintained ALT levels in the absence of NUC treatment in the first and second trimesters of pregnancy and did or did not develop a postpartum ALT increase (four patients per group). Combining all time points studied, gut microbiota of those with postpartum ALT increase differed significantly from those of four patients without postpartum ALT increase (PCA, Permanova $p<0.003$).

In conclusion, the composition of the fecal microbiota was patient- rather than time point-specific and differed among patients with and without postpartum ALT increase.

Poster #30

Gasilina, Anjelika
NHLBI

Microtubule-binding Myosin-X (Myo10) is required for efficient dendritic cell migration in complex environments

Anjelika Gasilina, Paniz Rezvan Sangsari, Nicole Y Morgan, John A Hammer

Anjelika Gasilina - National Heart Lung and Blood Institute, Paniz Rezvan Sangsari - National Institute of Biomedical Imaging and Bioengineering, Nicole Y Morgan - National Institute of Biomedical Imaging and Bioengineering, John A Hammer - National Heart Lung and Blood Institute

Activation of naïve T cells by antigen-presenting dendritic cells is a critical determinant of efficient adaptive immune responses. Following pathogenic antigen capture, dendritic cells use a CCL19/CCL21 chemokine gradient to guide their migration from the primary sampling site to the closest lymph node. During migration dendritic cells must negotiate passage through basement membranes, extracellular matrices, cell junctions, and branched vessel networks, all while maintaining speed, persistence and directionality. Efficient navigation through these complex microenvironments involves coordinated movements of the cell's nucleus, actin-rich extensions, and microtubule cytoskeleton, which together regulate space exploration, path finding, and pore size selection. In this study we are using imaging to define the contribution that the MyTH4/FERM domain-containing molecular motor myosin-X (Myo10) makes to dendritic cell migration. Using murine bone marrow-derived and splenic dendritic cells, we show that Myo10 is dispensable for dendritic cell differentiation, maturation, and CCL19/CCL21 chemokine sensing, but is essential for efficient directed cell migration in confined and complex environments. Specifically, Myo10-depleted dendritic cells exhibit increased velocity but impaired directionality and persistence during directed migration in both confined 2D environments and complex branched networks. In complex networks especially, Myo10-depleted dendritic cells are ineffective at using their nucleus as the pore size and path selector, and they cannot resolve competing leading edges. These deficiencies result in cell entanglement and fragmentation. Current efforts are directed at determining whether Myo10 requires its microtubule-binding MyTH4 domain to control dendritic cell navigation through complex environments. Delineation of the core mechanisms underlying dendritic cell migration may guide the development of dendritic cell-based therapeutic interventions.

Poster #31

Gauthier, Thierry
NIDCR

TGF-beta promotes glycolysis through PFKL in activated macrophages and exacerbates sepsis by disrupting blood coagulation

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The metabolism of macrophages plays a key role in their immune responses, but the mechanisms by which the metabolism is regulated remain largely unknown. We show here that TGF-beta induces a unique phenotype of macrophages and regulates the glycolysis of macrophages independently of inflammatory cytokine production. Specifically, TGF-beta increased expression and activity of phosphofructokinase-1 liver type (PFKL) in macrophages and thus promoted their glycolysis during cell activation, yet paradoxically suppressed the production of proinflammatory cytokines in the same macrophages. The upregulation of glycolysis was mediated by an mTOR-c-MYC dependent pathway, whereas the inhibition of cytokines was ascribed to downregulated activation of the major pro-inflammatory transcription factors, namely AP-1, NF□B and STAT1. Importantly, we found that in LPS-induced sepsis model, TGF-beta enhancement of macrophage glycolysis led to a decreased survival in mice by increasing blood coagulation. Analysis of cohorts of patients with sepsis and covid-19 revealed that the expression of PFKL, TGF-□ receptors TGFBRI and coagulation factor F13A1 in monocytes positively correlated with the progression of the disease. Thus, TGF-beta is emerging as a critical cytokine regulating macrophage metabolism and could serve as a therapeutic target in patients with sepsis and Covid-19.

Poster #32

Ghabdan Zanluqui, Nagela
NINDS

Venous plexus-associated lymphoid hubs support meningeal humoral immunity

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There is increasing interest in how immune cells in the meninges, the membranes that surround the brain and spinal cord, contribute to homeostasis and disease in the central nervous system. The outer layer of meninges, the dura mater, contains both innate and adaptive immune cells and functions as a site for B cell development. In this study, we identified previously undescribed lymphoid structures surrounding fenestrated venous plexi in the dura mater. We found the most elaborate immune organization, including lymphatic vessels, surrounding the rostral-rhinal confluence of sinuses. We termed this structure that interfaced with the skull bone marrow and a comparable venous plexus at the skull base, the rostral-rhinal venolymphatic hub. This hub emerged during development in mice at P8/9 before formation of the superior sagittal sinus. Single cell RNA sequencing demonstrated that rostral rhinal hub hosts a diverse array of resident innate and adaptive immune cells during steady state. Immune aggregates were also present in this structure during homeostasis and expanded with age or following challenge with systemic or nasal antigens. Following intranasal VSV infection, the rostral-rhinal hub supported local germinal center (GC) reactions consisting of T follicular helper cells as well as GC B cells that underwent proliferation, somatic hypermutation, class switch, and conversion into plasma cells locally. These data demonstrate lymphoid architecture around vascular plexi in the dura mater that can sample antigens and rapidly elaborate local humoral immune responses to protect the meninges and underlying brain parenchyma from pathogens.

Poster #33

Golec, Dominic
NIAID

PI3K-delta shapes Th2 lineage restriction through coordination of IL-2 signaling, Foxo1 inactivation and epigenetic remodeling

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Activated PI3K-delta syndrome (APDS) is a primary immunodeficiency caused by heterozygous activating mutations in PI3Kd, resulting in recurrent respiratory infections and allergic diseases, including asthma. Helper CD4 T cells are critical in orchestrating adaptive immunity; their differentiation into distinct lineages is instrumental in maintaining balanced responses. Given that APDS patients show an inability to clear respiratory pathogens (Th1) and development of allergic diseases (Th2), we hypothesized that hyperactivated PI3Kd (Pik3cdE1020K) may alter CD4 T cell differentiation. Using in vitro polarization of murine naïve CD4 T cells, we observed increased production of IFNg from Pik3cdE1020K under Th1-inducing conditions and marked increases in IL-4 and IL-13 production under Th2-conditions. However, Pik3cdE1020K Th2 cells also expressed high levels of T-bet and aberrantly produce Th1 cytokine IFNg. Strikingly, this dysregulation was also observed in vivo; Pik3cdE1020K CD4 cells show pronounced alterations following house dust mite (HDM) induced allergic-airway inflammation, with impaired Th2 and inappropriate increased Th1 differentiation. scRNAseq analysis of HDM-induced CD45+ Pik3cdE1020K lung cells revealed a global enrichment of IFNg response signatures. We linked this unstable Th1 and Th2 differentiation to hyper-responsiveness to IL-2, with inhibition of IL-2 restoring normal differentiation of Pik3cdE1020K CD4 T cells. Downstream of IL-2, we found increased phosphorylation and inactivation of transcription factor Foxo1 in Pik3cdE1020K CD4 T cells; RNA-seq further revealed altered expression of Foxo1-regulated genes. Forced expression of Foxo1 in Pik3cdE1020K Th1 and Th2 limited excessive IFNg and T-bet expression and rescued altered differentiation, whereas CRISPR-mediated targeting of Foxo1 recapitulated the phenotype of Pik3cdE1020K T cells. Further transcriptomic analysis of Pik3cdE1020K Th1 and Th2 cells revealed differential expression of genes involved in histone methylation. Accordingly, Pik3cdE1020K CD4 exhibit altered chromatin modifications, including H3K27 and H3K4 trimethylation. Together these data suggest that balanced regulation of PI3Kd plays a critical role in enforcing Th1 and Th2 lineage identity.

Poster #34

Gribonika, Inta
NIAID

Host colonization with cutaneous commensals induces humoral immunity via the formation of dermal tertiary lymphoid organs

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The skin is one of the largest barrier sites which harbors a plethora of microorganisms that shape the local tissue homeostasis and cellular immunity. Whether the skin microbiota promotes humoral responses, and what is the role of such responses remains unknown. Here we show that colonization with common skin commensal Staphylococcus epidermidis profoundly modulates cutaneous immunity that results in serum responsiveness with antigen-specific T-dependent IgG antibodies. Such response is uncoupled from inflammatory signals and is associated with the induction of dermal tertiary lymphoid structures that closely resemble classical germinal centers. Of note, such responses can occur in a tissue-autonomous manner as evidenced by the preserved antibody responses in mice deficient in professional lymphoid organs or impaired skin-to-lymph node migration. Our data also uncover a non-redundant role for Langerhan's cells in the generation of commensal-specific antibodies. Further, our work proposes a model by which commensal-specific antigens are captured in the hair follicle leading to the generation of T follicular helper cells that further expand the local B cell pool in an IL-21-dependent manner. We also found that commensal-induced antibodies can protect the host against subsequent infection with the same microbe demonstrating the importance of this line of defense in maintaining tissue barrier integrity. Collectively, our observations highlight the autonomous potential of skin organizational flexibility and for the first time demonstrate that B cells represent an indispensable local cutaneous compartment that actively maintains skin immunity.

Poster #35

Harrison, Mitra
NIAID

Assessment of Key Elements Required for Efficient Induction of an HIV-1-Specific Neutralizing Antibody Response

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The immunogenicity of HIV-1 Envelope (Env) glycoprotein, the sole neutralizing determinant on HIV-1 virions, is limited by its structural instability and extensive glycan shielding. Stabilized trimers can elicit neutralizing antibody responses but require many immunizations and the response is short-lived. In contrast, replicating vectors can more rapidly induce durable neutralizing antibodies. To understand the parameters of a live virus infection that confer a durable and broad neutralizing antibody response, we made Newcastle disease virus (NDV) virus-like particles (VLPs) expressing the HIV-1 Env, Influenza HA, or SARS-CoV-2 Spike. Influenza HA and SARS-CoV-2 Spike VLPs rapidly induced neutralizing antibodies (median ID50 2787 and 447, respectively) by week 8 after IM injection of rabbits. The introduction of stabilizing mutations in Env to maintain a native-like structure resulted in a high valency particle, but neutralizing antibodies were not induced. Formulation with AS01 adjuvant and packaging of a toll-like receptor stimulating RNA (RNA40) into the VLP induced modest neutralizing antibody titers that peaked at 14-22 weeks (median ID50 302 and 40, respectively). However, neutralizing antibodies fell below the level of detection by week 41. We observed the greatest improvements in durability and breadth upon increasing total dosage and with escalating dose immunization. Rabbits immunized with an escalating dose of HIV-1 VLPs with RNA 40 formulated with AS01 developed a neutralizing antibody response, peaking at week 24 (median IC50 869). Sera from 5 of 6 rabbits within this group neutralized at least 9 of 10 diverse HIV-1 pseudoviruses. Our results indicate that numerous features of a live virus infection, including conformation, spike density, TLR stimulation, total dose, and the presentation of viral glycoproteins over a prolonged period, can be combined to overcome the poor immunogenicity of HIV Env and suggest features to engineer into immunogens that might stimulate a broad and durable neutralizing antibody response.

Poster #36

Hemani, Humza
NIA

Longitudinal somatic mutation analysis of individual human CD8+ T cells by a UMI-based single cell mutation detection (USCMD) method

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Somatic mutation contributes to the developing cancer and aging. However, the degree of somatic mutation and its potential impact on human CD8+ T cell aging have not been analyzed. DNA-based single-cell mutation detection methods have been developed but are hindered by the limited number of cells examined and compounded by errors from PCR amplification and sequencing methods. Here, we developed a UMI-based scRNAseq mutation detection (USCMD) method that allowed us to examine 5000-10,000 cells per sample with reduced PCR and sequencing errors. We applied USCMD to analyze the scRNAseq data from human CD8+ T cells that were collected with 2-3 visits of each donor with total of 8 donors (aged from 30 to 69 years at first visit with an average of 9 years from first to last donation). We found that some novel missense mutations were associated with either gain or loss of their carrying cells as well as an increase or decrease in mRNA levels of the mutated genes compared to the unmutated genes. Together, this USCMD method improves the accuracy of mutational analysis of scRNAseq data and allows parallel transcriptional assessment of mutated and unmutated cells to understand their potential functional consequences.

Poster #37

Hirofumi, Shibata
NIAID

CRISPR screening for cytotoxic granule degranulation pathway in CD8 T cell
Hirofumi Shibata and Pamela L. Schwartzberg

Laboratory of Immune System Biology, NIAID, NIH

Degranulation of perforin-containing cytotoxic granules is an important factor for NK cells and CD8 cytotoxic T lymphocytes (CTLs) to kill infected cells or tumors. Defects in this pathway cause the primary form of hereditary hemophagocytic lymphohistiocytosis (HLH), a fatal hyper-inflammatory condition, which is known as familial HLH (FHL) syndrome, one of inborn errors of immunity (IEI) caused by immune dysregulation. Its causative genes include UNC13D (FHL3) encoding munc13-4, STX11 (FHL4) encoding syntaxin-11, and STXBP2 (FHL5) encoding munc18-2; these molecules are involved in the intracellular trafficking of cytotoxic granules and their fusion to the plasma membrane, followed by the delivery of their contents into the target cell through immunological synapses. However, other components essential for cytotoxic granule degranulation for CTLs are not well understood.

To identify indispensable molecules for cytotoxic granule degranulation, we established an efficient in vitro CD8 amplification and degranulation system to perform a CRISPR-screening. Using an optimized sgRNA-Thy1.1. retroviral system, we could obtain near 80% transduction efficiency of CD8 cells, which then could be differentiated in IL-2 to obtain mature CTLs.

Preliminary experiments showed that many proteins involved in FHL are relatively stable and that complete knockout of these molecules by CRISPR required amplification of knockout cells over long (15d) culture periods. After transduction with a gRNA library targeting PIP3 binding proteins, which are important for cell adhesion, subcellular trafficking and T cell activation, cells were harvested at short (Day 8) and medium-timepoints (Day 15) and to evaluate the frequency of gRNAs expression in the degranulated and non-degranulated fractions.

To expand to genome-wide screening, we have also cloned a library of sgRNAs into our high efficiency retroviral vector. Once promising candidate genes are identified, we will use individual knockouts to confirm their functions and cross-reference for disease associations using patient databases.

Poster #38

Ikeuchi, Tomoko
NIDCR

Fibroblasts specific response in the oral inflammatory disease periodontitis

Tomoko Ikeuchi, Hannah Brooks, Teresa Wild, Drake Williams, Tae Sung Kim and Niki Moutsopoulos

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Periodontitis is one of the most prevalent inflammatory diseases and strongly affects the quality of life in humans. In the disease, oral mucosal inflammation leads to destruction of tissues surrounding teeth, primarily bone, which causes tooth loss. Dysbiotic microbial biofilm is considered as one of the triggers of periodontitis, however removing the biofilm does not resolve all the inflammation. Interestingly, the oral cavity is a unique environment with mucosal and bone-related immunological aspects: the oral mucosa responds to the change of environment including pathogens and the subsequent inflammation causes bone loss. We have seen that the host response to the environmental shifts (dysbiosis or damage) activates immune cells and results in bone loss. Not only immune cells, but fibroblasts are also known as a major player of the microenvironment pathogenesis especially in the cancer field. Here, we hypothesized that fibroblasts in the oral cavity may engage inflammatory responses in periodontitis. In order to understand the fibroblast responses during pathogenesis, we performed scRNA-seq in mouse oral mucosal tissues using the experimental periodontitis model. Our work characterizes fibroblast subpopulations in oral mucosal tissues in mice, inflammatory responses in fibroblast subpopulations at early time points, and the role of the stromal compartment in the pathogenesis of periodontitis.

Poster #39

Ireland, Derek
FDA

Neonatal mouse model of SARS-CoV-2 and variants of concern to evaluate therapeutics.
Ireland, Derek D.C., Manangeeswaran, Mohanraj, Mendoza, Mirian, Logan Kelley-Baker and Verthelyi, Daniela I

FDA/CDER/OPQ/OBP/DBRRIII

Since first reported in 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is rapidly acquiring mutations, particularly in the Spike protein, that can modulate pathogenicity, transmission and antibody evasion leading to successive waves of COVID19 infections despite an unprecedented mass vaccination necessitating continuous adaptation of therapeutics. Small animal models can facilitate understanding of host-pathogen interactions, target selection for therapeutic drugs, vaccine development and the safety of therapeutics. We and others have shown that the expression of human ACE2 is required to ensure susceptibility to SARS-CoV-2 infection in mice. This study developed a model of SARS-CoV-2 infection to study pathogenesis and the immune response to infection in neonates. Strikingly, challenge of neonatal hACE2tg mice with SARS-CoV-2 Variants of Concern, which are defined by mutations in the Spike proteins (SARS-CoV-2-α, -β, ϒ, Δ or Ω), resulted in more rapid and severe disease with severe neurological damage, even with significant lower inoculation titres. Further, this model provides a platform in which the safety and efficacy of SARS-CoV-2 therapeutics, particularly monoclonal antibodies targeting the Spike protein can be evaluated. Prophylactic treatment with monoclonal antibodies targeting the receptor binding domain (RBP) of this protein resulted in protection from infection. These studies at BSL-3 are also used to validate studies using VSV-based pseudoviruses expressing the SARS-CoV-2 spike protein from Variants of Concern, which can be utilized at BSL-2.

Poster #40

James, Alyssa
NIAID

Damaging OSMR variants differentiate severe non-allergic pruritus from atopic dermatitis in humans

Alyssa E. James, Mehul Sharma, Yihui Liu, Christina Michalski, Michael P. O'Connell, Kate Del Bel, Sheryce Lassiter, Judy Bandoh, Julie Niemela, Henry Lu, Ashish Sharma, Bhavi Modi, Wingfield Rehmus, Pascal Lavoie, Margaret McKinnon, Kevin Conlon, Milos M
NIH/NIAID, British Columbia Children's Hospital, University of British Columbia, DLM/CC/NIH, Emory University, NCI/NIH, University of Mississippi Medical Center, University of Michigan, Ann Arbor, Columbia University, USAF, Memphis Dermatology Clinic, Finnish Institute for Health and Welfare, University of Helsinki/Helsinki University Hospital, University of Virginia, Walter Reed National Military Medical Center, University of Iowa, Icahn School of Medicine at Mount Sinai

Oncostatin-M receptor (OSMR) is a member of the GP130 superfamily and co-receptor for OSM and IL-31, cytokines associated with pruritus. OSMR up-regulation is associated with inflammatory skin conditions and IL-31-signaling is implicated in chronic hives and atopic dermatitis (AD). Despite this, loss-of-function variants in these pathways including recessive loss-of-function variants in IL6ST encoding GP130 have been associated with AD and elevated IgE. We examine the clinical impact of damaging variants in OSMR and identify a divergence in the role of OSMR in the pathogenesis of itch and atopy.

Three individuals with biallelic loss-of-function variants in OSMR were identified with moderate-severe AD despite minimal pruritus; two were homozygous [OSMR c.1307T>A (p.V436D)] and the third was a compound heterozygote [OSMR c.1307T>A (p.V436D); c.1046C>A (p.A349D)]. Primary dermal fibroblasts (DF) demonstrated reduced surface expression of OSMR - a finding recapitulated via forced over-expression of mutant constructs in vitro - and reduced pSTAT3/5 after stimulation with OSM or IL-31.

Three additional kindreds were identified with gain-of-function variants in OSMR co-segregating with autosomal dominantly inherited intense pruritus. IgE-mediated allergic diseases including AD were notably absent. One individual [OSMR c.1838T>C (p.L613S)] also presented with lymphocytic variant hypereosinophilic syndrome that transformed into a cutaneous T cell lymphoma. The most severely affected individual was homozygous [OSMR c.1891G>A (p.V631M)] and presented with diffuse lichen simplex chronicus and associated subepidermal amyloid deposition; his two affected children, heterozygous for the variant, had less severe symptoms and no detectable amyloid. The final individual [OSMR c.2170C>T (p.P724S)] also presented with adult-onset eosinophilic esophagitis. DF lines demonstrated increased pSTAT3/5, pMEK, and pERK1/2 after OSM or IL-31 stimulation.

Together these findings demonstrate that while OSMR-mediated signals contribute to cutaneous pruritus, severe AD may develop in the absence of IL-31 signaling, challenging the importance of this pathway - and potentially itch - in the pathogenesis of AD.

Poster #41

Javaid, Ayesha
NIDCR

Quantitation and Characterization of the Immune Cell Response within COVID-associated Pernio Lesions

Ayesha Javaid, B.S. (1), Ana Costa da Silva, Ph.D. (1), Lisa Arkin, M.D. (2), Jacqueline Mays, D.D.S., Ph.D. (1).

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(2) School of Medicine and Public Health, University of Wisconsin-Madison, Department of Dermatology

During the COVID-19 pandemic, cases of COVID-associated pernio, also known as COVID toes, surged globally. Pernio is a rare inflammatory condition associated with type 1 interferon-related disorders including lupus and the interferonopathies. Affected patients develop pain and swelling, followed by red-violaceous discoloration in the fingers and toes, typically with onset during colder weather months. Pernio emerged in 2020-2021 in patients who frequently reported recent exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), suggesting a viral linkage. Interestingly, most pernio patients were otherwise asymptomatic without extracutaneous symptoms, and with low rates of PCR or seroconversion, suggesting that the phenomenon might confer resiliency to typical manifestations of COVID-19 infection.

As part of a multicenter collaboration, we analyzed the local immune response in COVID-associated pernio finger and toe biopsies. Previous studies reported increased expression of IFN-1 in pernio and COVID-affected tissue. Using tyramide chemistry and traditional immunohistochemistry, we detected increased expression of CD303, a marker for plasmacytoid dendritic cells (pDCs), a main producer of IFN-1 during viral infections, and MXA, a key product of the IFN-induced antiviral response in pernio in comparison to controls (pre-2019 normal, pernio, and psoriasis skin). We also investigated IFNg, an antiviral cytokine, HLA-DR, a marker of immune cell activation, and CD3, a T cell marker. Tissue sections were imaged on a multispectral Leica SP8 confocal microscope, then immune cell expression was counted in each tissue using manual and positive color pixel counting tools on FIJI software. All numbers were normalized by the total tissue section area (um^2). Our analysis detected an increased infiltration of pDCs exhibiting robust IFN signaling along with activated T cells COVID-associated pernio effector sites. Interestingly, many activated cells are neither pDCs nor T cells, suggesting a role of other immune and stromal cells in the pathogenesis of COVID.

Poster #42

Jiang, Jiansheng
NIAID

Structures of Antibodies and Nanobodies in Complex with Spike/RBD: The Vital Role of CDR Loops in Capturing Epitopes

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Thousands of structures of SARS-CoV-2 and antibodies, either determined by X-ray crystallography or CryoEM, have been determined and deposited in the Protein Data Bank since 2020. We investigated 267 antibodies and 58 nanobodies that are in complex with the spike protein or its receptor-binding domain (RBD). We have identified 23 Frequently Contacted Sites (FCS) on the RBD surface and revealed the critical role of the complementarity determining region (CDR) loops in recognizing epitopes. The clustering analysis reveals many binding motifs associated with CDR loops. This structural information provides insights for structure-based next-generation vaccine design and the development of therapeutic strategies against future variants of this type of disease.

Poster #43

Jones, Madalyn
NIAID

CD8+Helios+ T cells: A unique functional T cell subpopulation?
Madalyn Jones, Ethan Shevach

National Institute of Allergy and Infectious Diseases, National Institutes of Health

The zinc finger protein, Helios, is a member of the Ikaros transcription factor family and is expressed in CD4+ T cells, CD8+ T cells and in some NK cells in both mice and humans. While it has been found to have a role in the homeostasis and suppressive function of CD4+ Foxp3+ T regulatory cells (Treg), its function in CD8+ T cells and NK cells is not well characterized. We have demonstrated that ~10-40% of CD8+ T cells from normal donors express Helios. Expression of Helios in CD8+, but not CD4+ T cells, can be upregulated by TCR stimulation in the presence of TGF□. Thus far, extensive studies have not shown a correlation between Helios expression and any other surface or intracellular markers. Mouse CD8+Helios+Ly-49+ T cells have been reported to inhibit the activation of B cells in germinal centers and have been claimed to be the CD8+ counterpart to CD4+Treg. While these studies have been performed in mice there is very little data to suggest that human CD8+Helios+ T cells exhibit T suppressor function. We are using a variety of methods including multiparameter flow cytometry, scRNA-seq analysis, and a CRISPR/Cas9 gene editing approach to attempt to answer the numerous questions surrounding the function of Helios in human CD8+ T cells.

Poster #44

Kaczanowska, Sabina
NCI-Bethesda

Myeloid-based approaches for cancer immunotherapy

Sabina Kaczanowska, Rosandra Kaplan

Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health

Myeloid cells are key regulators of the immune system and can dampen the antitumor response against cancer. Immune suppression is a major hurdle in cancer immunotherapy for solid tumors. We comprehensively characterized the immune population dynamics in the pre-metastatic niche and identified a core program of myeloid-mediated immune suppression. We hypothesize that targeting this myeloid-mediated immune suppression program could facilitate antitumor immune activation and be a successful immunotherapeutic approach. To take advantage of the large infiltration of myeloid cells into tumor and metastatic sites, we developed the genetically engineered myeloid cell (GEMy) technology to deliver therapeutic factors and restore antitumor immunity. We demonstrated that interleukin 12-secreting GEMys can functionally rebalance the dysregulated metastatic microenvironment in cancer and result in remarkable tumor responses in pre-clinical models that we are currently translating into the clinic. Further, while chimeric antigen receptor T cells (CAR-Ts) have demonstrated exceptional responses in leukemia and lymphomas, their efficacy in solid tumors has been limited. To understand the contributions of the peripheral immune milieu to CAR-T expansion, we evaluated patient samples from a Phase I clinical trial of GD2-CAR-Ts in children and young adults with osteosarcoma and neuroblastoma by multi-dimensional proteomic and sequencing analyses. We identified transcriptional programs and unique monocyte populations associated with good versus poor CAR-T expansion in pre-treatment patient apheresis. These data suggest key T cell-extrinsic factors underlying CAR-T function and identify myeloid populations that can be modified to improve immunotherapy outcomes for solid tumor patients.

Poster #45

Kang, Byunghyun
NIAID

Segmented filamentous bacteria (SFB) drives increased T cell-dependent polyclonal IgA and IgG2b responses in Peyer's patches by enhancing cDC-T cell interactions and the conversion of Th17 to Tfh cells.

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Segmented filamentous bacteria (SFB, Candidatus savagella) are commensal bacteria that preferentially colonize the ileum, particularly over Peyer's patches (PPs). SFB colonization of germ-free mice induces IgA responses by unclear mechanisms. We found that SFB-colonized SPF mice also have higher baseline IgA levels and develop more potent IgA responses to oral immunization and reovirus infection than uncolonized mice. PPs are the major site for T-cell-dependent, and a minor site for T- cell-independent IgA B-cell differentiation. CCR6+ B-cells and dendritic cells (DCs) interact in the PP subepithelial dome region (SED) to initiate IgA B-cell isotype switching, followed by further B-cell development in germinal centers. Using single cell mRNA sequencing of PP cells, we showed that IgA+, but also IgG1+ and IgG2b+ B-cells are present in the germinal center B-cell population, and that SFB colonization increased the numbers of IgA+ and IgG2b+, but not IgG1+ PP B-cells. Enhanced class-switching was dependent on CCR6, T cells, Tfh cells, and DC-expression of MHC class II, as well as CD40-CD40L interaction and to some degree IL-6. SFB colonization broadly affected the B-cell repertoire. Furthermore, an increased frequency of CCR6-expressing dome B-cells and T-cells together with a significant increase of activated CD11c+SIRPa+BST2-CD11b+ dome resident DCs with high levels of MHCII and CD86 by scRNAseq and flow cytometry in SFB+ compared to SFB- PP is consistent with increased initiation of class switching in the PP SED. Finally, our preliminary results from scRNAseq and flow cytometry suggest that SFB colonization bolsters the conversion of Th17 into Tfh cells by increasing CCR6+ activated T-B interaction in the SED. Together these data contribute to our understanding of how commensal microbes affect IgA responses to infection and vaccination.

Poster #46

KAUL, ZENIA
NIAID

IL-2 rescues impaired T cell function upon loss of Itk via metabolic reprogramming
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IL-2 inducible T cell kinase (Itk) is a critical component of T cell receptor (TCR) signaling required for activation of PLCgamma and downstream transcription factors (NFAT, AP1, NF-KB) that regulate expression of effector molecules. Loss of Itk impairs T-cell activation by modulating the strength or duration of TCR signal. We and others have previously reported that Itk-/- mice have defects in T cell development with decreased proliferation and impaired differentiation to multiple CD4 T helper lineages. Most severe defects are seen in the differentiation of Th9 cells, which produce the inflammatory cytokine, IL-9. We find that under Th9 differentiation conditions Itk-/- cells initially appear similar to WT cells but subsequently fail to sustain expression of activation markers and IL-9. Th9 differentiation could be rescued by inclusion of IL-2, a potent T cell growth factor. Itk-/- CD4 T cells show decreased mTORC1 signaling and do not maintain expression of IRF4, c-Myc and multiple nutrient transporters—including the Glucose transporter 1 (GLUT1), transferrin receptor (CD71), and amino acid co-transporter (CD98), all of which were rescued by IL-2. As expression of many nutrient transporters was low in Itk-/- cells, we used SCENITH—a flow cytometry-based technique that profiles metabolism through its effects on protein translation. We found that WT Th9 cells were primarily glycolytic but Itk-/- T cells were more dependent on mitochondrial respiration and exhibited less protein synthesis. Inclusion of IL-2 in Itk-/- cultures rescued metabolism and protein synthesis so that metabolic profiles resembled those of WT cells. Our results suggest that metabolic fitness and flexibility are central to dictating the functional/effector fate of CD4 T cells and that this requires the Tec kinase Itk and IL-2-mediated pathways, providing novel insight into mechanisms by which IL-2 can rescue T cell functional defects.

Poster #47

Kim, Yong-Hee
NIAID
Treg cell depletion in adult mice results in activation of antigen-presenting cells prior to fatal autoimmune disease

Yong-Hee Kim, Ethan M. Shevach

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Studies in mice expressing the diphtheria toxin receptor (DTR) exclusively on regulatory T (Treg) cells (Foxp3-DTR mice) demonstrated the critical importance of Treg cells in maintaining normal immune homeostasis. Adult Foxp3-DTR mice injected with DT begin to die from day 10 after DT treatment. Marked expansion of almost all immune cell types was observed prior to death. The purpose of the present studies was to examine in-depth changes in lymphocytes and antigen-presenting cells at early time points after Treg depletion to determine the most critical cell types controlled by Treg in the steady state. Complete depletion of Treg was observed 2 days after treatment, but profound activation of CD4(+) and CD8(+) T cells as measured by induction of CD44 expression and vigorous proliferation as measured by Ki-67 incorporation were not seen on day 2 but seen on day 6 of DT treatment. Curiously, most of the CD44(hi)Ki-67(+) T cells were CD25(-) and plasma levels of IL-2 or intracellular levels of IL-2 were not increased suggesting that early activation of CD4(+) and CD8(+) T cells was not the result of a burst of IL-2 production. On the other hand, the percentages of both IFN-gamma and TNF-alpha-producing T cells were increased and elevated concentrations of IFN-gamma and TNF-alpha were detected in the plasma on day 6. Plasma levels of CXCL9 and CXCL10 were also elevated consistent with the elevated concentration of IFN-gamma. Increased expression of CD80, CD86, CD40, and PD-L1 could be detected on CD11c(+) dendritic cells (DCs) on day 6. Taken together, these studies suggest that disruption of the regulation of Treg-mediated control of DC activation represents the first step in the complex autoimmune disease seen after Treg depletion.

Poster #48

Krishnamurthy, Siddharth
NIAID

Nutritional and Microbiota Synergy Control Enteric Coronavirus Infection

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To establish infection, enteric viruses must adapt to the intestinal millieu such as the microbiome and metabolites. However, mechanisms viruses use to adapt to this environment remain poorly understood. Such adaptation is particularly important, for coronaviruses in which there are strain dependent tropisms. While classical strains of mouse hepatitis virus (MHV) infect systemically, numerous unstudied MHV strains, like MHV-Y, productively infect the intestinal tract. Here, we develop a novel enteric model of coronavirus infection to explore how coronaviruses adapt to the intestinal environment.

Following oral infection with MHV-Y, Peyers patches and mesenteric lymph node (mLN) contained infectious virus under various nutrition conditions. However, colonic infection of MHV was highly dependent on the diet fed to the mouse, specifically diet enriched in fibers were required for colonic infection. Removal of the microbiota via antibiotic or gnotobiotic conditions had no impact on viral titers in the Peyers patches and mLN but prevented colonic infection. Acetylated starch restored colonic viral titers in antibiotic treated mice, supporting the idea that infection of MHV in the colon, but not other tissues, depended on short chain fatty acids produced by the microbiota in response to fiber. Comparative genomics of multiple enteric MHV strains revealed that enteric MHV strains encode a functional hemagglutinin esterase (HE), unlike most non-enteric MHV strains. HE encodes for an envelope protein that binds to 4-O acetylated sialic acid (Neu4,5Ac2) and has been shown to increase neurovirulence of neurotropic MHV strains. Confocal imaging revealed that Neu4,5Ac2 is abundantly expressed in the colonic lamina propria, and that its expression is abrogated in the context of antibiotics.

We propose that MHV enterotropism is controlled by the nutritional status and microbiota of the host as together, they allow expression of additional attachment factors to be expressed in the target tissue.

Poster #49

Krug, Laurie
NCI-Bethesda

Vaccination with a Replication-Dead Gammaherpesvirus Protects against Wild-Type Virus Replication, Reactivation, and Disease in Mice

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The gammaherpesviruses are oncogenic viruses that establish lifelong infections. While several vaccine strategies to limit gammaherpesvirus infection and disease are in development, there are no FDA-approved vaccines for Epstein-Barr virus or Kaposi sarcoma herpesvirus. Using murine gammaherpesvirus-68 (MHV68), a mouse model for gammaherpesvirus pathogenesis studies, we previously developed a codon-shuffling-based complementation strategy that enabled the production of replication-dead MHV68 (RDV). This method prevents recombination with the helper gene and reversion to virulence in the RDV vaccine due to WT virus contamination. We generated an RDV lacking expression of the essential replication and transactivator protein (RTA) to arrest viral gene expression early after de novo infection. Inoculation with RDV-RTA exposes the host to intact virion particles and leads to limited lytic gene expression in infected cells. Prime-boost vaccination of WT C57BL/6 mice with RDV-RTA elicited virus-specific neutralizing antibody and effector T cell responses in the lung and spleen tissues. In vaccinated mice challenged intranasally with WT MHV68, there was near complete abolishment of virus replication in the lungs 7 days post-challenge and virus reactivation from spleens 16 days post-challenge. IFNAR-/- mice, which lack the type I interferon receptor, exhibit severe disease upon infection with WT MHV68. RDV-RTA vaccination of IFNAR-/- mice prevented wasting and mortality upon challenge with 2x106 PFU of WT MHV68. These results demonstrate that prime-boost vaccination with a gammaherpesvirus that is disabled for lytic replication offers protection against acute replication, reactivation, and severe disease upon WT challenge.

Poster #50

Kulalert, Warakorn
NIAID

The neuroimmune CGRP/RAMP1 axis functionally tunes adaptive immunity to the microbiota
Warakorn Kulalert, Michel Enamorado, Alexandria Wells, Verena Link, Ai Ing Lim, Olena Kamenyeva, Juraj Kabat, Nicolas Bouladoux, Alexander Chesler, Isaac Chiu, Yasmine Belkaid

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The somatosensory nervous system surveils external stimuli at barrier tissues, regulating innate immune cells under infection and inflammation. The roles of sensory afferents in homeostatic adaptive immunity to the microbiota, however, remain elusive. Here, we identified a novel mechanism for direct neuroimmune interaction between commensal-specific T lymphocytes and skin-innervating sensory neurons through the neuropeptide calcitonin gene-related peptide (CGRP). Intravital imaging revealed that commensal-specific T cells are intimately associated with cutaneous nerve fibers in vivo. Correspondingly, we observed upregulation of the neuropeptide CGRP co-receptor, receptor activity modifying protein 1 (RAMP1), in defined subsets of T lymphocytes induced by commensals, but not in T cell populations elicited by cutaneous infection or inflammation. We then demonstrated that the neuroimmune CGRP/RAMP1 signaling axis acts cell-autonomously in commensal-reactive T cells to promote T cell migration and accumulation in the tissue, while simultaneously constraining production of key cytokines involved in barrier function. We proceeded to show that neuroimmune crosstalk via CGRP/RAMP1 in microbiota-induced T cells controls the skin epithelium physiology under homeostasis. Our characterization of the CGRP/RAMP1 signaling axis at this microbiota-neuroimmune juncture exemplifies the evolutionary partnership and ongoing dialog between the immune and nervous compartments at barrier tissues. Furthermore, the ability of the somatosensory neurons to participate in the highly specific and potentially long-lasting adaptive immune response to the microbiota underscores the functional versatility and plasticity of commensal-specific T lymphocytes that can be swiftly tuned to diverse arrays of sensory modalities ranging from thermosensation to noxious pain under steady state and pathology.

Poster #51

Kumta, Katie
FDA-CBER

Differential inhibition of the Type I IFNs by soluble IFNAR2

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Type I Interferons (IFN-I) are cytokines that promote transcription of antiviral proteins, making them key players in immune responses to viral infection. In addition to IFNβ, there are 12 human subtypes of IFNα, all of which signal through the IFNAR1/2 heterodimer and activate transcription of interferon-stimulated genes (ISGs) via the JAK/STAT pathway. There are known differences in receptor complex stability among Type I IFN, with IFNα1 and IFNβ demonstrating the lowest and highest affinities respectively. Whether there are qualitative differences among IFN-I is controversial, and it is unclear why there are multiple type I IFN genes. Soluble IFNAR2 (sIFNAR2) is an alternative splice product of the IFNAR2 gene that acts as a decoy receptor and inhibits interferon signaling. We explored whether sIFNAR2 reveals qualitative or quantitative differences among IFN subtypes by measuring STAT1 phosphorylation, ISG transcription, and antiviral activity in response to five IFN-I subtypes of varying affinities for sIFNAR2. We show that the extent of inhibition by sIFNAR2 of IFNs correlated with IFNAR2 binding affinity. Thus, while high affinity IFNs such as IFNβ and IFNα14 demonstrated strong inhibition of STAT phosphorylation and transcription by 1 μg/mL of sIFNAR2, inhibition of cellular responses to IFNα1 was not observed. Thus, sIFNAR2 provides a mechanism by which low affinity IFN-I may compete with higher affinity IFN for activation through the IFNAR1/2 heterodimer.

Poster #52

Lam, Khiem
NCI-Bethesda

Tumor-intrinsic factors dictate beneficial effect of microbiota-targeted therapies

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Gut microbiota impacts antitumor immunity and recent studies have shown that manipulating microbiota via fecal transplant can rescue immune checkpoint blockade (ICB) response in refractory melanoma patients. However, not all patients benefited from this approach. This leaves us with the question of: What makes patients responsive to microbiota-targeted therapies? We hypothesize that tumor-intrinsic factors (e.g. neoantigen load and immune microenvironment) contribute to the microbiota effect on anticancer response. To test this, we performed preclinical microbiota manipulation studies using dietary intervention. We found that mice fed high-fiber diet (FD) had improved spontaneous tumor control and response to ICB. Mechanistically, FD-induced changes in microbiota composition skewed the tumor innate immune repertoire towards an antitumor profile. Downstream of the innate compartment, FD reduced intratumoral Tregs and exhausted CD8+ T cells and increased activated CD4+ T cells. We further assessed the effects of FD across 12 mouse syngeneic models including tumor subtypes. Although we observed an overall beneficial effect of FD across several models, not all responded to the same extent, and were accompanied by different changes in the tumor immune profile. Moreover, some tumors showed little to no response. To address whether tumor neoantigen burden (TNB) impacts the degree of FD response, we correlated the amount of non-synonymous (NS) mutations (a TNB proxy) with FD effect on tumor growth. We found a positive association between NS mutations and FD response, suggesting a role for tumor immunogenicity in regulating FD effects. Furthermore, artificially increasing tumor immunogenicity via ovalbumin transduction in the tumor cell line resulted in a greater in vivo FD effect. Our findings suggest that tumor-intrinsic factors such as TNB and immune profile influence the beneficial effect of microbiota on antitumor immunity. Further identification of these factors and their mechanisms of action will help refine the use of microbiota-targeted therapies in the clinic.

Poster #53

Lee, Ha-Na
FDA-CDER

Differential effects of therapeutic antibodies targeting the Ebola glycoprotein on rVSVΔG-EBOV-GP-induced acute and chronic ocular diseases

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Ebola virus disease (EVD) survivors experience ocular sequelae, which are frequently associated with cataracts and vision loss. Previously, we developed a BSL2-complement rVSVΔG-EBOV-GP (or VSV-EBOV) infection model and showed that a subcutaneous inoculation with VSV-EBOV (1000 TCID50) in neonatal mice leads to infection of neurons in retina, causing severe retinitis. Here, we demonstrate that our BSL-2 animal model can be used to test the therapeutic effects of anti-EBOV-GP antibodies in acute retinitis and long-term ocular sequelae by using polyclonal Ab (SAB-139), antibody-dependent cell cytotoxicity (ADCC)-mediating non-neutralizing monoclonal antibody (REGN3478), neutralizing monoclonal Ab (REGN3481) or the combination of REGN3478 and REGN3481. Treatment of those anti-EBOV-GP antibodies at 3 days post infection (dpi) dramatically reduced viral titers in the blood, and improved survival. In acute infection, REGN3481 or the combination of REGN3478 and REGN3481 were more effective at reducing viral load in the eyes, downregulating the immune and inflammatory responses and minimizing retinal damage compared to SAB-139 or REGN3478 alone. Surprisingly, treatment of REGN3481 as a single therapy failed to reduce the risk of cataract development as determined at 3-6 months post infection whereas none of infected mice treated with the combination regimen with REGN3478 and REGN3481 showed cataracts. Overall, treatment with anti-EBOV-GP antibody not only improved survival and viral clearance, but also reduction of long-term retinal pathology and degeneration required a combination of therapeutics. Together, our findings suggest that this neonatal VSV-EBOV infection system can be used to assess the therapeutics targeting EBOV-GP in the eyes, and enables studies that explore the concomitant use of multiple therapeutic approaches.

Poster #54

Lee, Sang
NIAID

MHCII- dermis resident macrophages orchestrate localized ILC2-eosinophil circuitries to maintain M2-like properties in cutaneous leishmaniasis

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Tissue-resident macrophages (TRMs) are critical for tissue homeostasis/repair, and their development is governed by the tissue niche-specific signals. Although the importance of these nurturing signals for TRMs to perform tissue-specific functions has been extensively studied, it remains poorly addressed how TRMs maintains its anti-inflammatory properties during tissue inflammation. We previously showed the importance of eosinophil-TRM cooperative interactions for M2-like properties of dermal TRMs during leishmaniasis; eosinophils provide IL-4 to dermal TRMs, and IL-4-stimulated dermal TRMs produce CCL24 in turn which functioned to recruit more eosinophils. Here we identified two independent subsets of dermal TRMs, MHCII+ MRlow and MHC-MRhi populations. The MHC-MRhi subset of dermal TRMs is the main producer of CCL24, and showed more M2-like transcriptional profiles than the MHCII+ MRlow subset. MHC-MRhi dermal TRMs are also the sole source of TSLP to activate innate lymphoid cell 2 (ILC2), which produces IL-5 to amplify eosinophil-TRM cooperative interactions. Both selective depletion of IL-5+ ILC2 or genetic ablation of TSLP from dermal TRMs diminishes localized TH2 circuitries to maintain M2-like dermal TRMs, and disease progression was ameliorated. Thus, we demonstrate that M2-like dermal TRMs actively maintain themselves by orchestrating both ILC2 and eosinophils via producing TSLP and CCL24 respectively during infection.

Poster #55

Li, Jiangyuan
NIA

Predicting the CD8+ TCRs recognizing a dominant influenza virus (IAV) epitope
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Influenza A virus (IAV) is a major human pathogen that causes seasonal infections worldwide. IAV-specific CD8+ T-cell response provides an essential part of immune protection against IAV infections. In HLA-A2+ humans, matrix protein M1 residues 58–66 (GILGFVFTL, referred to as GIL) is a dominant epitope and TCRs recognize GIL/HLA-A2 were enriched in TRBV19 gene usage. Over a thousand of distinct TCR sequences have been identified from GIL-specific CD8+ T cells (vdjdb.cdr3.net). We trained an ensemble random forest (RF) model on these GIL-specific TCR sequences and other CD8+ TCRs identified as non-GIL control. We then used the RF-model to test TCR sequences from GIL-tetramer positive CD8+ T cells identified in our influenza vaccine study and identified TCRs with high and low binding scores. To validate the accuracy of this RF model, we selected 56 TCRs with binding scores ranging from 0.1-1 (with 1 being highest binding confidence) and expressed them in a human T cell line (NJ76 cells). We demonstrated that the GIL-specific sorted CD8+ TCRs with model specificity scores greater than 0.90 have good binding with GIL tetramer and induced reporter activity upon stimulation with GIL/HL-A2 monomer in vitro. Our results show that machine learning (RF) model can predict accurately the binding specificity of CD8 TCRs based on their □□TCR sequences and will serve as a valuable tool to not only to determine the antigen binding specificity of TCRs but also predicting the potential CD8+ T cell response against the antigen/pathogen.

Poster #56

Liman, Nurcin
NCI-Bethesda

Death Receptor 3 is a co-stimulatory molecule for iNKT cells that potentiates their agonistic activation and triggers systemic inflammation

Nurcin Liman, Jung-Hyun Park

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The full activation of T cells requires the concomitant signaling from the TCR and from secondary co-stimulatory molecules, such as CD28. Unlike conventional T cells that are generated by weak TCR engagement in the thymus, invariant NKT (iNKT) cells are generated upon strong agonistic TCR signaling. iNKT cells are a small population of thymus-derived T cells with innate-like phenotype and function that play a disproportionally important role in immune regulation and surveillance. Thus, it has been a long-standing question whether iNKT cells would still require co-stimulation for their activation, and, if so, what these co-stimulatory molecules might be. Here, we show that stimulation with the agonistic ligand alpha-GalCer is indeed sufficient to activate iNKT cells, but co-ligation of the cytokine receptor Death Receptor 3 (DR3) dramatically boosts both the magnitude and scope of their activation, resulting in systemic production of pro-inflammatory cytokines and leading to severe thymic involution. DR3 is a member of the TNF receptor superfamily that we found to be expressed on all iNKT cells in peripheral tissues, although at varying degrees. While DR3-deficiency does not affect thymic development or homeostasis of iNKT cells, we found that DR3 expression was dynamically regulated on iNKT cells, suggesting distinct responsiveness to DR3 co-stimulation depending on differentiation or activation status of iNKT cells. Because DR3 ligation alone did not suffice to activate iNKT cells, and DR3 exerted its effects only in the context of TCR co-stimulation, these results strongly support DR3 being a new bona fide co-stimulatory molecule for iNKT cells. Considering the interest of utilizing iNKT cells in adoptive T cells therapies and as anti-tumor bioreagents, employing DR3 as a tool to boost iNKT cell immunity will open new venues in translational and clinical research.

Poster #57

Lin, Qiaoya
NCI-Bethesda

IFN-gamma targets tumor vascular endothelial cells causing impaired perfusion and tumor growth suppression in adoptive T cell therapy

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CD8 T cell-secreted interferon-gamma (IFNγ) is a key factor in controlling adoptive T cell therapy (ACT) outcome. While direct effects of IFNγ on tumor cells have been well characterized, little is known about IFNγ effects on non-tumor cells (e.g., stroma and blood vessels) that could indirectly impact the therapy. We aim to dissect spatiotemporal effects of IFNγ in the tumor microenvironment in ACT and the underlying mechanism of the therapy. An ACT using ex vivo-activated ovalbumin (OVA) specific OT-1 T cells suppressed the growth of MCA-205-OVA-GFP fibrosarcoma or MOC2-SIINFEKL oral squamous cell carcinoma in wild-type mice. However, in IFNγR1-deficient hosts, the ACT failed, indicating that non-tumor cells were crucial targets of IFNγ. Furthermore, the efficacy was also abrogated in bone-marrow chimeras, generated by transferring the wild-type bone-marrow to lethally irradiated Tie2-Cre-Ifngr1flox/flox mice, that lack IFNgR1 expression specifically in endothelial cells. This indicates that IFNγ action on endothelial cells was indispensable. Early after T cell transfer (1.5 days), the fraction of OT-1 T cells infiltrating the tumor was small (0.098% of CD45+ cells), but high IFNγ production (36.3%) resulted in the peak IFNγ concentration (47.3 pg/ml) in the whole tumor tissue in a 7-day observation period. In contrast, the highest accumulation of OT-1 T cells with low IFNγ production (2.9%) was observed on day 4.5. Intravital imaging revealed that at day 1.5, IFN-γ-RES-YFP-OT-1 T cells with a high level of IFNγ secretion (strong YFP+ signal) localized mainly along the tumor vessels. CD31+ endothelial cell density decreased on day 4.5, significantly reducing blood perfusion in the entire tumor, as observed by near-infrared imaging after IR800-albumin infusion. In summary, our study demonstrates that endothelial cell targeting of CD8 T cell-derived IFNγ is responsible for tumor clearance in ACT, impairing blood perfusion. These results provide novel insights into the mechanism of ACT.

Poster #58

LIN, BIN
NIAID

NEMO Exon 5 Skipping led to a systemic autoinflammatory syndrome via excessive cell death

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NF-κB essential modulator (NEMO, encoded by IKBKG) is an essential gene in immune response, development, and cell death regulation. Mutations in NEMO have been associated with 3 Mendelian phenotypes with developmental defects and/or immunodeficiencies but a systemic inflammation phenotype was rarely reported. Here we identified a group of 20 patients (15 females and 5 males) with systemic inflammation caused by 10 different splice variants resulting in exon 5 skipping of NEMO. Common clinical features include panniculitis with systemic inflammation (100%), ectodermal dysplasia (83%), hepatosplenomegaly (77%) and B-cell lymphopenia (80%). We named this disease as NEMO deleted exon 5 autoinflammatory syndrome (NEMO-NDAS). Cell death was observed in skin and liver biopsies. Moreover, enhanced levels of soluble TNFR1 and TNFR2 were detected in serum compared to healthy controls, which further supported a role of cell death in the pathology. To understand the disease mechanism, U937 cell line with NEMO exon 5 skipping was created by CRISPR editing. This mutant cell line is highly susceptible to TNF induced cell death compared to the wildtype U937 cells. The cell death can only be partially rescued by RIPK1 inhibitor Nec1s but fully rescued by the combinations of Nec1s and CASP8 inhibitor Z-IETD-FMK, which indicates a RIPK1- and CASP8-dependence with additional factors. NEMO-NDAS mutations cause partial loss-of-function in NEMO-mediated TBK1 activation, IKKα/β activation and NFκB target gene expression. The cell death in mutant cells can be rescued by TNF inhibitor Adalimumab or anti-TNFR1 antibody in a dose-dependent manner and in some conditions can be further enhanced by co-administration of the RIPK1 inhibitor Nec1s. Our study showed that NEMO exon 5 skipping mutations lead to susceptibility to TNF induced cell death that is RIPK1- and CASP8-dependent, which provide novel therapeutic options for treating these patients.

This work was supported by the NIH IRP of NIAID.

Poster #59

Liu, Lunhua
FDA-CBER

Age specific differences in murine alveolar endothelial cells to pathological conditions

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Neonatal pulmonary viral infections can have diverse outcomes ranging from bronchiolitis and pneumonia in respiratory syncytial virus infection to more subdued response in SARS-CoV-2 infection. In this study, we compared the murine lung alveolar endothelial cell (MLEC) responses of neonatal and adult mice to toll-like receptor agonists Poly I:C, ssRNA40 or R848 activated macrophages, which can mimic SARS-Cov2 induced innate cell activation, as well as the ability of neonatal serum to modulate endothelial cell responses. We found that when neonatal and adult MLECs were cocultured with macrophages activated with TLR agonist, significantly decreased TNFα and IL6 and elevated IL-10 were detected in macrophages. Macrophage co-culture with adult MLECs downregulated ACE2 and VEGFR2 regardless of the stimulation conditions. In neonatal MLECs, stimulation with Poly I:C or ssRNA40 downregulated ACE2 expression when macrophages were not present. In the presence of macrophages, the expression of ACE2 in neonatal MLECs increased with Poly I:C stimulation but decreased with ssRNA40 stimulation. Addition of neonatal mouse serum to MLECs increases the reactive oxygen species but downregulates multiple inflammatory signal pathways. Concurrently, significantly decreased expression of VE-cadherin and Shp2 were detected in neonatal mice exposed MLECs. In addition, neonatal serum compromised MLEC barrier integrity whereas adult mouse serum did not. Morphologically, neonatal mouse serum, but not adult mouse serum triggered the formation of lipid droplets in MLECs by upregulating the expression of scavenger receptors SR-BI and CD36. Taken together, neonatal and adult MLECs differentially regulate cytokine-secretion from activated macrophages, and neonatal serum breaks the tight junction formation between MLECs by down regulating Shp2 and VE-Cadherin expression and induces the formation of lipid droplets in MLECs through receptors SR-BI and CD36. The differences in neonatal and adult MLEC responses to pathologic conditions may reflect the age specific pulmonary manifestations to viral infections.

Poster #60

Lopes, Amelie
NCI-Bethesda

Interrogating the role of the immune microenvironment in brain metastases response to immunotherapy using new preclinical melanoma models

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Brain metastases (BrM) remain an intractable deadly complication for advanced melanoma patients and efficient therapeutic strategies are desperately needed. The tumor microenvironment (TME) plays an important role in response to therapy. However, studies addressing the contribution of the TME to therapy efficacy in the context of BrM are lacking, in part due to the scarcity of appropriate preclinical models. Here, we characterized the TME and response to immune checkpoint blockade (ICB) of two novel immunocompetent BrM models representative of mutant-RAS human melanoma subtypes: BR1 (mostly brain-tropic) and BR3 (widespread metastases). Using a new machine learning approach to quantify metastatic burden we showed that BR1 BrM are sensitive to ICB with a better response to anti-PD-L1/anti-CTLA4 combination therapy as compared to monotherapies. In contrast, BR3 BrM are resistant to both mono- and combination ICB therapy. Interestingly, we found that ICB efficacy on extracranial BR3 metastases is organ-dependent emphasizing the key role of the metastatic microenvironment in response to therapy. Characterization of the BrM immune microenvironment by high-parametric flow cytometry revealed a significant recruitment of immune populations essential for an effective anti-tumor response such as dendritic cells, natural killer cells, and T-cells uniquely in ICB-sensitive BR1 BrM, while neutrophils were enriched in ICB-resistant BR3 BrM. Moreover, we uncovered phenotypically distinct microglia populations exclusively present in BR1 BrM that positively correlated with T cell infiltration. Consistent with this finding, single-cell RNA sequencing revealed upregulation of genes encoding for T-cell-attracting chemokines and antigen presentation in the BR1-associated microglia. Altogether, our data emphasizes the importance of characterizing the BrM TME. Our unique BrM models, mirroring the diversity of patients' response to ICB, provide a robust platform to optimize BrM therapy. Deciphering the contribution of the newly identified BR1 BrM-associated microglia to ICB efficacy will be crucial to identifying novel therapeutic targets.

Poster #61

Lotspeich-Cole, Leda
FDA-CBER

Sustained antigen delivery improves germinal center reaction and increases antibody responses in neonatal mice

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Laboratory of Bacterial Polysaccharides, FDA/CBER/OVRR/DBPAP

Germinal centers (GCs) are crucial for development of protective immune responses to vaccination, supporting development of high-affinity plasma cells and memory B cells. Neonates are known to have limited germinal center formation after vaccination and therefore require multiple doses of most pediatric vaccines. Prolonging antigen presentation can overcome weak responses to vaccination by enhancing germinal center initiation. Although sequential dosing in adult mice was shown to increase antibody production through prolonged antigen retention in lymph nodes and enhanced germinal center formation, studies have not been conducted to examine if this strategy overcomes the weak germinal center development observed in neonatal mice. Utilizing a neonatal mouse model, we investigated immune responses to three tetanus conjugated pneumococcal type 14 (PPS14-TT) vaccine dosing regimens. Five-to-seven-day-old C57Bl/6 mice were given one of three vaccine regimens: a single 0.2 µg bolus dose on Day 0; four doses of 0.05 µgs on Days 0, 2, 4, and 6; or four escalating doses of 0.02, 0.04, 0.06, and 0.08 µgs on Days 0, 2, 4, and 6 respectively. Germinal center responses after vaccination were measured by ELISA, ELISpot, and flow cytometry. Neonatal mice receiving four sequential doses of PPS14-TT vaccine have increased in PPS14-specific IgG1 antibody titers compared to neonates receiving a bolus dose. Correspondingly, they also have significantly more PPS14-specific antibody secreting cells. In support of a model where prolonged antigen availability enhances GC development, we observed a significant reduction in T follicular regulatory cells (TFR) seven- and ten-days post-vaccination, corresponding with an increase in Foxp3- T follicular helper cells (TFH). TFH cells are critical for GC B cell support, and we observed an increased proportion of GC B cells in mice receiving sequential doses of the vaccine at day 10. These data demonstrate that extending antigen availability can overcome weak neonatal GC responses to vaccination.

Poster #62

Lubkin, Ashira
NIAID

Candida albicans pathogenesis in the context of mucosal type II interferonopathy
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Interferon-gamma (IFNg) is a powerful cytokine that is crucial for adequate host defense and thus is important for maintaining health in the face of the constant threat of pathogens. However, unchecked IFNg can cause autoimmunity in several organs and can even be directly toxic to host cells. Indeed, excessive IFNg, or type II interferonopathy, occurs in several diverse disease states, including STAT1 gain-of-function mutations, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), Downs syndrome, and AIDS. We have recently shown that in APECED, which is caused by deficiency of autoimmune regulator (AIRE), excessive IFNg leads to greater susceptibility to oral Candida albicans infection. C. albicans is both the most common human fungal pathogen and a normal member of the human microbiome. The ability of C. albicans to morph between commensal and pathogenic states is tied to its complex transcriptional regulation of virulence traits in response to environmental cues. Thus, the fact that C. albicans causes disease in APECED patients suggests that that the excessive IFNg in the oral mucosa leads C. albicans to transition to a more virulent state. We are using host-pathogen RNAseq to delineate the transcriptional response of C. albicans to excess IFNg. Further, we have found that during infection of oral epithelial cells, C. albicans becomes more invasive in the presence of IFNg. Thus, we are also using a metabolomic approach to investigate soluble mediators released by these cells in response to IFNg. These experiments will shed light on C. albicans virulence regulation in response to autoinflammatory conditions. Further, this study will provide new insights into the dualistic nature of IFNg — providing protection against many pathogens, while facilitating disease from a specific pathogen. These findings will have implications that go beyond APECED, to include the many conditions in which tissue-specific interferonopathy plays a role.

Poster #63

Maeng, Hoyoung
NCI-Bethesda

Evidences of immune response against epitope-enhanced peptide when vaccinated with peptides or peptide-pulsed DCs targeting TARP in patients with biochemically recurrent prostate cancer

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Background: TARP was first described by the Pastan Lab. It is expressed in >90% of prostate cancer. Two TARP-targeting vaccines were developed at the Vaccine Branch: peptides with adjuvant and peptide-pulsed autologous DCs. For the peptide platform, HLA-A*0201-restricted Wild Type 27-35 (TARP27-35WT) and Epitope-Enhanced 29-37(9V) (TARP29-37(9V)EE) were injected. Dendritic cells (DCs) were pulsed with peptides. The first-in-human study of two vaccines in biochemically recurrent prostate cancer (BCRPC, N=42) was separately reported (Wood et al, OncoImmunol 2016). Presented study focuses on vaccine immunogenicity.

Methods: Patients enrolled on Protocol 09C0139 were vaccinated on weeks 0, 12, 18, and 24 after 1:1 randomization between vaccine platforms. TARP-specific immune responses were assessed after vaccination. T cell responses were assessed by PBMCs that were cryopreserved. The MSKCC nomogram was used to calculate PSA slope. Decrease in PSA-slope log value by week 24 or week 48 was defined as clinical “response”. Thawed PBMCs were in vitro stimulated (IVS) for 7 days with APCs pulsed with TARP27-35WT, TARP29-37EE, or TARP29-37WT. PBMCs were tested for tetramer-specific T cells by flow cytometry and for cytokine responses by ELISPOT.

Results: Both peptide vaccines and DC vaccines induced TARP-specific T cells. The TARP29-37EE performed as hypothesized to induce specific T cells that can recognize both WT and EE sequence. ELISPOT assay results were analyzed and organized in descending order by the number of spots. All 6 top responders who had the strongest IFN-gamma ELISPOT response (>1000 to at least one peptide) were “responders”. ELISPOT responses were more frequent when immunized with DCs than peptides.

Conclusions: This first-in-human TARP vaccine study showed immunogenicity in BCRPC without any safety concerns. The EE peptide induced specific T cells that can recognize the WT counterpart. The ability of strong IFN-gamma ELISPOT response among the responders may suggest a protective role of vaccine-induced T cells.

Poster #64 has been withdrawn.

Poster #65

Majumdar, Shamik
NIAID

Ackr1-deficient mice are protected from lethal SARS-CoV-2 challenge

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To date, there have been more than 613 million confirmed cases of COVID-19, with over 6 million deaths worldwide. In severe cases, the causative agent of the COVID-19 pandemic, SARS-CoV-2, induces acute respiratory distress syndrome. To study the roles of chemokine pathways during COVID-19 pathogenesis, we performed an infection screen in mice deficient in chemokine ligands and receptors using the mouse-adapted strain of the virus. Weight loss and death were recorded for 2 weeks after infection of wild type mice, and mice genetically deficient for Ccr2, Ccr5, Ccr6, Cxcr3, Cxcr6, Cxcl10 or the atypical chemokine receptor Ackr1. Acute weight loss was observed in all infected mouse strains; however, Ackr1-/- mice uniquely displayed markedly increased survival (12% mortality [N=57] compared to 42% mortality in wild type mice [N=53]). ACKR1 (also known as the Duffy antigen receptor for chemokines) is a non-signaling receptor expressed on endothelial cells, erythrocytes and a subset of neurons that binds to a wide range of chemokines thereby controlling their availability as a scavenger and shaping chemotactic gradients. Comprehensive expression analysis revealed Ackr1 as the most highly induced chemokine receptor in infected lung at day 4 post-infection. Of note, COVID-19 mortality is disproportionately low in West Africa, overlapping with the geographic distribution of genetic deficiency of erythrocyte ACKR1 due to a single nucleotide polymorphism in the ACKR1 promoter that interrupts binding of the transcription factor GATA-1. This mutation was putatively fixed in the population under the selective pressure of Plasmodium vivax infection, which requires ACKR1 to enter erythrocytes. Further studies are required to understand the modulation of SARS-CoV-2 pathogenesis by Ackr1 and its potential to explain the trajectory and limited mortality of Covid-19 in Africa and to serve as a potential therapeutic target.

Poster #66

Mak, Nelly
NCI-Frederick

Evolutionary divergence in the IFITM genes of bat and avian species compromises antiviral function: implications for reservoirs of zoonotic viruses

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Interferon-induced transmembrane proteins (IFITMs) are antiviral proteins that provide cell-intrinsic immunity against viruses including influenza A virus, HIV-1, and coronaviruses. Polymorphisms in human IFITM3 are associated with severe outcomes of Influenza A virus and SARS-CoV-2 infections. A critical determinant of the antiviral activity of human IFITM3 is an amphipathic alpha-helix that has been shown to bind membrane cholesterol, insert into membranes, and modulate membrane fluidity. As a result, IFITM3 inhibits virus-cell fusion, blocking virus entry. Bat and avian species serve as reservoir hosts of pathogenic viruses with zoonotic potential, with horseshoe bats reported to be the origin of SARS coronaviruses. Characterizing immune irregularities that predispose certain species to serving as virus reservoirs is an emerging field of immense public health importance. Here, we characterized the IFITM gene repertoires in animals, with an emphasis on the amphipathic helix. We found that the amphipathic helices of bat IFITMs are structurally and functionally distinct from that of human IFITM3. By producing chimeric human/bat IFITM3 constructs, we found that an IFITM amphipathic helix from the greater horseshoe bat (Rhinolophus ferrumequinum) has lost its ability to restrict influenza A virus entry. This loss of function is associated with altered helical structure and reduced cholesterol binding potential in vitro. We further show that the amphipathic helices of duck and chicken IFITM3 exhibit little to no cholesterol binding activity, which may explain their reduced antiviral activity against influenza A virus. Our results demonstrate that natural variation in the amphipathic helix alone is sufficient to ablate the antiviral activity of IFITMs in reservoir species. Overall, divergence of the amphipathic helix and the subsequent loss of antiviral activity of bat and avian IFITMs may predispose these species to serve as virus reservoirs. These findings contribute to our understanding of the processes shaping zoonotic transmission and virus emergence in humans.

Poster #67

Maltez, Vivien
NIAID

Hijacking Suppression: Anti-CD40 Converts Regulatory T Cells Into Type I Effectors
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Pancreatic ductal adenocarcinoma (PDA) is an aggressive and heterogeneous cancer that is refractory to current treatments. To address this issue, we employ a mouse PDA-derived tumor clone library where individual clones elicit a spectrum of unique immune cell infiltration profiles. We found that the addition of anti-CD40 to the standard anti-PD1, anti-CTLA4 immunotherapy cocktail results in tumor regression only in tumor clones with high numbers of infiltrating T cells.

Our question is: what is the mechanism of action for anti-CD40 that empowers therapeutic responsiveness?

Our approach will leverage high multiplex microscopy and quantitative image analyses, enabling us to decipher the complexities of cellular behaviors, interactions, and phenotypes within an intact tumor microenvironment (TME). C57Bl/6 mice were subcutaneously implanted with tumor cells into both flanks.

We found that our therapy uniquely alters regulatory T cells (Tregs) in the TME, severely depleting, reprogramming, and restricting the remaining Tregs to the tumor periphery within 48 hours of anti-CD40 administration. In order to determine the fate of these cells, we used tamoxifen-inducible Foxp3 lineage tracing mice to label all Foxp3–expressing cells prior to therapy initiation. Strikingly, we found that many of the lineage marked Tregs no longer expressed Foxp3. These ExTregs now had high levels of Tbet and IFN□, and had evidence of cognate antigen recognition, as assessed via imaging of NFAT1 nuclear translocation in situ. Single and combination treatments in the absence of anti-CD40 failed to induce this ExTreg phenomenon. Blockade of MHC-II, IL-12, or IFN□ or tumor implantation into Batf3 KO, IL12p40 KO, or IFN□ KO mice also ablated this phenomenon. These data reveal a unique mechanism by which anti-CD40 amplifies the anti-tumor immune compartment through the concurrent depletion and conversion of immunosuppressive Tregs to an effector population.

Poster #68

Manangeeswaran, Mohanraj
FDA-CDER

BSL2-compliant lethal mouse model of SARS-COV-2 and variants of concern to evaluate therapeutics targeting the Spike protein

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Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) variants with increased ability to evade host immune response and increased transmission necessitates continuous testing of novel anti-SARS-CoV-2 therapeutics. The major bottleneck for testing large number of candidate therapeutics is the lack of small animal models that can be used under BSL-2 conditions. To generate the first BSL2-compatible in vivo system we used replication competent, GFP tagged, recombinant Vesicular Stomatitis Virus where the VSV glycoprotein was replaced by the SARS-CoV-2 spike protein (rVSV-SARS2-S). We show that infection of neonatal but not adult, K18-hACE2 transgenic mice (hACE2tg) leads to infection of the lungs and brains. Infection with rVSV-SARS2-S resulted in neuronal infection and encephalitis with increased expression of Interferon-stimulated Irf7, Bst2, Ifi294, as well as CxCL10, CCL5, CLC2, and LILRB4 in the brain and is uniformly lethal. Prophylactic treatment with anti-SARS-CoV-2 spike protein (RBD domain) monoclonal antibody resulted in 100% protection from lethal infection against rVSV-SARS2-S demonstrating the role of SARS-CoV-2 spike protein in infection and disease. Most importantly, our studies show that tropism, disease course and lethality mediated by the SARS-CoV-2 spike protein is comparable to SARS-CoV-2 infection under BSL-3 conditions. To demonstrate adaptability of the model to emerging variants of concern (VOC), we showed that rVSV-SARS2-S viruses expressing spike proteins from VOC (rVSV-SARS2-Spike-□, rVSV-SARS2-Spike-β, rVSV-SARS2-Spike-ϒ or rVSV-SARS2-Spike-Δ) resulted in rapid lethality (4 vs 10 days) compared to rVSV-SARS2-S. This highlighted the key role of Spike protein in determining the disease course in this model. We propose that rVSV-SARS2-S viruses can be an effective surrogate for the BSL-3 virus to test the potency of therapeutics targeting the spike protein of current or future SARS-CoV-2 VOC under BSL-2 conditions.

Poster #69

Mansoori, Mohammad
NIAID

Tregs suppress antigen-specific CD8+ T cells in vivo by depleting pMHC-I complexes from Dendritic Cells

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T Regulatory cells (Tregs) regulate antigen-specific immune responses by using a variety of mechanisms. Recent studies have shown that antigen-specific Treg can suppress CD4+ effector T cells in vitro and in vivo by depleting peptide-MHC-II complexes from the DC surface. It remains unclear as to how Treg suppress CD8+ T effectors, particularly in vivo. To explore Treg-mediated suppression of CD8+ T cells, we generated CD4+ iTregs from OT-II mice specific for Ova323-339 in association with I-Ab and determined their capacity to suppress CD8+ T cells from OT-I mice specific for Ova257-264 (SIINFEKL) in association with H-2Kb. CD4+ OT-II iTreg suppressed the in vitro proliferation of OT-I T cells in the presence of DCs pulsed with both Ova323-339 and Ova257-264 or with DCs singly pulsed with each peptide. Interestingly, the expansion of OT-I CD8+ T cells in vivo was not suppressed by OT-II iTreg when the two peptides were presented on separately pulsed DCs, but was suppressed when both peptides were presented on the same DCs. OT-II Tregs depleted the Kb-SIINFEKL complexes from the DC surface in vitro by a process of trogocytosis only in the presence of their cognate antigen. The uptake of Kb-SIINFEKL complexes by OT-II Treg was not secondary to leakage of free SIINFEKL from the DC surface as OT-II Treg from MHC-I deficient mice were as efficient in uptake of pMHC-I as Treg from wild type mice. Multiphoton imaging microscopy in vivo reveals a close interaction between Tregs and CD8+ T cells suggesting that suppression of CD8+ T cells in vivo requires close proximity between Treg and responder CD8+ T cell may involve an artificial synapse between Treg and CD8+ T cell.

Poster #70

Manthiram, Kalpana
NIAID

Trisomy 8-associated Autoinflammatory Disease (TRIAD) is Characterized by Dysregulated Myeloid Cells

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Constitutional trisomy 8 mosaicism has been associated with a Behçet's-like inflammatory disease, but immunologic features and treatment responses are not well-characterized. Here, we characterize a cohort of 20 individuals with trisomy 8 mosaicism (T8M) and inflammatory disease. Congenital dysmorphologies included camptodactyly, vertebral defects, strabismus, hydronephrosis, corneal opacities, macrocytosis, and bleeding diathesis due to platelets dense granule defects. Two participants had myelodysplasia (MDS). The majority of patients had recurrent fever and severe oral ulcerations, while nearly half had genital ulcers or rash similar to Behcet's disease. Colchicine, apremilast, and IL-1 and TNFa inhibitors were effective therapies. Two participants improved following hematopoietic stem cell transplant. With ddPCR on sorted cell populations, we found that cells from the myeloid lineage (monocytes and neutrophils) had a significantly higher percentage of cells with trisomy 8 compared to those from the lymphoid lineage (T and B cells) in both the peripheral blood and bone marrow, suggesting that an extra copy of chromosome 8 may confer a survival advantage preferentially to myeloid cells. By flow cytometry, we found that participants with T8M had more classical monocytes compared to healthy controls. During flares, inflammatory genes associated with activated neutrophils and monocytes were upregulated in whole blood. Single cell RNAseq revealed differences in monocyte subsets compared to healthy controls; using chromosome 8 gene expression to identify which cells were trisomy 8 and which were normal (disomy) in each individual, we found that trisomy 8 monocytes had differential expression of genes involved in apoptotic, oncogenic, and inflammatory pathways. Our findings suggest that patients with T8M are at risk for a distinct autoinflammatory disease characterized by mucosal ulcerations and recurrent fever which we propose calling trisomy 8 associated autoinflammatory disease (TRIAD). T8M is characterized by dysregulated myeloid cell function, thereby increasing patients' risk for TRIAD and MDS.

Poster #71

Maul, Robert
NIA

Variable gene splicing promotes AID activity during somatic hypermutation

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Activation-induced deaminase (AID) is recruited to B cell immunoglobulin genes to introduce point mutations and single-strand DNA breaks during somatic hypermutation (SHM) and class-switch recombination (CSR). During both mechanisms, AID recruitment is intricately linked to the process of RNA polymerase II transcription. During CSR, transcription of the IgH switch region promotes AID access at DNA secondary structure sites, which induce RNA polymerase pausing and RNA processing. However, similar DNA structural elements are not found in the variable gene suggesting a different mechanism for AID recruitment to this area. To address this mechanism, several labs have dissected different components of the variable gene, replacing the VDJ exon, promoter, and J-intron. While replacing these components has shown not defects in SHM, our lab has shown that inserting bacteriophage DNA sequence between the promoter and leader exon drastically reduced AID activity at transgenes (1). In combination with the work from Hein and Radbruch who knocked out switch region intronic splices sites (2), this suggests that proximity of the promoter to initiation of splicing events in the variable gene may contribute to AID function. To address this question, we generated a unique VDJ allele removing sequences important for leader to V and J to C splicing and analyzed mutation frequencies in the VDJ and J-intron. This data shows that splicing is a significant component of AID recruitment to the variable gene.

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Poster #72

Mayer, Christian
NCI-Bethesda

Distinct sites and mechanisms of apoptosis prior to B lymphocyte activation limit autoimmune disease

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B lymphocytes rearrange their B cell receptor (BCR) in the bone marrow before migrating to the periphery where transitional 1 (T1) B cells generate long-lived mature B cells. Random rearrangements also create self-reactive and/or polyreactive BCRs which are silenced by receptor editing, apoptosis or anergy. We determined the rate, site, and function of apoptosis during physiologic B cell development. Rosa26-INDIA apoptosis indicator mice revealed pronounced apoptosis of peripheral T1 cells and comparably little apoptosis in the bone marrow. Single-cell BCR cloning and antibody profiling demonstrated limited clonal deletion of self-reactive/polyreactive B cells. Instead, most T1 cells died by neglect. Conditional anti-apoptotic Bcl-2 expression revealed two mechanisms protecting female mice from fatal autoimmune disease – suppression of B cell expansion and hypergammaglobulinemia requiring apoptosis prior to B cell activation and censoring of autoantibody production requiring apoptosis after B cell activation. These findings shed light on B cell development and autoimmunity.

Poster #73

McGuire, Tomi
NCI-Bethesda

Loss of tissue resident macrophages protects against tumor, but hampers immune initiation and tissue repair

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Tissue resident macrophages play critical roles in the peritoneal cavity initiating immune responses, resolving inflammation, and repairing tissue damage. In homeostasis, resident macrophages constitute 90% of the total macrophage compartment in the cavity. Previous work from our lab has shown that chronic inflammation results in permanent loss of resident macrophages and recruitment of inflammatory macrophages that replenish the niche. Yet, the functional consequences of this macrophage remodeling remain unclear. To address this, we examined the phenotypic, transcriptional, and functional profile of cavity macrophages in homeostatic or chronic inflammatory conditions. Phenotypically, macrophages in chronic inflammation had a distinct, inflammatory profile compared to the resident macrophages observed in homeostasis or acute inflammation. Single cell RNA sequencing (scRNA-seq) revealed that this shift in programming was paralleled at a transcriptional level, with metabolic reprogramming and enrichment in pathways related to interferon response, rather than the homeostatic pathways characteristic of cavity resident macrophages (e.g., complement system, wound healing, and coagulation). Functionally, we saw that the remodeled macrophage compartment poorly initiated immune responses against a microbial challenge with reduced neutrophil recruitment following zymosan-induced peritonitis. Remodeling also hampered liver tissue repair, and mice succumbed to chemical-induced liver damage. In the context of ovarian cancer, which metastasizes to the peritoneal cavity, resident cavity macrophages are thought to support tumor progression. When we challenged animals with ovarian tumor, we saw that remodeling actually improved tumor control. Blocking inflammation recovered resident macrophages, reducing animal susceptibility to liver damage and improving neutrophil recruitment. scRNA-seq showed that recovered macrophages were enriched in resident-like pathways, and shifted towards resident-like metabolic programming. Our findings demonstrate that macrophage functional remodeling during chronic inflammation has beneficial and harmful consequences for health depending on context. Understanding the underlying mechanisms will be critical for the rational design of macrophage-targeted therapies.

Poster #74

Mendoza, Mirian
FDA-CDER

Developing a new immunocompetent mouse model for Dengue virus infection

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Antibodies play a key role in blocking viruses from infecting cells and increasing their clearance. However, some viruses, like Dengue, can infect cells more readily when bound by antibodies of sub-optimal specificity or levels, resulting in more severe infections, this is called antibody dependent enhancement. Currently there are no immune competent animal models to examine whether therapeutic antibodies could elicit ADE. Dengue is the virus infection where ADE is more clearly observed, therefore, we developed the first immune competent mouse model of Dengue. This study shows that immunocompetent neonate C56BL/6 mice challenged subcutaneously with DENV develop viremia followed by high levels of virus in the CNS and eyes starting at 6 days post infection. The mice exhibit clear signs of neurological disease such as unsteady gait, ataxia, and tremors and succumb to infection 9-12 days after challenge. The infection in the CNS is associated with significant increase in mRNA expression for interferon-inducible genes, chemokines, antigen presentation and activation, complement as well as apoptosis which correlate with the level of infection. Moreover, immunohistochemistry and flow cytometry demonstrate an increase in infiltrating immune cells in the CNS of DENV infected mice. Currently we are using this model to study the impact of immune modulators alone or in combination with mAbs and small molecules on disease progression and survival to establish the model as a tool to test the impact of different product quality attributes on anti-DENV therapeutic safety and efficacy.

Poster #75

Mody, Drashty
NIDCR

Characterization of ZG16b protein as a potential biomarker for salivary gland damage from onset of chronic graft vs. host disease

Drashty Paresh Mody, Ana Costa da Silva, Jacqueline Mays

NIH, NIDCR, Oral Immunobiology Unit

The oral cavity is a frequent target of chronic Graft versus Host Disease (cGVHD) following allogenic hematopoietic stem cell transplant (allo-HSCT). It is challenging to detect early cGVHD induced damage to exocrine tissues, such as salivary glands, prior to irreversible tissue fibrosis. Recent work in our laboratory used shotgun proteomics to identify putative salivary biomarkers in saliva at onset of salivary gland cGVHD. Saliva samples from patients affected with oral cGVHD post allo-HSCT had a drop in protein content of zymogen granule 16b (ZG16B) which was recapitulated in immunohistochemical staining of human minor salivary gland biopsy sections. Loss of ZG16b correlated with infiltration of CD45+ cells. IHC data suggested localization of ZG16b protein to acinar cells. These cells frequently co-stain with MUC7, a common marker for serous acinar cells, which was probed after single cell RNAseq data from health labial minor salivary glands showed high expression of ZG16b in serous and serous mucous acinar cell populations.

Little is known about the function of this ZG16b in acinar cells or of its homolog pancreatic adenocarcinoma up-regulated factor (PAUF) in other cells. Ongoing work is being done to probe binding partners for ZG16b including endogenous proteins, components of the oral microbiome and immune cell receptors. In parallel, the utility of salivary ZG16b as a biomarker for cGVHD and general salivary gland damage is being tested. This work will bring clarity to the role of this protein in exocrine tissues and allo-immune disease.

Poster #76

Moon, Sookjin
NIEHS

Membrane protein Flotillin-2 regulates T cell activation and division by increasing T cell receptor signaling threshold

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The threshold for T cell receptor (TCR) activation plays a pivotal role in directing appropriate T cell immunity and in avoiding aberrant responses, but the role of T cell membrane proteins in regulating signaling threshold remains poorly understood. Here, we report that Flotillin-2 (Flot2), a membrane-anchored scaffolding protein, increases the TCR signaling threshold to inhibit TCR signaling-mediated responses upon suboptimal stimulation by dendritic cell (DC)-presented antigen. Flot2 ablation induced increased TCR signaling in OVA-specific OTI CD8+ T cells in response to sub-optimal OVA variant peptides presented by wild-type (WT) DCs. Flot2-deficient OTI CD8+ T cells also showed increased expression of T cell activation markers and more proliferation than WT OTI CD8+ T cells. This enhanced TCR signaling, activation, and proliferation compared to WT counterparts was particularly evident under suboptimal peptide stimulation. By contrast, enhanced signaling, activation, and proliferation were not observed in Flot2-null CD8+ T cells activated by plate-bound anti-CD3/soluble anti-CD28, suggesting that T cell Flot2 determines the TCR signaling threshold in collaboration with a required DC signal. Dividing Flot2-deficient OTI CD8+ T cells activated by DC-presented peptide showed higher emergence of CD8hi and T-bethi populations, which are considered pre-effector daughters with greater activation phenotype. Immunofluorescence analysis further revealed that Flot2-deficient OTI CD8+ T cells responding to suboptimal DC-presented peptide stimulation showed asymmetric partitioning of membrane CD8, which is the initiation of the CD8hi population. Taken together, our study suggests that Flot2 increases the TCR signaling threshold, thereby suppressing T cell activation, proliferation, and initiation of early fate bifurcation producing effector progenitors in the context of suboptimal TCR stimulation. These results have important implications for improving T cell reactivity and health outcomes in diseases characterized by poor antigenicity.

Poster #77

Morales-Sanchez, Abigail
NCI-Bethesda

Reversion of thymus involution rescues old mice from fatal Toxoplasma gondii infection

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5. Laboratory of Molecular Biology and Immunology, National Institute on Aging

The thymus is a specialized lymphoid organ where T-cells develop and mature. Thymic epithelial cells (TEC) make up the thymic microenvironments that support the generation of a functionally competent and self-tolerant T-cell repertoire. Infections, starvation and aging, among other factors, cause thymus involution with loss of T-cell generation and decreased frequency of naïve T-cells in secondary lymphoid organs. We developed a model to experimentally reverse thymus involution through an inducible expression of Myc (iMyc), using Psmb11-Cre mice in which Cre activity is specific for TEC. In this model, over-expression of Myc in aged TEC drove thymic growth, and partially restored frequencies of CD4+ and CD8+ naive T-cell populations to the levels observed in young adult mice. Old mice with a rejuvenated thymus showed significantly better survival upon Toxoplasma gondii infection. During the acute infection, old mice showed a decreased frequency of parasite-specific T-cells and increased serum levels of IFNg, IL6 and TNFa. Preliminary analysis of single-cell RNA-Seq showed a high expression of exhaustion markers in effector CD4 and CD8 T-cells of old mice that was diminished in iMyc mice. We are now investigating the mechanisms responsible for the pathogenic immune responses seen in aged individuals, with the goal of understanding how improved thymic function modifies immune responses.

Poster #79

Murphy, Caitlin
NEI

Exposure to Commensal Microbiota Promotes Ocular Autoimmunity in Retina-Specific T Cell Receptor Transgenic Mice

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Autoimmune uveitis is a group of intraocular inflammatory diseases driven by retina-specific T cells targeting the neuroretina, and is one of the leading causes of blindness. Using a spontaneous mouse model of autoimmune uveitis (R161H) in which CD4 T cells express a transgenic R161H T cell receptor, we have shown that gut commensal microbiota are involved in activating retina-specific T cells and triggering uveitis.

To investigate how a germ-free (GF) host responds to microbiota exposure and how this affects retina-specific T cell gene expression, R161H TCRa-/- mice were rederived into GF conditions. GF mice were cohoused with SPF mice for 4 weeks (exGF). Fecal samples were collected weekly to examine changes in the gut microbiome by 16S amplicon sequencing. Disease progression was monitored twice weekly by fundoscopy. At the endpoint, eyes were collected for histopathology, and CD4 T cells from lymph nodes, eyes, and intestinal lamina propria were analyzed by flow cytometry and RNAseq for immunophenotype and gene expression.

Exposure to commensal microbiota strongly promoted disease development. After 1 week of cohousing, disease scores of exGF mice reached the severity of age-matched SPF mice and were significantly higher than those of GF mice. Flow cytometry analyses revealed an increased percentage of CD4 T cells expressing the proinflammatory cytokines IL-17A and IFN-g in exGF mice. Ongoing analysis of exGF gut microbiome changes may help identify microbes affecting disease outcomes. RNAseq data will provide insights into phenotypic differences among retina-specific T cells in different tissues, and may uncover novel pathways involved in uveitis development.

Poster #80

Murray, Page
NIAID

Prostaglandin E2 signaling via EP4 on macrophages protects against acute colitis by preserving intestinal barrier function

Page Murray, Elizabeth Emmanuel, Erik Karmele, Brian Kelsall

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The inflammatory bowel diseases (IBD), Crohn’s disease and ulcerative colitis, are increasing in incidence and cause significant morbidity, however disease pathogenesis is poorly understood. Prostaglandin E2 (PGE2) may protect against intestinal inflammation. Disruption of PGE2 synthesis is associated with gut inflammation and the gene for the PGE2 receptor EP4 was associated with Crohn's disease by GWAS. EP4 is highly expressed on intestinal macrophages, cells essential for immune homeostasis, suggesting PGE2 signaling on macrophages may prevent intestinal inflammation.

To address this, we induced colitis by feeding dextran sulfate sodium (DSS) to CX3CR1CRE/+EP4fl/fl (Cre+) mice, which lack EP4 on macrophages, and to their Cre- wildtype littermates. Cre+ mice developed worse colitis than the Cre- littermates based on weight loss, colon length, histology, in addition to increased rectal bleeding that implies major intestinal barrier disruption, a key mechanism of colitis. Cre+ mice have higher total gut and endothelial permeability early during inflammation compared to Cre- littermates, further supporting intestinal barrier disruption as a mechanism of increased disease severity in Cre+ mice.

To address the protective mechanisms of PGE2, we performed scmRNA sequencing on colon cells of the Cre+ and Cre- mice following DSS treatment. We identified monocyte and macrophage populations, with macrophages clustering into CD11c+CD80+ macrophages expressing inflammatory cytokines, and CD11c+ CD80- and CD169+ CD11c- macrophages associated with inflammation resolution and wound-healing. During colitis there was a progressive increase in the proportions of inflammatory monocytes and a loss of CD11c+ CD80- and CD169+ CD11c- (non-inflammatory) macrophages that was accelerated in Cre+ mice. Further, the proportion of CD11c+ CD80+ (inflammatory) macrophages increased in Cre+ compared to Cre- littermates. These data are the first detailed scmRNA analysis of immune cell populations in DSS colitis and suggest that PGE2 signaling through EP4 may protect non-inflammatory macrophage populations during inflammation, contributing to maintenance of the intestinal barrier.

Poster #81

Nagai, Motoyoshi
NIAID

The importance of dietary factors in regulating oral tolerance

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Oral tolerance is one of the most critical systems to regulate allergic disorders. Orally ingested antigens are first taken up by CX3CR1 high macrophages and transferred to CD103+ dendritic cells (DCs) in the small intestine. These DCs promote differentiation of antigen-specific regulatory T (Treg) cells by presenting antigens, together with producing retinoic acids and TGF-β. However, how dietary factors regulate the induction and maintenance of oral tolerance remains obscure. To address this, we temporarily shut down the supply of diet by fasting before the oral administration of food antigen. We found that fasting canceled the induction of oral tolerance and exacerbated asthma and allergic rhinitis. Fasting decreased the number and attenuated the function of CX3CR1high macrophages and CD103+ DCs in the gut and suppressed the differentiation and proliferation of antigen specific Treg cells. Furthermore, we demonstrated that the supply of carbohydrates and specific amino acids, especially arginine, is indispensable for oral tolerance induction. These observations imply that prior food intake and nutritional signals are critical in maintaining immune homeostasis via induction of tolerance to ingested food antigens.

Poster #82

Natarajan, Kannan
NIAID

Mechanistic Aspects Of Tapasin Mediated Antigen Presentation Revealed By Structure Of A Tapasin/MHC-I Complex.

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T cell immunity to viruses and cancers is crucially dependent on the cell surface display of major histocompatibility complex class I (MHC-I) molecules selectively loaded with high affinity peptides. Peptide loading occurs in the ER within a multimolecular assembly known as the peptide loading complex (PLC). A critical component of the PLC is tapasin, a 45 kDa membrane glycoprotein in whose absence the expression and stability of MHC-I are greatly reduced. To determine the mechanistic basis of tapasin-mediated peptide loading, we determined the crystal structures of human tapasin in complex with HLA-B44:05 as well as tapasin complexed to each of two well-characterized antibodies. The tapasin-stabilized peptide receptive state is characterized by distortions of the peptide binding groove, destabilization of the □2-microglobulin interaction, and rearrangements of the membrane proximal Ig domains, all of which are reversed on high affinity peptide binding. Additionally, the structural footprints of the anti-tapasin antibodies confirm previous functional assays and, together with the tapasin/MHC-I complex, reveal dynamic aspects of tapasin function. Insights gained from this ensemble of tapasin structures and mutagenesis data on the tapasin/MHC-I interface will be presented.

(Supported by the Intramural research program of the NIAID/NIH)

Poster #83

Nelson, Christine
NIAID

IL-10 suppresses T cell expansion while promoting tissue-resident memory cell formation during SARS-CoV-2 infection in rhesus macaques

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SARS-CoV-2 infection has a broad spectrum of outcomes, ranging from asymptomatic to fatal, but little is known about the host factors that promote or prevent lung inflammation during COVID-19. We used non-human primates to study the role of pro- and anti-inflammatory cytokines in the regulation of host defense and disease severity after SARS-CoV-2 infection. We first examined the kinetics of infection and host response after infection of rhesus macaques with SARS-CoV-2. As visualized by 18FDG-PET/CT imaging, foci of inflammation peaked ~day 3 and resolved by day 9 post-infection. Single cell RNA sequencing revealed a robust influx of activated inflammatory monocytes into the airways on day 3, which correlated with viral RNA levels. Antigen-specific T cell responses were first detected on day 7 post-infection and were preferentially localized to the airways. We next examined the role of IL-10 and IFNg during SARS-CoV-2 infection by blocking these cytokines early during infection in rhesus macaques. IFNg blockade tended to decrease lung inflammation based on 18FDG-PET/CT imaging and had no major impact on innate lymphocytes, neutralizing antibodies, or antigen-specific T cells. In contrast, IL-10 blockade transiently increased lung inflammation and enhanced accumulation of virus-specific T cells in the lower airways. However, IL-10 blockade also inhibited the differentiation of virus-specific T cells into airway CD69+CD103+ tissue resident memory cells (TRM). While virus-specific T cells were undetectable in the nasal mucosa of all groups, IL-10 blockade similarly reduced the frequency of total intravascular stain-negative TRM cells in the nasal mucosa. However, neither cytokine blockade regimen substantially affected viral replication, suggesting that IFNg and IL-10 have no major role in the early control of SARS-CoV-2 replication. In conclusion, IL-10 has a key role in suppressing local lung inflammation and the accumulation of SARS-CoV-2-specific T cells in the lower airways, while also promoting TRM at respiratory mucosal surfaces.

Poster #84

Oakley, Miranda
FDA-CBER

CD47 regulates parasite burden and promotes pathogenesis in murine malaria models

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CD47 is an anti-phagocytic (“don’t eat me”) signal that inhibits programmed cell self-removal; loss of this molecule by aging erythrocytes is associated with increased likelihood of macrophage phagocytosis. We have investigated the role of CD47 in malaria immunity and pathogenesis in murine malaria models. Previously, we demonstrated that absence of CD47 confers resistance to infection with Plasmodium yoelii 17XNL, a murine malaria that exhibits an aged-based preference for young erythrocytes. Next, we established that CD47 blockade with an anti-CD47 monoclonal antibody promotes survival and reduces the pathologic features of experimental cerebral malaria (ECM) during Plasmodium berghei ANKA (Pb-A) infection in C57BL/6 mice, a murine model of ECM. To delineate the immunological mechanism of CD47 regulation of ECM pathogenesis, we present studies comparing Pb-A infection in wildtype (WT) versus CD47 KO C57BL/6 mice. In CD47 KO mice, absence of CD47 resulted in partial but highly significant ($p<0.001$, log-rank) resistance to ECM; following infection with Pb-A parasites, 22/23 (95.6%) WT mice developed ECM by day 10 post-infection. In contrast, only 13/23 (56.5%) of CD47 KO mice succumbed to malaria during the cerebral phase of infection. Through flow cytometric analysis of brain sequestered and splenic immune cell subsets and cytokine profiling of serum, we show that absence of CD47 during Pb-A malaria is associated with a significant reduction in brain sequestered CD8+ T cells which are pathogenic during ECM, an increase in splenic CD107a+ NK cells, and alteration of a subset of cytokines. In addition, comparative analysis of WT versus CD47 KO brain tissue by immunohistology demarcates clear differences in pathologic features such as hypertrophied endothelial cells, presence of parasite hemozoin, macrophage infiltration, vasculopathy, and ring hemorrhages. A further understanding of the mechanism of anti-CD47 antibody-mediated protection from ECM may open avenues for novel immunologic-based treatment options against cerebral malaria in African children.

Poster #85

Oh, Yeuran
NIA

Modeling NF-κB regulation of the IL12b locus

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Nuclear Factor kappa B(NF-κB) is a transcription factor that regulates numerous genes in immune and inflammatory responses. The NF-κB family of proteins consist of five members: RelA/p65, RelB, c-Rel, p50/p105, and p52/p100, which can form fifteen potential homo- and heterodimer complexes. Unlike the best characterized RelA/p65, less is understood about the mechanisms and relative contribution of gene regulation by c-Rel. In macrophages, interleukin 12b(IL12b), which encodes the p40 subunit of IL12 and IL23, is an exemplary gene that is thought to be regulated with a major contribution by the c-Rel. In addition, the potential distal regulatory kappa B(κB) sites contain higher affinity motifs for c-Rel-containing dimers than RelA-containing dimers. Using fluorescence live-cell imaging and c-Rel and IL12b reporter mice, we observe the real-time single-cell dynamics of nuclear c-Rel and IL12b gene expression in bone marrow-derived macrophages induced by NF-κB activating ligands. Analysis of the live-cell imaging shows quantitative features of nuclear c-Rel and IL12b gene expression with different stimuli. Furthermore, a mathematical model of IL12b gene regulation by NF-κB provides more details and insights of the signaling pathway. We propose a mathematical model of dynamic gene regulation which includes actions of RelA- and c-Rel-containing dimers at κB sites proximal and distal to the IL12b locus. Our combined experimental and mathematical modeling approach will reveal quantitative insights that may provide a more comprehensive understanding of regulatory mechanisms underlying IL12b gene expression employed by NF-κB dimers.

Poster #86

Oh, Jihoon
NIDDK

Wild-derived microbiota modulate the host immune system and mitigates virus-induced hepatitis

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The microbiota of mammals co-evolved with their hosts over millions of years, resulting in a symbiotic host-microbe relationship that is critical to host physiology. At all phases of life, the microbiota and the host immune response shape each other. However, standard laboratory mice are lacking many of the microbes and pathogens that have co-evolved with mammals in the natural world.

To mitigate this problem, we previously established mouse models that combined the tractable genetics of C57BL/6 mice with the natural microbiota (bacteria, viruses, fungi, mites and protozoa) of wild mice. We reported that these mice serve as better models for human responses than conventional laboratory mice in two preclinical studies.

Here, we studied the effects of complex wild-derived microbiota on the immune system at different stages of life and their role in disease model. Using multiparameter flow cytometry, we demonstrate that mice with wild-derived microbiota displayed an enhanced effector immune phenotype. Compared to standard laboratory mice, mice with wild-derived microbiota displayed increased numbers of myeloid cells (including neutrophils, eosinophils, and monocytes), accelerated maturation of NK cell and B cells, and expanded memory T cell subsets. Compared to laboratory mice, mice with wild-derived microbiota also displayed higher serum levels of antibodies of various isotypes, including IgA, IgE, IgG, and IgM. NK cells showed increased degranulation and expression of cytotoxic molecules such as granzyme B, and PMA/ionomycin-stimulated T cells showed increased production of IL-2, IL-5, IL-13, and IFN-γ.

To explore the physiological significance of this elevated basal immune response, we infected mice with lymphocytic choriomeningitis virus (LCMV)-WE2.5 strain, which causes hepatitis. The presence of wild-derived microbiota was associated with low serum ALT and increased frequency and effector function of LCMV-specific T cells.

Collectively these observations demonstrate a heightened innate and adaptive immune response in the presence of wild-derived microbiota.

Poster #87

Okada, Reona
NCI-Bethesda

BET bromodomain inhibitor as priming agent for immune checkpoint blockade in neuroblastoma

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High-risk neuroblastoma, often marked by MYCN amplification and widely disseminated disease, is an aggressive pediatric malignancy and accounts for 15% of cancer-related deaths in children. Less than 50% of affected patients achieve long-term remission despite intense multimodal therapy, demanding novel therapies to improve the survival of these children. Immune checkpoint inhibitors (ICIs) have induced long-term survival in adults with multiple advanced-staged cancers, but clinical trials in children produced overall disappointing results, partially because pediatric cancers have generally low mutational burden compared to adults and are devoid of immune cells. In neuroblastoma, MYCN acts as a transcriptional master regulator and enforces an immunosuppressive tumor microenvironment (TME). We used a bromodomain and extra-terminal protein inhibitor, JQ1, to suppress MYCN. We demonstrate that JQ1 remodels the TME and opens a therapeutic window for PDL-1 diabody therapy in vivo. These promising results suggest that JQ1 may serve as a priming agent prior to immunotherapy in neuroblastoma and warrants further clinical validation.

Poster #88

Oliveira Silva Souza, Camila

NIAID

Heterogeneity of immune response during schistosomiasis in inbred mouse strains

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Schistosomiasis is a chronic helminth disease that can progress to severe fibrosis, hepatosplenomegaly and eventually death in some individuals but not others. This heterogeneity of immune responses and susceptibility to infection is associated with genetic factors that are poorly understood. Murine schistosomiasis provides a useful model for studying the role of genetic variation and its impact on the heterogeneity of immune response and immunopathology during schistosomiasis. First, we evaluated mortality and morbidity of different inbred strains of mice after S. mansoni infection. C57BL/6 mice are more resistant to infection than all other inbred strains of mice. Next, we compared C57BL/6 with the more susceptible Balb/c strain for immunopathology. Compared to C57BL/6 mice, spleen weight, size and cell numbers were greater in Balb/c mice at 7 wpi with S. mansoni. However, there is no difference in liver weight between mouse strains. In the blood, we observed lymphopenia and neutrophilia in the Balb/c mice. We speculated that infection of Balb/c mice could be associated with increased extramedullary hematopoiesis in the spleen. Compared to C57BL/6 mice, HSC, CMP and GMP progenitors, and their proliferation were significantly increased in the spleen from Balb/c mice, confirming the positive correlation between spleen weight and extramedullary hematopoiesis. Also, we observed increased neutrophils hematopoiesis which is correlated with increased GM-CSF production by CD4+ in the spleen of Balb/c mice. As expected, Balb/c mice displayed an increased Th2/Th1 ratio and increased Treg cells when compared to C57BL/6 mice. We hypothesize that these immunological phenotypes are associated with schistosomiasis mortality and gene regulatory elements (GREs) that differ between C57BL/6 and Balb/c mice regulates the immune response and the development of splenomegaly during S. mansoni infection. Future work is directed at identifying specific GREs that regulate these phenotypes and disease outcomes.

Poster #89

Oyesola, Oyebola
NIAID

Previous helminth infection enhances murine host resistance to SARS-CoV-2 through pulmonary macrophage dependent T cell activation.

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Helminth endemic regions are associated with reduced COVID-19 morbidity and mortality, raising the possibility that worm infections modulate disease pathogenesis and outcomes following exposure to SARS-CoV-2 (SCV2). Here, we show that prior infection with a lung migrating helminth, Nippostrongylus brasiliensis, enhances viral clearance and survival of human-ACE2 transgenic mice challenged with SCV2. This protection is associated with increased numbers of pulmonary SCV2-specific CD8+ T cells and CD8+ T cell depletion abrogates N. brasiliensis-mediated control of viral loads. In addition, single-cell transcriptomics and spectral cytometry revealed that prior N. brasiliensis infection skews the lung micro-environment towards a Type 2 and regulatory phenotype with pulmonary macrophages from mice previously infected with N. brasiliensis having increased antigen presentation modules suggesting a role in the rapid generation and recruitment of SCV2-specific CD8+ T cells. Indeed, pulmonary macrophage depletion ablated the enhanced viral clearance and diminished the enhanced N. brasiliensis infection driven T cell responses. These findings suggest that imprinting of immune cells by lung migrating helminths can limit disease severity during SCV2 infection.

Poster #90

Palmieri, Erika
NCI-Frederick

Distinct environments change metabolically upon inflammation

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Immune cells undergo major metabolic rewiring when activated by stimuli. We have previously demonstrated that proinflammatory macrophages specifically “commit” to a metabolic state where increased aerobic glycolysis sustains fast ATP production, independently of Oxidative Phosphorylation (OXPHOS). This is associated with a “broken” mitochondrial TCA cycle and accumulation of metabolites like citrate, succinate and itaconate, important for cellular functions. We have shown by assessing enzymatic activities and through metabolomics and carbon tracing studies that induced Nitric Oxide levels are responsible for this major mitochondrial reprogramming. Importantly, in endotoxin injected mice (M1 response), we have shown alterations in the metabolic signature of the peritoneal lavage fluid, where we documented regulation of citrate, α-KG, arginine metabolism and itaconate production. Parallelly in the plasma, we find energetic metabolites to be decreased compared to baseline, and arginine and glutamate deprived. Interestingly, in acute response both compartments upregulate similar metabolic pathways favoring glycolysis, antioxidant response and nucleotide biosynthesis for cell proliferation, while only later they reach distinct metabolic fingerprints. Our new data on M2 skewed inflammation, modeled by infection with parasite N. brasiliensis, enlarged our knowledge of changes in the niche. In this macrophage-driven condition we detect higher levels of arginase-derived metabolites, polyamines, and glycolytic intermediates in the lavage, consistent with anti-inflammatory and repair response. Moreover, via lipidomics we found that in M1 lavage, fatty acid (FA) and lipid synthesis signature is prevalent, and downregulation of beta oxidation of very long change FA is apparent, while in plasma all chain triacylglycerols (TG) and acyl carnitines accumulate, indicating preservation of oxidation but lipid macromolecule synthesis. Conversely, in M2 we show that TG are depleted in lavage after infection, suggesting utilization of FA. Our data propose that macrophage metabolically respond to the surrounding environment and at the same time, direct alterations of it, presumably to facilitate pathogen clearance.

Poster #91

Panda, Abir
NIAID

Inhibition of NK and myeloid cell inhibitory receptor interactions by anti-MHC-I augments innate and adaptive immunity in both mouse and man.

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Mouse NK cells recognize MHC-I antigens via stochastically expressed inhibitory Ly49 receptors that prevent NK cell activation via cytoplasmic ITIM. The loss of MHC-I expression on tumor cells (“missing self”) abrogates inhibitory signals, resulting in NK activation. We have identified an anti-mouse pan MHC-I mAb (M1/42) which blocks Ly49-MHC-I interactions, but not TCR-MHC-I interactions in vitro. Administration of this mAb in vivo markedly activated IFNγ-producing NK cells, independent of Fc receptors. NK cell-derived IFNγ primed APC to produce IL-12/-15/-18 cytokine cascades that further drove the proliferation of NK cells and memory phenotype (MP) T cells. Administration of M1/42 profoundly augmented innate and adaptive immunity against acute and chronic viral infection, responses against both PD-1-sensitive and -resistant transplanted tumors, and successfully constrained lung and liver metastases. Extending these observations to humans, we show that Fab fragments of the anti-pan-human MHC-I mAbs, W6/32 and DX17, markedly induced NK cell proliferation and IFNγ production in cultures of human PBMC. Additionally, administration of these Fabs to humanized mice (PBMC reconstituted NSG or NK cell reconstituted NOGIL-15) induced human NK and MP T cell proliferation and activation. W6/32 and DX17 potently blocked the interaction of leukocyte Ig-like inhibitory receptors LILRB1, B2, and B5 with human MHC-I, but had no effect on TCR-MHC-I interactions. Consistent with these observations, the crystal structure of a DX17 Fab/MHC-I complex reveals that the footprint of DX17 overlaps the LILRB binding site on MHC-I. These results strongly suggest that similar to the effects of blocking Ly49-MHC-I interactions in the mouse, inhibition of LILRB-MHC-I interactions in man may result in marked augmentation of anti-tumor and anti-viral immunity.

This work was supported by the Division of Intramural Research (DIR), NIAID, NIH.

Poster #92

Parvathaneni, Swetha
FDA-CBER

Unique features of IL-6 mediated germinal center responses to vaccines in neonatal mice

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The inability of neonates to develop CD4+Foxp3-CXCR5+PD-1+ T follicular helper (Tfh) cells contributes to their weak vaccine responses. Previously, we measured diminished Tfh and IgG responses when IL-6 was co-injected with a pneumococcal conjugate vaccine (PCV) in neonatal mice. This is in sharp contrast to adults, where IL-6 improves vaccine responses by expanding Tfh cells. In this study, we investigated the changes in IL-2 response in immunized neonates because recent reports suggest that in adult mice IL-6 promotes the expansion of Tfh cells by protecting them from IL-2 mediated suppression through the downregulation of IL-2Rβ expression on Tfh cells. Indeed, we found decreased IL-2Rβ expression on Tfh cells in IL-6 co-injected adult mice. In sharp contrast, co-injection of neonatal mice with PCV and IL-6 not only stimulated IL-2 production from CD4+ T cells but also significantly increased IL-2Rβ expression on Tfh cells. Reflecting the differences in IL-2Rβ expression on immunized adult vs neonatal mice Tfh cells, IL-2 stimulation increased phospho-STAT5+ Tfh cells in neonates and decreased in adults. Underscoring the detrimental role of IL-6 in neonates and in contrast to adult mice, PCV immunization of IL-6 KO neonatal mice resulted in increased Tfh generation accompanied by elevated antibody responses. Importantly, in the absence of IL-6, neonatal mice Tfh cell IL-2Rα and IL-2Rβ levels were decreased. We also observed that CpG containing PCV increased antibody responses in neonatal mice, which was accompanied by an increase in IL-21 producing Tfh frequency and a sharp decrease in IL-6Rα as well as IL-2Rα and IL-2Rβ levels on Tfh cells. The decrease in receptor levels translated into suppressed signaling because IL-6 and IL-2 stimulation resulted in diminished p-STAT3 and p-STAT5, respectively. These findings underscore age specific differences in IL-6 mediated vaccine responses and highlight the need to consider age related immunobiological differences in designing vaccines.

Poster #93

Parween, Farhat
NIAID

Chemokine localization determines non-redundant roles for their receptors in extravasation of human pathogenic type 17 Th cells

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We have shown previously that CCR2 is expressed on highly differentiated human Th memory cells as well as mucosal associated invariant T (MAIT) cells. CCR2+ T cells typically co-express a combination of other inflammation-associated chemokine receptors, including CCR6, which is expressed on all human Th17 and related (type 17) cells, and we found that the CCR6+CCR2+ human Th cell population is enriched in cells with a pathogenic cytokine and cytotoxic profile. Single-cell analysis revealed two subtypes of such CCR6+CCR2+ cells, either biased to produce GM-CSF/IL17-A or GM-CSF/IFN-γ. Pathogenic activity requires migration into inflamed tissue, and chemokine receptor redundancy is a critical issue in understanding mechanisms of leukocyte trafficking and in exploiting the chemokine system for therapeutic benefit. To unravel the roles for CCR2, CCR6 and other chemokine receptors expressed on pathogenic type 17 cells in extravasation we used flow chambers containing HUVEC activated with TNFa and/or IFNg. We found that cells within the CCR6+CCR2+ subset use CCR6 and other receptors (but not CCR2) to arrest on endothelial cells, whereas only CCR2 mediates transendothelial migration. CCR2 ligands are secreted by endothelial cells but fail to bind to the cells' luminal surfaces. Although transducing endothelial cells to express a surface-bound CCL2-CXCL9 chimeric chemokine enabled CCR2-mediated arrest, the chimeric protein inhibited transendothelial migration. These data show that the activation of one receptor, CCR2, is preserved for the critical step of transendothelial migration by the selective localization of ligands. These studies reveal the integration of transendothelial trafficking and a pathogenic effector profile in type 17 human Th cells and demonstrate redundant and non-redundant roles for the multiple chemokine receptors on these cells due to chemokine positioning. Our findings provide fundamental information that can inform more effective combinatorial inhibition or expression of chemokine receptors/ligands to regulate the migration of highly inflammatory T cells.

Poster #94

Patel, Shil
NCI-Bethesda

Investigation of a putative Bim cis-regulatory sequence in agonist and negative selection

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T cells are members of the adaptive immune system that develop in the thymus and feature a T cell receptor (TCR). Each developing T cell can make a clonotypic receptor allowing for a collection of cells that can recognize a large pool of antigens. Unfortunately, this comes at the cost of generating T cells that can react strongly to self-antigens. During development, strongly self-reactive cells undergo apoptosis (negative-selection) or diversion to immunoregulatory subtypes (agonist-selection). The pro-apoptotic molecule Bim is upregulated in such cells, but to higher levels in those that die compared to those directed to agonist-selection. What connects TCR signal strength to the outcome of deletion versus agonist-selection remains incompletely characterized. Our lab has shown that agonist-signaled thymocytes gain chromatin accessibility at a 3 kbp regions upstream of the Bim gene, that contains sequences matching the DNA binding motif of Nurr77, a TCR induced transcription factor. We hypothesize this region includes a cis-regulatory sequence that sensitizes cells receiving high TCR signaling to apoptosis, thereby tuning the balance between negative and agonist selection, and shaping the pool of agonist selected cells. To test the role of this region in thymic selection, we have generated a mutant mouse in which it has been deleted. With flow cytometry, we will assess these mice for changes in the size of the agonist-selected population and their expression of Bim. We will utilize single-cell RNA sequencing to identify changes in their transcriptome and TCR clonotype distribution. Lastly, we will combine transcription factor expression data with a search for their respective DNA binding motifs to identify which factors may drive changes in Bim expression through this cis-regulatory sequence. Taken together, the proposed project aims to delineate a mechanism by which developing T cells with strong self-reactivity in the thymus undergo negative versus agonist selection.

Poster #95

Patino Molano, Liliana
NIDCR

Differential methylation in Foxp3 locus between CD4 Tregs and CD8 Tregs

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CD4 and CD8 T cells play fundamental roles in the cell-mediated immunity. CD4+Foxp3+ regulatory T cells (Treg) are developed in the thymus and in the periphery in response to TCR stimulation in the presence of Transforming Growth Factor Beta (TGF-β) and IL-2. While the biology of CD4 Treg cells and their suppressive effects have been well characterized, the differentiation and maintenance of CD8 T cells expressing Foxp3 is however largely unknown. Here, we investigated the in vitro induction and maintenance of CD8+Foxp3+ regulatory T cells (CD8 Treg). CD8 Treg can be induced by TGF-β in the context of TCR stimulation, although the degree of Foxp3 induction is lower than CD4+ T cells. Importantly, compared to CD4+ Tregs, CD8 Treg cells showed a faster and greater reduction in Foxp3 expression, but increased the expression of IFN-γ and granzyme-B. In an inflammatory bowel disease model in mice, we also observed that in contrast to CD4 Treg cells, CD8 Treg lose the Foxp3 expression and consequently their suppressive function. These results suggest that CD8 Treg are not able to maintain the Foxp3 expression as CD4 Treg cells. We then investigated the underlying molecular mechanisms and showed that Tet 1, 2 and 3 enzymes were expressed higher in CD8 than CD4 in response to TGF-β treatment, suggesting a different epigenetic regulation between CD4 and CD8 T cells. Whole genome bisulfite sequencing revealed differences in the DNA methylation in the CNS1 region of the Foxp3 locus between CD4 and CD8 Treg cells. This change in methylation status could modify the binding of several transcription factors responsible for the transcriptional regulation. The understanding of Foxp3 maintenance in CD8 Treg will have implications to improve the immunotherapy in some tumors, where CD8+ Foxp3+ Treg cells have been reported to be increased.

Poster #96

Peluf, Victoria
NIAID

Temporal and tissue-coordinated requirements for the IL-12 response in Th1-mediated control of systemic infection

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Host protective IFN-gamma-mediated immunity during Toxoplasma gondii infection is thought to be stimulated by the interaction of the parasite protein profilin and TLR11/12 expressed on cDC1 and pDC in the bone marrow and spleen, respectively, resulting in IL-12 dependent Th1 differentiation. Thus, TLR11/12-/- animals provide a unique model for studying how pathogen-specific IL-12 responses contribute to Th1 immunity. As demonstrated previously, TLR11/12-/- mice display curtailed IL-12 production associated with acute mortality. Surprisingly, however, IFN-gamma levels measured at the site of infection and in the serum remain indistinguishable from those observed in infected wild-type animals. Nevertheless, TLR11/12-/- mice display a delay in bone marrow cell egress and lack of splenomegaly, a phenotype characteristic of T. gondii-exposed animals in which IFN-gamma signaling is absent. Moreover, daily administration of rIFN-gamma, in contrast to rIL12, failed to rescue TLR11/12-/- animals. These findings suggest that, despite the presence of the cytokine itself, the propagation of IFN-gamma signaling is impaired in T. gondii-infected TLR11/12-/- mice. In addition, wild-type animals treated with anti-IL-12p40 Ab showed a similar pattern of deficiency in IFN-gamma signaling and revealed that IL-12 is necessary only from day 3 until day 5 p.i. to promote host resistance. The IFN-gamma-inducible GTPase (Irgm1) is one of the downstream mediators of host protection during T. gondii infection and, using a Irgm1 reporter mouse strain, we showed that blocking IL-12 on day 3 p.i. causes an early decrease in frequency of Irgm1 pos cells and diminished expression of other IFN-gamma-inducible markers (e.g., Sca-1, CD69) in the bone marrow and spleen, but not at the site of infection. Taken together, our findings reveal a requirement for a synchronized and coordinated IL-12 response throughout the body that, when disrupted, leads to an irreversible impairment of IFN-gamma-mediated host immunity.

Poster #97

Peng, Dingkang
NIAID

Foxp3 M370I mutation allows the activation of T effector program in Tregs

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The transcription factor forkhead box P3 (Foxp3) dictates the development and functions of regulatory T cells (Tregs) in both thymus and peripheral tissues to mediate immune tolerance. Mice with a deficiency in Foxp3 manifest autoimmunity in multiple organs (spleen, liver, gut, lung and skin), which is quite similar to clinical manifestation of IPEX. The mutation in the 370th amino acid (Methionine to Isoleucine, M to I) of FOXP3 protein was identified in IPEX patients with a Th2 phenotype. To study the mechanism through which this mutation affects Treg development and functions, we generated M370I-Knock in (KI) mice via CRISPR-Cas9 technology. Interestingly, the homozygous female KI (Foxp3KI/KI) mice and the hemizygous male KI (Foxp3KI/Y) mice showed dramatic T cell activation accompanied by increased number of Th1-, Th2- and Th17-like Tregs. The KI Tregs were capable of producing various effector cytokines including IL-2, IFN-γ, IL-4, IL-13, and IL-17A. On the other hand, the expression of Foxp3 and CD25 was reduced in these cells. These results indicate that Foxp3 M370I mutation renders these Tregs to display an effector cell phenotype. Accordingly, we observed the upregulation of different transcription factors associated with effector T cells, including T-bet, GATA3 and TCF7. We also generated heterozygous KI female (Foxp3KI/GFP) mice by crossing Foxp3-KI with Foxp3-IRES-GFP. The heterozygous mice showed no evidence of autoimmunity, however, the KI Tregs expressed lower levels of Foxp3 and CD25 compared to the wildtype Tregs. This cell intrinsic defect was also confirmed by mixed bone marrow chimera experiments. Furthermore, we found that in the thymus of these heterozygous female mice, the KI Tregs had reduced portion of Eos- and CD25-expressing cells, indicating an early developmental defect of KI Tregs in the thymus. Such a developmental defect may result in a failure in silencing T effector program in these KI Tregs.

Poster #98

Pessenda, Gabriela
NIAID

Kupffer Cells Heterogeneity Contributes to Visceral Leishmaniasis Resistance

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Visceral leishmaniasis (VL) is caused by Leishmania (L. donovani/L. infantum) protozoan parasites. The human disease is transmitted by sand fly bites and is fatal in the absence of treatment. Macrophages in the liver, spleen, and bone-marrow are the main target cells for visceral Leishmania species. Kupffer cells (KCs) are the liver embryonic-derived resident macrophages (emKCs), characterized by Clec4f and Tim4 expression, and their sessile behavior within the liver sinusoids. In the murine VL model, KCs are important for both initial parasite growth and granuloma formation, the latter being associated with the eventual protective response. KC proliferation, migration, death, and their replacement by moKCs has not been investigated in VL. We tracked granuloma formation in C57BL/6 mice infected with L. infantum at 19- and 42- d.p.i., and found that KC proliferation was enhanced at 19 d.p.i., and that Clec4f and Tim4 expression was reduced at 42 d.p.i. Granuloma cores were mainly Clec4f+ at 19 d.p.i. but Clec4f- at 42 d.p.i. These cores contained mixed KC clonal lineages and were located outside the liver sinusoids. Evidence of KC apoptosis and ferroptosis was also observed at 42 d.p.i. Congenic parabiotic mice demonstrated KC populations that bore either congenic marker at 42 d.p.i, suggesting that some emKCs die during late infection and are replaced by Clec4f- moKCs. In BACH1-/- mice, in which ferroptosis is reduced, the frequency of emKCs Clec4f+ was enhanced at 42 d.p.i. Functionally, Clec4f- KCs expressed higher levels of iNOS, and CCR2-/- mice, which have reduced moKCs, showed higher parasite loads. Our data indicates that KCs migration to the liver parenchyma, facilitating their activation by other immune cells, together with their partial replacement by moKCs, results in their increased killing activity, and suggests that KC heterogeneity is an important hallmark of hepatic resistance in VL.

Poster #99

Pichler, Anrea
NIAID

PI3K: a key driver of effector differentiation under conditions of T cell exhaustion

Andrea C Pichler, Jennifer L Cannons,Julie Really, Dominic Golec, Dorian McGavern, Pamela L Schwartzberg

NIAID, NIH

During chronic infection and cancer, prolonged antigen exposure drives a state of T cell hyporesponsiveness, called exhaustion, causing T cell dysfunction, but also prevents immunopathology. Notably, a small subset of TCF-1-expressing progenitor Tex (pTex) cells, is required to maintain terminally exhausted T cells and for responses to immune checkpoint blockade cancer therapy. Understanding molecular mechanisms of exhaustion may help identify pathways for therapeutic applications.

Phosphatidylinositol 3-kinase-□ (PI3K□) is important for T cell signaling, differentiation, survival, and metabolism. We have found that TCF-1 expression is repressed in CD8+ T cells from patients with Activated PI3K Delta Syndrome (APDS) and a mouse model (Pik3cdE1020K/+ mice). These mice display increased effector CD8+ T cell expansion with impaired central memory responses in acute viral infections. How activated-PI3Kδ affects T cell exhaustion is unknown.

We found that half of Pik3cdE1020K/+ mice rapidly died upon chronic LCMV clone 13 infection. Nonetheless, the surviving Pik3cdE1020K/+ mice recovered more rapidly than WT. To evaluate Tex cell survival, differentiation, and function, we developed a high-dimensional, 37-color spectral flow cytometry panel. Pik3cdE1020K/+ mice showed decreased numbers and percentages of TCF-1+ pTex cells, but a large expansion of KLRG1+ effector-like cells, with increased cytokine production, suggesting these cells might induce early immunopathology yet increase viral clearance. Pik3cdE1020K/+ “terminal Tex” cells showed decreased levels of inhibitory receptors; produced more TNF-□ and IFN-□ compared to WT, suggestive of increased effector function; and downregulated Tox, a key transcription factor for epigenetic remodeling in exhaustion. Moreover, these “terminal Tex” cells induced death of recipient mice when transferred into infection matched animals. Our findings suggest that a carefully balanced activation of PI3Kδ is required to balance effector vs exhaustion differentiation and may provide insights for therapeutic strategies to reinvigorate effector functions in terminally exhausted T cells.

Poster #100

Pontejo, Sergio
NIAID

Mast cells enhance MCMV infection of macrophages: critical effects of heparin and macrophage scavenger receptors

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Most viruses rely on their binding to cell surface glycosaminoglycans (GAGs) to initiate cell entry. This early stage in viral infection constitutes a promising target for the development of universal anti-viral therapies based on GAG-binding peptides or soluble GAGs, such as heparin, that block the initial cell surface GAG-virus interaction. An important limitation of these studies is that they are often incomplete in the scope of target cell types, typically restricted to highly permissive fibroblast or epithelial cell lines. To address this gap in knowledge, we tested the effect of soluble heparin on the infectivity of mouse cytomegalovirus (MCMV) in different types of cells. Contrary to its well-established anti-viral properties in fibroblasts, we found that soluble heparin drastically enhanced MCMV infection of primary and immortalized macrophages. In addition, we proved that heparin altered the MCMV endocytic pathway in macrophages and promoted MCMV infection via macrophage scavenger receptors. Mast cells constitute the main source of soluble heparin in vivo. These cells respond quickly to antigens or pathogens in barrier tissues by releasing cytoplasmic granules loaded with histamine, cytokines, peptides and heparin, which represents >40% of the granule content. We found that mouse peritoneal mast cells degranulate within minutes upon MCMV peritoneal challenge, and that, consistent with our in vitro data, MCMV infectivity in peritoneal macrophages was highly impaired in mast cell-deficient Kit W-sh mice. Importantly, this macrophage infectivity defect in Kit W-sh mice was corrected when soluble heparin was co-injected with the virus. We are working on a model whereby upon mast cell degranulation and heparin release, MCMV infection is specifically directed to macrophages by promoting MCMV entry via macrophage scavenger receptors while blocking viral binding to cell surface GAGs on fibroblasts and other cell types.

Poster #101

Qin, Xu
NIAID

Robust, prolonged adaptive immune responses to SARS-CoV-2 in tonsils and adenoids of convalescent children

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SARS-CoV-2 infection triggers adaptive immune responses from both T and B cells. However, most studies focus on peripheral blood, which may not fully reflect immune responses in lymphoid tissues at the site of infection. Tonsils and adenoids are secondary lymphoid structures at the mucosal surface of the naso- and oropharynx, which contain cell populations not found in peripheral blood, including germinal center (GC) B cells, T follicular helper cells (Tfh), and tissue resident memory (TRM) cells. To evaluate both local and systemic adaptive immune responses to SARS-CoV-2, we collected peripheral blood, tonsils, and adenoids from 110 children undergoing tonsillectomy/adenoidectomy within the first year of the COVID-19 pandemic from September 2020-January 2021. We found 24 with evidence of prior SARS-CoV-2 infection, including neutralizing antibodies against multiple viral variants and SARS-CoV-2-specific GC and memory B cells in the local tissues. Using single cell BCR sequencing, we found that virus-specific BCRs were class-switched and somatically hypermutated supporting their emergence from GCs, with overlapping clones in the adenoids and tonsils. We also found expanded T cell clonotypes, most prominently among CD8+ T cells, in convalescent tissues and blood, including clones with CDR3 amino acid sequences that were identical to previously reported SARS-CoV-2-reactive TCRs, some of which were found in multiple tissues. Oropharyngeal tissues in COVID-19-convalescent children showed persistent expansion of GC B, Tfh, and effector CD8+ TRMs that were enriched for IFN-□-type responses, with particularly prominent changes in adenoids, and evidence of persistent viral RNA in tissues as long as 10 months after infection. Our results provide evidence for prolonged tissue-specific adaptive

immunity to SARS-CoV-2 in the upper respiratory tract of children weeks to months after acute infection.

Poster #102

Rahman, Shah Md Toufiqur
NIA

Decoding ligand-specificity using simultaneous monitoring of RelA and c-Rel signals in primary mouse macrophages

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Macrophages are innate immune cells which initiate inflammatory responses during pathogenic invasion via activation of NF-κB signaling. The temporal dynamics of NF-κB signaling may encode the pathogen-specific information and regulate ligand-specific gene expression. Existing data on this topic are mostly for one subunit of NF-κB, p65, also known as RelA, in cell lines overexpressing a fluorescent fusion protein. To monitor the stimulus-specific signaling dynamics of both subunits of the canonical NF-κB system, we have generated a double knock-in reporter mouse model expressing two endogenous subunits of NF-κB: mEGFP-RelA and mScarlet-c-Rel. We used high-throughput live-cell fluorescence microscopy to simultaneously monitor signaling dynamics of both NF-κB subunits in primary mouse bone marrow-derived macrophages (BMDMs). Quantitative analysis reveals ligand-specific temporal patterns of RelA and c-Rel signaling dynamics in individual single cells. For all six different TLR ligands used in this study, both the RelA and c-Rel show non-oscillatory responses where the RelA signaling occurs faster. However, following TNF-α stimulation, both NF-κB subunits show oscillatory responses (period ~2.5 hours) but RelA signaling is still faster and much stronger than c-Rel. We fed both the RelA and c-Rel signals, or features of signaling dynamics to several machine learning (ML) classifiers and found that for all ML classifiers, signaling information conveyed by both NF-κB subunits improves the ligand-identification potential over that obtained from RelA signal alone. We have also evaluated the correlations among the RelA and c-Rel signaling features and found ligand-specific cross-correlation patterns. The findings of this research will reveal how macrophages use both NF-κB subunits in identifying different immunological assaults.

Poster #103

Rao, Indira
NIAID

Sympathetic regulation of skin microbiota-induced innate-like T cells

Indira Rao, Dean E Merrill, Michel Enamorado, Verena Link, Inta Gribonika, Yasmine Belkaid.

Laboratory of Host Immunity and Microbiome, NIAID.

Recent discoveries in microbiota-mediated neuroimmune crosstalk highlight how co-evolved systems collaboratively facilitate host fitness. However, in the skin – the largest and outermost protective barrier – little is known about the role of microbiota in neuro-immune regulation. We find that murine skin colonization with commensal Staphylococci induces significant accumulation of dermal IL-17A-producing Vg6+ gdT (Vg6 gdT17) cells in IL-1 and microbiota-dependent manner. Compared to sedentary Vg6 gdT17 from unassociated controls, intravital imaging shows that commensal-induced dermal Vg6 gdT17 form large highly motile aggregates. Transcriptomic analysis revealed significant differences between Vg6 gdT17 from commensal-colonized mice versus unassociated controls. Among these, the cells from colonized mice showed significant downregulation of sympathetic beta-adrenoreceptor (bAR) expression. Beta-AR signaling has recently been shown to impact conventional T cell motility in the skin. We observe that, upon systemic b2AR agonist administration, the highly dynamic commensal-induced dermal Vg6+ gdT17 clusters are rendered immobile, showing sympathetic control of gdT17 cell motility. Intriguingly, skin commensal exposure leads to systemic induction of Vg6+ gdT17 cells, implying a role for these cells beyond local surveillance. Further studies are underway to test the hypothesis that sympathetic regulation upon exposure to skin commensals promotes inter-organ gdT cell dissemination and impacts their functional potential. Overall, this work aims to uncover the mechanism(s) underlying microbiota-induced skin neuroimmune interactions in promoting organismal homeostasis.

Poster #104

Rappaport, Jessica
NCI-Bethesda

Characterization of metastatic burden of new immunocompetent preclinical melanoma brain metastasis models to optimize immunotherapy approaches

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Metastasis occurs when malignant cells spread from their primary location to distant sites in the body. Brain metastasis (BrM) is a deadly complication for cancer patients and severely lacks effective therapies. Many preclinical BrM models involve artificial disruption of the blood-brain barrier (BBB) and/or immunodeficient mice, which limits translation to the clinic. Here, we characterized two new immunocompetent melanoma brain metastasis models, BR1 and BR3. Both models were derived from a murine subcutaneous melanoma representative of mutant-RAS human melanoma subtypes and were injected intracardially. Whole brain and lungs were imaged using a stereomicroscope, and the metastatic burden was characterized by a new machine learning approach that we developed using the QuPath software interface. Metastases count, individual lesion size, and metastatic areas were measured. For both models the localization of the BrM is representative of what is observed in the clinic as the highest BrM burden is spread through the cortex with less and smaller BrM found in the cerebellum. We observed that BR3 is more aggressive showing the highest BrM burden. Additionally, while BR1 is highly brain-tropic, BR3 develops widespread metastasis. Further kinetic studies to characterize BrM development suggest that BR3 is faster to form micro-metastases. Indeed, while both BR1 and BR3 pigmented BrM are visible by eye 12 days after intracardiac injection, microscopic BrM are clearly identified by day 4 for BR3 and day 8 for BR1 using a stereomicroscope. The presence of micro-metastases was confirmed by immunohistochemistry staining of the tyrosinase-related protein-2, a specific melanoma antigen. In summary, we present and characterize two new relevant BrM models that involve immunocompetent mice with no artificial disruption of the BBB. They constitute a robust and much needed tool in this field to understand the contribution of the immune microenvironment and environmental factors to metastasis development and optimizing response to therapy.

Poster #105

Rivera, Claudia A.
NIAID

Endogenous retroviruses modulation of intestinal immune homeostasis and oral tolerance development

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Endogenous retrovirus (ERVs) result from accumulated germline infections by ancient exogenous retroviruses and constitute a sizable proportion of the mammalian genome. Recent findings support the idea that these elements control tissue immune threshold of activation and inflammation. Within this context, our lab has recently described that, in the skin, the beneficial immunity to the microbiota depends on endogenous retrovirus expression. How the crosstalk between ERVs and the microbiota controls tissue immunity at other barrier sites and how dysregulation of this dialogue impacts tolerance responses remain unknown. My preliminary data support the idea that antiretroviral (anti-RT) treatment (blocking the conversion of ERV into cDNA) specifically impairs gut regulatory T cells and compromises the induction of oral tolerance to food antigens. Further, our results demonstrate that ERV expression is highly conserved across the epithelium and that anti-RT treatment impairs type I IFN signaling in mucosal dendritic cells involved in the induction of regulatory T cells. We hypothesized that ERV sensing within the gut promotes the development of intestinal immune populations known to control inflammatory reactions and induce oral tolerance. This project utilizes pharmacologic and genetic approaches to modulate ERV expression or sensing at steady state and under inflammatory conditions. Decoding how ERVs control immunoregulation within the GI tract may have important clinical implications for the treatment of gut inflammatory disorders and in the control of food allergies.

Poster #106

Roberts, Lydia
NIAID

Utilization of multiomics to identify breakdowns in pulmonary vaccine efficacy

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Not all natural infections or vaccines elicit long-lived, protective immune responses. The underlying mechanisms responsible for waning immunity, particularly to pulmonary infections, are poorly understood. To address this outstanding question, we undertook a multiomics approach to compare two well characterized models of adaptive immunity driven by bacterial infection, Bordetella pertussis and Francisella tularensis. Survival of secondary infection of both organisms requires CD4+ T cells for immunity, however there are stark differences in the duration of protection engendered by each bacterium. Utilizing metabolomics, genomics, and traditional immunological techniques we determined how the metabolic landscape of the lung interplays with the secondary pulmonary T cell response to uncover the T cell intrinsic and/or extrinsic factors that contribute to longevity of the protective response. Single cell RNA sequencing and flow cytometric analysis revealed that CD4+ T cells favored proliferation immediately after secondary B. pertussis infection. In contrast, poor CD4+ T cell immunity following F. tularensis infection correlated with the expression of surface markers associated with terminal differentiation, weak proliferative capacity, and rapid production of large amounts of the IFN-γ. Metabolomics analysis revealed a depletion of tryptophan, an amino acid required for T cell proliferation in F. tularensis infected animals. Increased expression of indoleamine 2,3-dioxygenase 1 (IDO1), the enzyme required for breakdown of tryptophan, among pulmonary macrophages was confirmed by scRNA-Seq and Western blot analysis. Thus, we hypothesized that elevation of tryptophan levels among Francisella immune animals would improve vaccine efficacy. However, increased tryptophan resulted in higher bacterial burdens and an exacerbated innate response dominated by damage-causing neutrophils compared to controls. These data highlight how macrophage intrinsic metabolic responses diversely affect both innate and adaptive immunity and highlights the varied functionality of a single metabolite in different cell types within the immune pulmonary compartment.

Poster #107

Ruiz, Stormy
NIA

Does Transcriptional Regulation Target AID to Immunoglobulin Loci?

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Activation-induced deaminase (AID) is the critical enzyme required for somatic hypermutation (SHM) and class switch recombination (CSR). Upon exposure to antigen, B cells become activated and express AID, initiating the process of affinity maturation. While we understand how AID is targeted to the switch region to initiate CSR, relatively little is known about how AID is targeted to the variable region to initiate SHM. The difficulty lies in the requirement to study SHM in vivo using germinal center B cells, which is a small population of cells. To address this disparity, the lab has developed a knock-in mouse model called “JH1”, which utilizes endogenous VH-D-JH gene segments in a chromosomal arrangement such that upstream VH gene segments and downstream JH segments are intact and intervening DNA is deleted. This unique model retains natural chromosomal configuration, representing what is observed in endogenous B cells from the bone marrow. Additionally, I have utilized CUT&RUN technology on our low cell inputs, expanding our possibilities for probing many critical targets in precise populations of interest. The lab and others have shown that SPT5 (a subunit of the RNA Polymerase II stalling/elongation factor DSIF) is required for AID targeting, thus SHM. Promoter-proximal pausing occurs up to 2kb downstream of the V promoter and is necessary for AID activity as well. We hypothesize that RNA Polymerase II stalling/elongation regulation is important for AID targeting. I am currently querying well-known regulators of transcription, including p-TEFb and Paf1c which positively regulate RNA Pol II, NELF which negatively regulates RNA Pol II, and DSIF which has differential effects on transcription. Understanding how AID is targeted to the V region in germinal center B cells will help us to understand the elusive, yet indispensable process of SHM, which may be useful when fine-tuning humoral responses via vaccines and other treatments.

Poster #108

Sakai, Jiro
FDA-CBER

STAT5-regulated autocrine IL-6 signaling dictates IL-10-dependent regulatory functions of neonatal B10 cells

Jiro Sakai and Mustafa Akkoyunlu

Center for Biologics Evaluation and Research/ US Food and Drug Administration

Neonates have suboptimal immune responses to immunization compared to adults, resulting in higher susceptibility to microbial infection. B lymphocytes have a central role in humoral immunity to immunization and infection, and antigen recognition by BCRs is essential for the activation and differentiation of B lymphocytes. Neonatal B cells, however, do not respond to BCR activation as robustly as adult cells do. Our hypothesis is that unique features of neonatal BCR signaling may contribute to suboptimal humoral responses. The aim of this project is to decipher the neonatal BCR signaling pathways and identify those that may be responsible for suboptimal B cell functions.

Purified adult and neonatal B cells were stimulated with anti-IgM to crosslink BCRs. RNA-seq analysis revealed that gene sets of STAT3 and STAT5 signaling pathways were enriched in neonatal B cells. We found that STAT3 and STAT5 were highly phosphorylated in neonatal B cells compared to adult B cells. We found that BCR-mediated STAT5 phosphorylation induces rapid IL-6 production, which in turn activates STAT3 in an autocrine/paracrine manner. Moreover, IL-6-induced STAT3 activation led to the production of anti-inflammatory cytokine IL-10. Further underscoring the role of IL-6 in the induction of IL-10, B cells from IL-6-KO mouse did not secrete IL-10 in response to BCR activation. IL-10 is known to inhibit inflammatory cytokine production by macrophages. To assess whether the autocrine IL-6 is essential for IL-10-dependent immunosuppression, we incubated peritoneal macrophages with conditioned medium (CM) from adult B cells, wild-type neonatal B cells, or IL-6-deficient neonatal B cells. We found that CM from wild-type neonatal B cells suppressed TNF-α production by macrophages in an IL-10-dependent manner, whereas CM from IL-6-deficient neonatal B cells or adult B cells did not.

Our studies unveiled the essential role for STAT5-induced autocrine IL-6 signaling in IL-10-mediated immunosuppressive functions of neonatal B cells.

Poster #109

Salazar Cavazos, Emanuel
NCI-Bethesda

LEVERAGING THE STOCHASTICITY OF IMMUNE RESPONSES AGAINST TUMORS TO IDENTIFY THE SPARK T CELLS THAT INITIATE SUCCESSFUL CANCER IMMUNOTHERAPIES

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Thousands of patients have benefited from the growing use of cancer immunotherapies. However, the success of these therapies can be highly variable, with some patients experiencing complete tumor remission, while others suffer with progressive disease. Most studies trying to understand this variability have focused on differences in the genetic makeup of the tumors, the status of the patient's immune system, and/or environmental factors. Here, we explore a more fundamental contributing factor, namely that the heterogeneity of genetically identical cells could account for the stochasticity in the outcome of cancer immunotherapies.

To explore this possibility, we extracted mouse TCR-transgenic CD8+ T cells, and co-cultured them with antigen-expressing tumor cells. We used multiplexed in vitro assays and single-cell analysis to delineate the conditions necessary for observing large stochastic variations in the immune response. For example, we analyzed a large number of technical replicates of tumors/clonal T cells mixes to find stoichiometries when the number of replicates with anti-tumor reactions follows a Poisson distribution. We then developed a stochasticity-based framework to identify a rare population of naïve T cells ("Spark T cells") that is necessary and sufficient to spark anti-tumor immune reactions: stochastic fluctuations in the number of spark T cells account for the variability of these reactions. Our framework combines statistical modeling, high-throughput spectral flow cytometry and machine learning, to further define a gating strategy to identify the Spark T cell progenitor population.

We are currently performing experiments to test the efficacy and the functional significance of the identified immune population in vivo. We are also applying this framework to identify immune populations in human-derived TCR-engineered T cell blasts that have higher efficacy in initiating the response against tumor cells. We envision this framework being applied to identify other relevant immune cell types that act as catalysts for successful cancer immunotherapies.

Poster #110

Schrock, Dillon
NHLBI

LFA-1 ligation and tropomyosin promote the formation of the pSMAC actomyosin arc network in mouse CD8+ T cells

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CD8+ T cells are a critical arm of the adaptive immune system, able to kill virally-infected and transformed cells. Their function is critically dependent on their ability to form stable interactions with antigen-presenting cells (APCs). These interactions are mediated by a highly-organized structure at the T cell: APC interface termed the immunological synapse (IS). The IS itself is organized largely by the underlying actin cytoskeleton. Perturbation of either the Arp2/3-dependent branched network of the distal region or of the formin-derived arc network of the peripheral region dampens TCR signaling and impairs subsequent T cell activation.

Tropomyosins are actin-binding proteins that form head-to-tail polymers along the actin filament. Several tropomyosin isoforms associate preferentially with linear formin-generated filaments, such as those in the peripheral region of the IS, where they promote the recruitment and activation of myosin 2 and prevent cofilin-mediated severing. We have found that mouse CD8+ T cells express one such tropomyosin isoform, Tpm3.1/3.2, which colocalizes with the formin-derived pSMAC arc network. Disruption of Tpm3.1/3.2 function severely impacts synaptic organization and limits myosin 2A recruitment. Conversely, we find that ligation of the integrin LFA-1 by ICAM-1 significantly enhances both myosin 2A recruitment and synaptic organization. Our observations suggest a mechanism by which LFA-1 and Tpm3.1/3.2 cooperate to recruit myosin 2A to the pSMAC arc network, leading to enhanced synaptic organization and contractility. We hypothesize that this cooperation between LFA-1 and tropomyosin is necessary for developing the highly organized and mechanically active synaptic interface that is necessary for efficient target cell killing.

Poster #111

Segrist, Elisha
NIAID

Role of endogenous retroviruses in the control of immunity in the Female Reproductive Tract
Elisha Segrist, Yasmine Belkaid

Laboratory of Host Immunity and Microbiome, Metaorganism Immunity Section, NIAID

The FRT is a unique barrier: it controls infections but also tolerates foreign entities like sperm and the fetus. Endogenous retroviruses (ERVs) influence inflammatory and pathogen responses. In the skin, the commensal S. epidermidis induced ERV expression which drove a cGAS-STING dependent Type 1 IFN gene program. S. epidermidis colonization combined with a high fat diet massively upregulated ERVs and caused inflammation of the skin.

To uncover the influence of ERVs on immunity in the FRT, we will perform complementary in vitro and in vivo studies. We will generate an ERV reporter vaginal epithelial cell line to perform a pooled CRISPR knockout screen in the context of TLR stimulation. This will allow us to identify novel molecular immune mechanisms involved in regulation of ERVs in the vaginal epithelium. We will then use a murine model to uncover if a high fat diet or the vaginal microbiota influences expression of ERVs in the epithelium of the FRT in mice, and if this has functional consequences for susceptibility to virus infection or inflammatory conditions. We will also perform functional in vivo studies using a murine model of intravaginal infection with disparate viruses, herpes simplex virus 2 (HSV-2) and murine papillomavirus (MmuPV1). First, we will administer anti-retroviral drugs to repress ERV cDNA production and illicit immune cell changes and will then intravaginally infect with HSV-2 or MmuPV1. This will allow us to understand if ERV expression in the FRT has consequences for host control of local vaginal viral infection and viral spread from the reproductive tract. Overall, this study will give greater insight into the role of ERV sensing in the control of host immune responses in the FRT and how the vaginal microbiota and nutrition could alter host susceptibility to virus infection and virus transmission to offspring.

Poster #112

Sepahpour, Telly
FDA-CBER

Role of IRF-7 mediated Type I Interferon response in the protective immunity induced by LmCen-/- parasites against Visceral Leishmaniasis

Sepahpour, Telly, FDA/CBER/OBRR; Singh, Komudi, (NIH/NHLBI); Ireland, Derek, FDA/CDER/OPQ; Alshaweesh, Jalal, Nagasaki University, Japan; Hamano, Shinjiro, Nagasaki University, Japan; Ismail, Nevien, FDA/CBER/OBRR; Gannavaram, Sreenivas, FDA/CBER/OBRR

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Leishmaniasis is a neglected vector borne disease caused by the various strains of Leishmania parasites and is endemic across the world. The disease is transmitted to humans through the bite of infected sandfly and blood transfusions. Currently, there are no FDA-approved anti-leishmania vaccines or donor screening assays, while treatment options are limited. Recently, our laboratory developed a Leishmania major centrin (LmCen-/-) gene deleted strain as a live attenuated dermotropic vaccine candidate for leishmaniasis. Previous studies in parasitic and viral pathogenesis established that type I interferons (IFNs) are instrumental in the development of both innate and adaptive immune responses. However, the relevance of type-I IFN response in the development of protective immunity following immunization is not understood. In this project, we aim to elucidate the role of type I IFN response in protection following immunization with LmCen-/- parasites.

RNA from the whole blood from mice following infection with wildtype LmWT or LmCen-/- was analyzed by the NanoString Platform 4.0 to identify differentially expressed genes. To verify the results of the transcriptomic analysis, Flow Cytometry analysis was conducted on lymph node and spleen tissues of BALB/C mice that were immunized with LmCen-/- and challenged with virulent L. donovani at different time points. Cytokines from these tissues (IFN-alpha/beta, IL-2, IL-4, IFN-gamma, IL-10) were detected via ELISA.

Our results show that immunization with LmCen-/- induces consistent increases in type I interferon responses induced by an elevated expression of IRF-7 a regulator of type -I IFNs within the 24hr and 48hrs of immunization. The induction of a protective Th1 response as indicated by a strong IFN-g, IL-2 and TNF-a production and a reduced IL-10 was coincident with a gradual decline in the type-I IFN response as measured by IFN-alpha/IFN-beta in the LmCen-/- immunized mice indicating that type-I IFN and Th1 responses are negatively correlated.

Poster #113

Sharma, Rahul
NHLBI

BLOC1S1: An Unexpected Regulator of Th2 Cell-driven Inflammatory Responses

Rahul Sharma, Kaiyuan Wu, Kim Han, Jing Wu, Michael N Sack

Laboratory of Mitochondrial Biology and Metabolism, NHLBI, National Institutes of Health, Bethesda, MD 20892, USA.

BLOC1S1 is an adaptor protein that modulates endosome-lysosome function as a component of numerous multiprotein complexes. We postulated that its modulation in CD4+ T cells may help us uncover the role of endosome-lysosome biology in adaptive immunity. In primary CD4+ T cells BLOC1S1 cre-recombinase knockout resulted in the preferential induction of Th2 compared to Th1 and Th17 cytokine production following T cell receptor activation. As atopic dermatitis is an allergic inflammatory skin disease characterized by the production of the type 2 cytokines in the skin we evaluated the effect of CD4+ T cell BLOC1S1 KO vs controls in calcipotriol-induced atopic dermatitis in mice. The absence of BLOC1S1 exhibited exacerbated atopic dermatitis accompanied with significantly elevated plasma IgE, skin eosinophilia, elevated type 2 responses, and inflammatory pathology relative to wild-type mice. RNA-seq analysis of naïve control vs. BLOC1S1 KO CD4+ T cells, showed enrichment for transcription of genes involved in autophagy and lysosomes in the absence of BLOC1S1. Immunofluorescence imaging in response to Th2-specific differentiation showed excess asymmetrical distribution of LC3 positive autophagosomes in BLOC1S1 KO cells. This mouse model will now give us a platform to explore the role of endo-lysosomal biology in Th2 polarization and activation.

Poster #114

Shi, Guoli
NCI-Frederick

Rapalogs downmodulate intrinsic immunity and promote cell entry of SARS-CoV-2

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SARS-CoV-2 infection in immunocompromised individuals is associated with prolonged virus shedding and the evolution of viral variants. Rapamycin and its analogs (rapalogs, including everolimus, temsirolimus, and ridaforolimus) are FDA-approved as mTOR inhibitors in clinical settings such as cancer and autoimmunity. Rapalog use is commonly associated with increased susceptibility to infection, which has been traditionally explained by impaired adaptive immunity. Here, we show that exposure to rapalogs increases susceptibility to SARS-CoV-2 infection in tissue culture and in immunologically naïve rodents by antagonizing the cell-intrinsic immune response. By identifying one rapalog (ridaforolimus) that is less active in this regard, we demonstrate that rapalogs promote Spike-mediated entry into cells by triggering the degradation of IFITM2 and IFITM3 via an endolysosomal remodeling program known as microautophagy. Rapalogs that promote virus entry inhibit the mTOR-mediated phosphorylation of the transcription factor TFEB, which facilitates its nuclear translocation and triggers microautophagy. In rodent models of infection, injection of rapamycin prior to and after virus exposure resulted in elevated SARS-CoV-2 replication and exacerbated viral disease, while ridaforolimus had milder effects. Overall, our findings indicate that preexisting use of certain rapalogs may elevate host susceptibility to SARS-CoV-2 infection and disease by activating a lysosome-mediated suppression of intrinsic immunity.

Poster #115

Shissler, Susannah
NCI-Bethesda

Investigation of adult thymic epithelial cell progenitors using Foxn1 lineage tracing
Susannah C. Shissler1, Marieke Lavaert1, Jennifer E Cowan1, Yongge Zhao1, Parirokh Awasthi2, Raj Chari2, Michael J Kruhlak3, and Avinash Bhandoola1

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The development of T cells is dependent on the thymus and thymic epithelial cells (TEC). Cortical (c)TEC facilitate early T cell development and positive selection and medullary (m)TEC coordinate negative selection and enforce central tolerance. Significant heterogeneity is evident within TEC compartments, but the relationships between these subtypes, and the identity of the cells maintaining the thymus in adult mice remain elusive.

The transcription factor Foxn1 is required for formation of the thymus. Postnatally, Foxn1 is known to regulate genes involved in TEC function such as genes involved in antigen processing and presentation. We hypothesized that adult TEC progenitors express Foxn1. To test this hypothesis, we developed a Tamoxifen-inducible, fate mapping system under the control of Foxn1 (Foxn1-Cre-ERT2). Preliminary results demonstrate that induction of Foxn1-Cre-ERT2 in adult mice efficiently fate maps cTEC as well as both MHC-Hi and MHC-Lo mTEC populations. The proportion of labeled TEC persists long term (out to 15wk) in all TEC compartments, including the MHC-Hi subset of mTEC that is known to be a short-lived population. The stable, long-term labeling of the MHC-Hi mTEC compartment indicates that the progenitor that maintains this population expresses Foxn1 in adult mice and is traced in our system.

To identify the putative progenitor populations we will apply two parallel approaches: bioinformatics and microscopy. To identify putative progenitor populations, we will characterize labeled cell populations with scRNASeq and CITESeq in a time series analysis. To determine whether single progenitors are bipotent or lineage restricted, we will utilize the Confetti reporter system and Ce3D full-lobe imaging to assess the clonal contribution to TEC compartments in situ. Our experiments will thus identify TEC progenitors that express Foxn1 and are labeled in Foxn1-Cre-ERT2 mice, and will clarify lineage relationships between these labeled TEC progenitors and their putative downstream progeny.

Poster #116

Silva, Lakmali Munasinghage
NIDCR

Fibrin is a critical regulator of neutrophil effector functions at the oral mucosa

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Objectives: Herein, we focus on the role of fibrin, a proinflammatory molecule, excessive deposition of which can cause chronic inflammation and severe tissue damage via unknown mechanisms. Fibrin removal, fibrinolysis, is achieved by the proteolytic activity of plasmin. The critical role of defective fibrinolysis becomes evident in patients with the autosomal recessive disease: type I plasminogen (Plg) deficiency. Indeed, mucosal lesions in humans and mice with Plg deficiency are also characterized by excessive fibrin deposition. We hypothesized that insufficient plasmin-mediated fibrinolysis is a key contributor to mucosal immunopathology of periodontal disease.

Methods: We sought to understand the mechanistic link between mucosal fibrin deposition and immunopathology by using an array of genetically engineered mouse models, including Plg-/-, and Plg-/-Fgg390-396A (mutation in the aMb2 integrin binding site on the fibrin gamma-chain).

Results: We demonstrate that (i) Plg-deficient mice develop spontaneous and severe periodontal bone loss with persistent extravascular fibrin deposits compared with littermate controls, (ii) Plg-deficient mucosal lesions have a significantly increased neutrophil infiltration, (iii) abrogating the engagement of fibrin to neutrophils through the aMb2 leukocyte integrin receptor was sufficient to prevent Plg deficiency-associated periodontal bone loss, (iv) fibrin-aMb2-neutrophil engagement activated neutrophil effector functions, including the production of reactive oxygen species and neutrophil extracellular traps, and (v) removal of extracellular DNA by DNase I treatment and blocking NETosis via depletion of neutrophil elastase, controlled periodontitis in Plg-/- mice.

Conclusion: Our work uncovers fibrin as a critical regulator of neutrophil effector functions within the oral mucosal tissue microenvironment and suggests fibrin-neutrophil engagement as a pathogenic instigator and therapeutic target in oral mucosal disease, periodontitis.

Poster #117

Songkiatisak, Preeyaporn
NIA

Studying the role of NF-κB in Alzheimer's Disease in vitro using hiPSC-derived brain models

Preeyaporn Songkiatisak1, Mohammad Aqdas1, Shah Md Toufiqur Rahman1, Kyu-Seon Oh1, Myong-Hee "Mia" Sung1

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Neuroinflammation is a key pathological driver of various neurological diseases, including Alzheimer's disease (AD). NF-κB signaling pathway is generally recognized as an important regulator of inflammation and aging. Aged microglia may undergo cellular senescence partly influenced by NF-κB. Our previous in vitro data indicated that most microglia cells formed a tight cell cluster, termed "clustered microglia". The rest was a subpopulation of microglia with smaller cell size and high motility, termed "free-roaming microglia". In microglia from aged animals, the composition of clustered versus free-roaming subsets was shifted toward a higher prevalence of free-roaming microglia where c-Rel is expressed and the canonical NF-κB signaling is more sustained. To study NF-κB signaling in the context of neuroinflammation, we are developing 3D models, using iPSC neurons derived from donors of various age groups and co-culturing them with primary microglia from a double knock-in mice in which their endogenous RelA (p65) and c-Rel are labeled with distinct fluorescent proteins. Live cell imaging and biomarker assessment of microglia and neurons activated by Amyloid β (1-42) will uncover important molecular mechanisms associated with age-dependent interactions between neurons and resident macrophages of the young and aged brains.

Poster #118

Stassenko, Elizabeth
NCI-Bethesda

SLP-76 interaction with PLC-y1 fine-tunes TCR signal strength for appropriate thymocyte selection and peripheral T-cell activation

Elizabeth Stassenko, Junya Wada, Mariah Lee, Wenmei Li, Hidehiro Yamane, and Lawrence E. Samelson

LCMB, CCR, NCI, NIH

T cell receptor (TCR) signal strength is a key determinant regulating T-cell fate at various stages of thymocyte development and mediating the differentiation of peripheral T cells into distinct effector/memory T cell subsets. We previously demonstrated by in vitro reconstitution that TCR engagement results in the formation of a heterotetrameric complex composed of LAT, Gads, SLP-76, PLC-y1, and that this complex is essential for proper activation of PLC-y1 to transduce TCR-mediated signals. The interaction of the SLP-76 proline-rich region (PRR) with the PLC-y1 SH3 domain is weak, though these two domains are highly conserved among a wide variety of mammalian species. Therefore, we hypothesized that the low affinity of the SLP-76 PRR for PLC-y1 might be evolutionarily favored to prevent excess TCR signals during thymocyte development and peripheral T-cell activation. To test this hypothesis, we developed mice carrying a conditional T-cell specific knock-in mutation in the SLP-76 PRR, determined previously to have 2.5-fold enhanced affinity for PLC-y1, by CRISPR/Cas9-mediated gene editing and the Cre/LoxP system. The cellularity of total thymocytes and the composition of double negative/double positive/single positive for CD4 (SP4) or CD8 (SP8) populations were not affected by CD4Cre-induced SLP-76 mutation (mSLP-76/CD4Cre). Notably, the SLP-76 mutation significantly increased the frequency of cleaved caspase-3-expressing SP4 and SP8 thymocytes that had received TCR signals. Moreover, the numbers of medullary SP4 and SP8 most mature thymocytes were markedly diminished in mSLP-76/CD4Cre mice. Moreover, the SLP-76 mutation rendered CD8+ T-cells more sensitive to OVA-altered peptide ligands with weaker affinity for OT-I TCR. Our data indicate that the delicate interaction between SLP-76 and PLC-y1 fine-tunes TCR signal strength for appropriate selection of SP4 and SP8 thymocytes. We are currently investigating the effect of the SLP-76 PRR mutation on effector/memory T-cell subset differentiation in the periphery.

Poster #119

Steffke, Emily
NCI-Bethesda

ChAdOx1 and MVA Vaccines for the Treatment of a P1A-Expressing SB28 Glioblastoma Model in C57BL/6 Mice

Emily Steffke1,2, James McAuliffe1, Vinnycius Pereira Almeida1, Laurine Noblecourt1, Amanda Wicki1, Taijun Hana2, Hideho Okada3, Gary Kohanbash4, Carol Leung1, Masaki Terabe2, Benoit Van Den Eynde1

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Glioblastoma is an extremely aggressive and difficult cancer to treat, which may partly be due to its limited ability to induce T cell responses. However, using vaccines to generate tumor-specific T cells may provide a meaningful benefit to patients when combined with other therapies. Here, we investigated whether ChAdOx1 and MVA vaccines could generate therapeutically effective CD8+ T cell responses against a model antigen, P1A, expressed in SB28 glioblastoma cells syngeneic to the C57BL/6 mouse strain. We demonstrate that heterologous prime-boost vaccination with ChAdOx1/MVA vaccines targeting P1A can generate a large magnitude of CD8+ T cells specific for P1A43-51, a novel epitope presented by H-2Db MHC class I haplotype. P1A43-51-specific CD8+ T cells can recognize P1A-transfected SB28 glioblastoma cells in vitro when MHC Class I expression is upregulated in the tumor cells. Furthermore, prophylactic vaccination with ChAdOx1/MVA-P1A significantly prolonged survival of C57BL/6 mice subcutaneously challenged with P1A-expressing SB28 tumors when the vaccines are co-administered with anti-PD-1 and □-galactosylceramide, an iNKT cell agonist. This triple combination of therapies also provides a survival advantage in the therapeutic setting. Future work will investigate the efficacy of this vaccination strategy on intracranial orthotopic SB28 models.

Poster #120

Sultana, Sabrina
NIAID

Determining the function of the long non-coding RNA H19 and microRNA mir675 in fetal hematopoiesis

Sabrina Sultana, Xiuhuai Liu and Stefan A. Muljo

Laboratory of Immune System Biology, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

H19 is one of the first long intergenic non-coding RNAs (lincRNAs) to be discovered and is epigenetically imprinted. Phylogenetic analysis of sequence covariation suggests that H19 RNA adopts secondary structure via conserved base-pairing. We became interested in H19 because it is expressed in fetal hematopoietic stem and progenitor cells (HSPCs) but not in the counterparts in adult bone marrow. Furthermore, we found evidence that H19 lincRNA is directly bound by Lin28b which is a key regulator of fetal hematopoiesis. H19 lincRNA also harbors the precursor for a microRNA, miR-675 and this should be one of its vital functions. The high expression of H19 in fetal liver HSPCs suggests that it might regulate fetal hematopoiesis. To begin studying the function of H19 in vivo, we deleted H19 and Mir675 from the mouse germline. To determine whether the function of H19 is due to miR-675, we will analyze H19 KO and miR-675 KO mice in parallel.

Poster #121

Sung, Mia
NIA

Double Knock-in Reporter Mice Reveal NF-kappaB Trajectories in Signaling, Immune Cell Development, and Aging

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NF-κB is a transcription factor whose regulatory control reaches far beyond the immune system. In vitro studies suggest that mapping the spatiotemporal complexity of NF-κB signaling in primary cells and in vivo is essential to understanding its function in health and disease, but the lack of tools to directly monitor NF-κB protein components has hindered such efforts. To address this need, we generated reporter mice with the endogenous RelA (p65) or c-Rel labeled with distinct fluorescent proteins and a double knock-in with both labeled subunits. Overcoming hurdles in simultaneous live cell imaging of RelA and c-Rel in cells from the reporter mice, we found that the quantitative features of signaling reflect the identity of activating ligands, differ between primary and immortalized cells of the same type, and shift toward c-Rel in microglia from aged brains. We also identified an unexpected depletion of nuclear RelA:c-Rel heterodimers in stimulated cells. Quantitative same-cell measurements in these mice revealed a trajectory of subunit expression in several immune lineages, with a surprising downregulation at key cell maturation stages. These data begin to reveal the power of these reporters in gaining deeper insights to NF-κB-linked biology, with the spectral complementarity of the labeled NF-κB proteins enabling diverse applications from single-molecule analyses to in situ identification of cells in active inflammatory states.

Poster #122

Swanbery, Nathan
NCI-Bethesda

Enforced expression of Myc and T58A-mutant Myc in lineage-traced thymic epithelial cells

Nathan J. Swanbery1, Susannah C. Shissler1, Marieke Lavaert1, Jennifer E. Cowan1, Avinash Bhandoola1

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The thymus is responsible for T cell development, in which thymic epithelial cells (TEC) coordinate T lymphopoiesis. Cortical (c)TEC support T lineage commitment and positive selection, whereas medullary (m)TEC delete self-reactive T cells to enforce central tolerance. Both mTEC and cTEC contain several distinct subtypes that were only recently characterized. Thus, precursor-product relationships among TEC subsets are not presently well understood. To elucidate TEC precursor-product relationships, we've developed and acquired Tamoxifen-inducible Cre-ERT2 systems under subset-restricted genes for lineage-tracing within TEC (Foxn1) or mTEC subsets (Ccl21a and Aire). With these models, we can induce targeted RFP expression in adult TEC subsets to map precursor-product relationships, providing insight into how the adult thymus is maintained.

We also wish to understand the cellular origins of thymomas, a diverse group of cancers originating from TEC. In human thymomas, increased expressions of Myc have been observed. In mice, overexpression of Myc in TEC is shown to increase thymus size and TEC numbers, while maintaining morphology and function. We hypothesize that enforced expression of Myc or stabilized T58A-mutant Myc in lineage-traced adult TEC subsets will expand the targeted population and may eventually result in transformation. If the targeted population is a precursor for other subsets, then multiple Myc-expressing TEC subsets may show population expansion

In these experiments, we will examine thymus size, function, and morphology in our models via flow cytometry and microscopy analysis to assess normal, enhanced, or oncogenic characteristics. We predict that our combined Myc and reporter expressions will expand and label targeted TEC subtypes and progenies. Conversely, perhaps only some or none of the precursor or product populations will expand. Regardless of whether our hypothesis is correct, our experiments will provide insight into precursor-product relationships in TEC subtypes that contributes to better understanding both Myc-driven thymic hyperplasia and a model for thymoma origins.

Poster #123

Taylor, Joshua
NIA

Unmasking arterial-resident autoreactive B cells involved in atherosclerosis and peripheral artery disease progression

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1Laboratory of Molecular Biology and Immunology, NIA, NIH; 2Graduate Program in Immunology, Johns Hopkins University School of Medicine

Atherosclerosis is characterized by the progressive buildup of cholesterol-rich plaques within the arterial wall. This activates the immune system, including B cells which secrete antibodies into the plasma. In diseased mice, a subset of these circulating antibodies binds self-antigens released from necrotic cells within plaque, which we suspect increase inflammation. To identify local immune responses, we isolated activated B cells from aortas of atherosclerotic ApoE-deficient mice on a high-fat diet and the tibial arteries from humans following amputation as a consequence of peripheral artery disease (PAD). single-cell sequencing of the B cell receptors was performed to obtain antibody heavy and light chain pairs. We identified, selected, and expressed 35 mouse and 48 human antibody pairings that are over-represented in comparison to matched splenocytes (mouse) or circulating B cells from non-PAD systemic lupus erythematosus patients (human). Epitope determination was performed by human proteome array, and 14 mouse and 21 human potential protein targets were identified with significant affinity and specificity. The 12 more promising mouse antigens were inspected by immunohistochemistry to confirm presence in inflamed plaques, and successful purified recombinant expressions of these 35 proteins were used to interrogate autoreactive antibody titers in human and mouse diseased sera by ELISA. ApoE-deficient mice were immunized with the 6 most prevalent antigens with adjuvant alone as control. Measurement of serum antibody titers showed strong, rapid, and early onset of antigen-specific antibody responses in the immunized group compared to the control group, and strikingly we saw decreased tricuspid valve plaque accumulation in these animals. Likewise, human antigen ELISAs comparing antigen-specific serum antibody titers in PAD patients to healthy donors identified two hits, AZIN1 and CASQ1, which are now being further investigated by histology and longitudinally using past serum samples from high-titer patients, a unique benefit provided by the Baltimore Longitudinal Study on Aging.

Poster #124

Teijeiro, Ana
NIAID

Breast cancer remodels the bone marrow immune microenvironment to favor metastasis
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Breast cancer is the most diagnosed cancer, with an estimated 2.3 million new cases (11.7%). Bone is the most common site of breast cancer metastasis, occurring in 70% of patients, and an indication of short-term prognosis, with a 10% 5-year survival rate. Understanding the environmental and host factors involved in the induction of metastatic stimuli is essential to develop therapies that extend patient's lifespan. Bone colonization by cancer cells is a stepwise process including the formation of a premetastatic niche and relies on interactions between cancer cells and the bone microenvironment. The bone microenvironment comprises numerous cell types, such as osteoclasts, osteoblasts, mesenchymal stem cells, haematopoietic cells, immune cells, and adipocytes. Calcium, growth factors, and chemokines facilitate bone metastasis. However, how defined components of the immune system control bone metastasis remains poorly understood. Further, how physiological stressors such as nutrition contribute to pro- or anti-metastatic bone immunity is also unclear. We have previously showed that caloric restriction induces T cell migration to the bone marrow and remodels the bone microenvironment, favoring T cell function and a protective environment. Using mouse models of breast cancer, we found that mammary tumors profoundly transform the bone marrow immune microenvironment to support metastasis. Mammary tumors induce bone lymphoid cell loss, with significant reduction in CD4 and CD8 T cells and NK cells. On the other hand, mammary gland tumors are associated with enhanced innate immune cells such as monocytes and eosinophils within the bone marrow. Of note, targeting the bone microenvironment with dietary restriction restores the homeostatic immune pool and contributes to an anti-tumor bone immunity, supporting the idea that nutritional interventions are potential therapeutic strategies to target bone metastasis. The results presented here suggest that breast cancer cells crosstalk with the bone and remodel the immune microenvironment to favor tumor cell colonization.

Poster #125

Thacker, Seth
FDA

Protein aggregate morphology and presence of IIRMI can impact immunogenicity.

Seth G Thacker, Cheng Her, Logan Kelley-Baker, Derek D C Ireland, Mohanraj Manangeeswaran, Daniela Verthelyi

FDA/CDER/OPQ/OBP

Immunogenicity has been shown to negatively impact safety, PK, and efficacy. A critical quality attribute that has been associated with immunogenicity risk is protein aggregation. Multiple factors such as mechanical stress, light, metal ions, silica particles, pH, and liquid-solid interphases can impact on the formation of aggregates and it is generally accepted that the stress impacts on the size, charge, and cohesion of the protein aggregates. Despite advances in aggregate characterization, it is still unclear what are the critical attributes of protein aggregates that impact on their immunogenicity risk. For example, it is not known whether particles in the low nm size are more likely to induce an immune response than those in the um range. Correlating the properties of protein aggregates with their immunogenicity has been difficult because it is hard to isolate specific types and sizes of protein aggregates. To address this, we generated aggregates using different stress conditions (stirring, end over end rotation and heat) and characterized the resulting aggregates for size and shape as well as the innate immune response they elicited using in in vitro cell-based assays and in vivo. We found that stirring and rotational mixing stress yielded distinct aggregates capable of eliciting a defined pattern of innate immune activation in vitro and in vivo. Further, we demonstrate that the response to protein aggregates is magnified by the presence of trace amounts of microbial impurities resulting in increased ADA rates in macaques. This study provide evidence that both the quantity and quality of protein aggregates should be considered when performing a immunogenicity risk assessment as some types of aggregates are more immunogenic than others.

Poster #126

Thornton, Angela
NIAID

Acquired Lipodystrophy is Mediated by a Treg Specific Deletion of Helios

Angela M Thornton (1), Vinay Penna (1), Oksana Gavrilova (2), and Ethan M Shevach (1)

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(2) MMC, NIDDK, NIH

The selective deletion of the transcription factor, Helios, in Regulatory T Cells (Treg) leads to systemic immune activation characterized by a Th1 phenotype, hypergammaglobulinemia, and enhanced germinal center formation. We also observe acquired lipodystrophy, hepatic steatosis, and insulin resistance. Further analysis revealed a significant lymphocytic infiltrate in both the inguinal and perigonadal adipose tissue, indicating autoimmune mediated destruction of the white adipose tissue (WAT). Additionally, we demonstrate that the destruction of adipocytes is dependent on CD8+ T cells as Heliosfl/fl x Foxp3Cre mice crossed to B2m deficient mice (lacking Class I MHC) maintain normal adipose tissue volume. Flow cytometry analysis of the infiltrate demonstrates that the destruction of the adipose tissue is mediated by CD8+ T cells through both cytotoxic and Fas-L dependent mechanisms.

Preliminary analysis of the adipose tissue Treg suggests that the Treg fail to acquire or maintain an effector state in the absence of Helios. Thus, Helios deficiency in Treg disrupts immune homeostasis in the adipose tissue, leading to the expansion and activation of CD8+ T cells, the destruction of the tissue and metabolic disease.

Poster #127

Ticas Rodas, Carlos
NIA

Follicular B cells from old mice are hyper-responsive and inhibit antigen-specific antibody responses

Carlos J. Ticas, Robert W. Maul, and Patricia J. Gearhart

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During aging, immune cells acquire multiple defects that accumulate over time in a process known as Immunosenescence. To study intrinsic defects in B cells, we used an adoptive transfer model with follicular B cells from either young (FOY, 8-12 weeks) and old (FOO, >22 mo.) donor mice being transplanted into a young uMT mouse which is deficient in B cells. After immunization with Nitrophenyl (NP)-chicken gamma globulin, FOO B cells form a larger amount of germinal center (GC) cells which have divided more rapidly than their FOY counterparts. However, these FOO cells produced lower amounts of NP-specific antibodies after immunization. To investigate this defect further, we performed single cell RNA sequencing (scRNAseq) on naïve and d14 GC cells using both FOY and FOO donors. Comparison of naïve B cells reveals minor differences between the two groups. Interestingly, Ccr6 was up regulated in naïve FOO cells but down regulated in FOO GC cells suggesting a pre-activation state in FOO that is lost after antigen encounter and differentiation. Repertoire analysis revealed that FOO GC cells do not select for the canonical NP-specific IgH V-gene 1-72. Furthermore, FOO B cells which do express the 1-72 V gene lack the well documented high-affinity CDR2 mutation (W33L) known to increase antibody affinity 10-fold. These findings show that old mice produce pseudo-activated B cells that are hyper-responsive to immunization, blocking proper selection within the germinal center reaction.

Poster #128

Tolnay, Mate
FDA-CDER

Lymphocytes sense antibodies through human Fc receptor-like proteins: emerging roles in mucosal immunity

Mate Tolnay

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Members of the Fc receptor-like (FCRL) family modulate B and T cell responses, yet their functional roles remain enigmatic. FCRL3 promoter polymorphism has been associated with several major autoimmune disease risk, indicating physiologic importance. Providing essential functional context, human FCRL3, FCRL4, and FCRL5 have recently been identified as receptors of specific antibody forms, revealing novel ways lymphocytes can interact with antibodies. We reported that FCRL3 is a secretory IgA receptor, whereas the FCRL4 ligand was defined as dimeric IgA. Therefore, FCRL3 and FCRL4 are able to distinguish the mucosal and systemic origin of IgA-containing immune complexes, respectively, with clear implications in guiding mucosal responses. Secretory IgA can signal mucosal breach through FCRL3, driving the functional plasticity of regulatory T cells towards inflammatory to help control invading pathogens. Conversely, recognition of dimeric IgA by FCRL4 on memory B cells located in mucosa-associated lymphoid tissues could promote tolerance to commensals. Memory B cells that accumulate under conditions of chronic antigen presence frequently express FCRL4 and FCRL5, and antibody ligands could provide functional feedback to the cells. FCRL5 apparently recognizes the age of the IgG molecule, using deamidation as a molecular clock, conceivably playing regulatory roles in chronic antibody responses. A framework of FCRL3, FCRL4, and FCRL5 operating as sensors of antibodies in immune complexes is proposed. Sensing the spatial origin and age of immune complexes can shape lymphocyte functional attributes and inform their participation in mucosal immune responses. The potential contributions of FCRL3 and secretory IgA to the pathogenesis of autoimmune diseases will be discussed.

Poster #129

Torres Juarez, Flor
NIAID

Metabolipidomic profiling of omega-3 and omega-6-derived bioactive lipid mediators in lungs of Mtb infected mice and nonhuman primate granulomas

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Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis (TB), responsible for more than a million deaths worldwide each year. Cytokines are key soluble protein mediators in conferring host resistance to TB. Biologically active fatty acid lipid mediators have also emerged over the last decade as important contributors to host resistance. Arachidonic acid (AA) derived eicosanoids are integrated into TNFa, IL-1 and type I interferon (IFN) cytokine networks by which they modulate infected-cell death and lung pathology. However, in addition to omega-6 fatty acid derived AA, omega-3 derived eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid derivatives also exert potent pro-resolving and biologic activities and it is unknown whether these lipid mediators are dynamically regulated in the lung after Mtb infection. Here, we measured changes in AA, EPA and DHA derived lipid mediators with liquid chromatography tandem mass spectrometry (LC-MS/MS) based metabolipidomic profiling in lungs of Mtb infected mice and TB granulomas from nonhuman primates. We found that cysteinyl leukotrienes and protectin conjugates in tissue regeneration (PCTR) were differentially and significantly changed after Mtb infection in mouse lungs. While leukotriene E4 (LTE4) and PCTR2 levels were increased, LTC4 and PCTR1 levels were reduced. In addition, resolving E1 (RvE1) was significantly increased. Importantly, in Il1r1-/- lungs levels of AA and EPA were significantly reduced, indicative of global changes in fatty acid metabolism in addition to eicosanoids the absence of IL-1r1. IL-1 and type I IFNs represent opposing inflammatory pathways and cross-regulate each other in TB. When we compared the metabolic profile of susceptible Il1r1-/- mice with resistant Ifnar1-/- mice, levels of LTE4 and neuroprotectin D1 (NPD1) negatively correlated with the bacterial burden across all mouse strains. To directly ask whether LTE4 or NPD1 could modulate host resistance to Mtb we are currently investigating mice deficient in receptors for cysteinyl leukotrienes and NPD1.

Poster #130

Trichka, Josephine
NCI-Bethesda

The ESCRT protein CHMP5 controls skeletal muscle homeostasis and coordination of myeloid cell-mediated tissue repair

Josephine Trichka and Stanley Adoro.

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Macrophages are required for maintenance of tissue homeostasis through their ability to regulate tissue metabolism, immunity, and inflammation, and to promote repair after injury. In skeletal muscle, the coordinate activity of resident and monocyte-derived macrophages facilitates crucial crosstalk between satellite cells, fibroadipogenic precursors, and myofibers that drives regeneration of damaged muscles. Dysregulation of this crosstalk contributes to conditions such as myopathy, inefficient wound healing, and sarcopenia. Despite their central role in muscle homeostasis and regeneration, the tissue-intrinsic factors and mechanisms that coordinate the recruitment and activity of these macrophages is poorly understood. In this study, we investigated how skeletal muscle homeostasis is regulated by the endosomal-sorting complex required for transport (ESCRT) protein CHMP5, which has recently emerged as a regulator of mammalian tissue development. CHMP5 (charged multivesicular body protein 5) was initially characterized as a member of the ESCRT family of proteins that coordinate membrane scission events in eukaryotic cells. However, recent studies by us and others have revealed non-canonical roles for CHMP5 in development wherein CHMP5 promotes the stability of client proteins required for cellular differentiation and cell fate decisions in both hematopoietic and non-hematopoietic tissues. Using an in vitro model of myogenesis, we found that CHMP5 knockdown impaired the differentiation of C2C12 myoblasts into myotubes. Furthermore, when wild-type bone marrow-derived macrophages were cocultured with CHMP5 knockdown C2C12 myoblasts, they failed to polarize into M2-like macrophages. In vivo, mice with muscle-specific CHMP5 heterozygous deletion (CHMP5het mice) displayed altered locomotion compared to littermate controls and diminished myeloid cell presence in muscle following cardiotoxin-induced injury. 12 days post-injury, histologic and flow cytometric analyses of muscles from CHMP5het mice revealed less regenerative progress compared to littermate controls. These data suggest a critical function for CHMP5 in promoting muscle homeostasis both in the steady state and in the context of injury and inflammation.

Poster #131

Valentina, Ottaviani
NIDCR

Dissecting the role of TGF-b in the skin

Ottaviani V., Chen W., N. Joller

NIDCR, NIH and Department of Quantitative Medicine, University of Zurich

The skin is outermost barrier in the body. Its only breaches are represented by hair follicles, which represent the only point of entrance for all the skin-sitting pathogens and commensals. Therefore, they can be defined as the most immunologically active sites of the skin. As such, tight regulation is needed at these sites and it is achieved not only through immune cells, but also non-hematopoietic cells like keratinocytes and fibroblasts. Example of immune cells are regulatory T cells, which are not only activated by, but also produce large amounts of TGF-b, a multifunctional cytokine, present in the body in 3 isoforms (TGF-b1,2 and 3). These isoforms have been reported to be fundamental for skin development during organogenesis and additional findings report about their role in driving cell localization in the skin as well as involvement in tissue repair. Though, little is known about their immunological role in the skin. Upon generating a working protocol to separate skin layers and isolate cells from them, we were able to start to define the cell composition of each layer both at steady state and during inflammation. Moreover, we could detect the three TGF-b isoforms in each layer at both gene and protein level further discriminating between hematopoietic and non-hematopoietic sources. Finally, the importance of TGF-b in the skin was confirmed by experiments on TGF-b receptor I depleted mice. For the future, we are planning on defining exactly which cells secrete and respond to TGF-b and confirm these findings by generating conditional KO mice. Additionally, the role of TGF-b will be assessed in world-wide skin diseases.

Poster #132

Valtierra Alvarado, Monica
NIAID

Obese Mice Have Attenuated Inflammatory Responses Following Infection with Bordetella pertussis

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Obesity is a global health problem that has been reported to predispose individuals to an increased risk of developing more severe infections compared to the regular weight host. However, these studies have been largely restricted to viral-mediated diseases and the impact of obesity following bacterial infection is less well understood. Pertussis is a highly contagious respiratory disease that, despite being vaccine-preventable, is still responsible for a significant source of morbidity and mortality worldwide. With a growing population of obese hosts, it is essential to understand how this metabolic condition may impact susceptibility and immunity to this bacterium. Here, we utilized a diet-induced obesity (DIO) mouse model to determine the effect of obesity on the outcome of infection with B. pertussis (Bp). Following intranasal infection, we did not observe any differences in bacterial burdens among regular weight (RW) and obese mice. However, despite similar bacterial loads we did observe significant differences in markers of inflammation that suggested obese mice had an attenuated inflammatory response compared to RW animals. Specifically, on day 14 after infection, we observed lower numbers of inflammatory monocytes in the lungs from DIO mice and lower levels of the activation markers MHC-II and CD86 among alveolar and interstitial macrophages from DIO compared to controls. Consistent with a dampened cellular response, DIO mice had significantly lower concentrations of a broad array of pro-inflammatory cytokines and chemokines (IL-12p40, IL-1β, IL-6, IFN-γ, TNF-α, MCP-1, and CXCL-1) in their lungs compared to RW mice after the Bp challenge. Collectively our approach revealed that although obesity had no impact on the control of bacterial replication it was associated with an attenuated inflammatory response in the lungs of mice. Together our data suggest that obesity favors improved resolution of inflammation following Bp infection that is independent of the hosts ability to control bacterial replication.

Poster #133

Wahlsten, Madison
NCI-Bethesda

Deep learning a model of cytotoxic T cell activation in the tumor microenvironment
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Cytotoxic CD8 T cells recognize antigens presented on major histocompatibility class I (MHC-I) molecules expressed on the surface of other cells, including tumors, leading to T cell activation and killing. The level of T cell activation, indicated by surface marker expression, cytokine production, and killing activity, is modulated by many factors including the quality and quantity of presented antigens. Immunotherapy treatments such as checkpoint blockade inhibitors have been successful in several cancers such as non-small cell lung cancer and melanoma, but limited in other types of cancers (e.g., pancreatic or prostate carcinomas) owing to differences in tumor antigenicity. Previous work from our lab has shown that the quality of an antigen for T cell activation can be encoded in a single parameter derived from cytokine dynamics produced in ex vivo co-cultures between antigen presenting cells (APCs) and T cells. Here we built a model that can capture the quality of tumor antigen seen by an individual T cell. Using a custom robotic platform, we generated high-throughput kinetics of T cell activation by analyzing cells at various timepoints. We performed spectral flow cytometry to measure the expression of up to 30 surface markers and intracellular signals per cell. Typical datasets comprise over ten million cells, characterized by 25-30 features over 72 hours, across many conditions. To analyze these content-rich datasets, we designed a machine-learning based model that can classify the antigen seen by an individual cell using marker expression values from flow cytometry with high accuracy (area under the receiver operating characteristic curve > 0.8). By leveraging the multifactorial nature of T cell activation at the single cell level, we aim to provide an in vivo-relevant classification of T cell activation, as well as insight into perturbations that could be applied to immunotherapies to achieve better responses in more patients.

Poster #134

Wang, Yihui
FDA-CBER

The Comparison of the Duration of Immunity Induced by Pertussis Vaccines and Infection in a Baboon Model

Yihui Wang and Tod Merkel

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Whooping cough is a highly contagious, acute respiratory illness caused by the Gram-negative bacterial pathogen Bordetella pertussis, a strict human pathogen with no other known reservoir. Disease is often mild in older children and adults but can be severe or fatal in young children and infants. It is endemic worldwide and a major cause of vaccine-preventable deaths despite high vaccine coverage rate. A steady increase in the number of reported pertussis cases has been evident over the last 20 years. The apparent correlation of this resurgence and the replacement of whole-cell pertussis (wP) vaccines with acellular pertussis (aP) vaccines suggests the current aP vaccines may not control pertussis in the population as well as the wP vaccines they replaced. Some evidence indicates the aP vaccines may induce protection with shorter duration of immunity relative to wP vaccines. However, interpretation of existing data is confounded by differences in aP and wP-vaccinated populations and the exposures they experience.

There is increased interest in developing next-generation pertussis vaccines that induce longer lasting protection. However, immunological correlates predicting durable and protective immunity remain unknown. Studying duration of immunity in a human population is problematic due to potential asymptomatic exposures and the difficulty in sample accessibility. In an ongoing, long-term study, using a baboon model we are comparing the duration of immunity and immunological correlates of protection in a controlled population comparing aP and wP vaccines as well as immunity induced by infection. Extensive sampling was undertaken around the vaccinations to characterize the vaccine-mediated immune responses. Animals enrolled in the study will be followed for years with sampling twice yearly during medical examinations. With strict control of Bordetella exposure, the data generated from the study will provide a clearer understanding and a basis of comparison for the duration of aP, wP, and infection-induced immunity.

Poster #135

Warner, Blake
NIDCR

Single Cell and Spatial Transcriptomics Identifies Altered Cellular Neighborhoods in the Salivary Glands of Sjogren's Disease Patients

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Sjogren's Disease(SjD) is a systemic autoimmune disorder targeting the lacrimal and salivary glands. Bulk transcriptomic tissue-level approaches cannot uncouple disease-specific changes in cellularity and expression state simultaneously. We hypothesize that integrated single-cell (sc) and spatial transcriptomics can unravel pathogenic cell-cell interactions and identify viable drug targets.

SjD(n=15) and healthy (HV,n=18) minor salivary glands (MSG) were used for scRNAseq(~250,000 cells); SjD(n=25) and HV(n=25) MSG were used for 10X Visium spatial transcriptomics (~15,000 spots). Flow cytometry, HiPlex in situ hybridization (ISH), and immunohistochemistry (IHC) were used for orthologous confirmation. Altered utilization of pathways in single cells was assessed and visualized across 7000 annotated pathways from KEGG, GO, Reactome, and mSigDB. Spatial transcriptomics was used to corroborate the altered cellularity and cellular networks using Cell2location inferred from scRNAseq identities. Cellular co-occurrence and cellular neighborhood analyses were performed.

Our scRNAseq dataset from MSG defined SjD changes in cell populations and transcriptional states. SjD SG had significantly more inflammatory cells and loss of acinar cells. Disease- and cell-type-specific differentially expressed genes (DEG) were identified including increased expression of MHC class I, B2M and HLA-B, and interferon (IFN) stimulated genes. Specific transcriptional states correlated with anti-SSA positivity, but not focus score. T cells functional annotation analysis revealed enrichment of pathways including: 'positive regulation of the Type I IFN response', 'T cell receptor signaling', and 'response to IFNγ signaling'. A T cell activation assay, CD8+T-cells from SjD MSG were significantly more cytotoxic than in HV. Spatial transcriptomics confirmed profound cellularity-dependent architectural and transcriptional changes and disease-specific cell-cell interactions (e.g., T cells:antigen presenting cells, T cells:acinar cells) and cellular neighborhoods.

Our findings link the loss of acinar cells with the presence of CD8+T-cells and illustrates disease-specific immune cell interactions. In summary, these data pinpoint pathogenic cell populations and cell-cell communications that may be directly targetable for therapeutic intervention.

Poster #136

Wells, Alexandria
NIAID

Adaptive immunity against ancient retroelements controls the tissue threshold of activation
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The skin is the body's largest and outermost barrier organ, and where microbiota-T cell communication is essential to promote protective immune responses. Integrated retroviral elements, known as endogenous retroviruses (ERVs), comprise up to 8% of the human genome. We previously found that innate sensing of ERVs was necessary to generate immune responses to the skin microbiota. Here, we hypothesize that ERV-specific CD8 T cells constitute a novel class of immune cells present in the skin at steady state, and that they may contribute to barrier immunity. We first established a model of sterile injury where ERVs can be reactivated specifically in the skin, independently of the microbiota. Topical application of brain-heart infusion broth dissociated in tween detergent robustly reactivated ERVs, and recruited CD8 T cells to the skin in an ERV dependent manner. To identify ERV antigen presenting cells we analyzed ERV expression by RNAseq, revealing Langerhans cells (LCs) have the most abundant ERV expression amongst skin dendritic cells. Using mice depleted of LC, we found that CD8 T cell responses observed in the context of sterile injury were highly dependent on LC. These observations supported the idea that LCs drive CD8 T cell responses to sterile injury by presenting ERV antigens. To identify ERV-reactive T cells, we selected peptides from our RNAseq that were uniquely expressed in LCs, but not expressed in thymic epithelial cells. Several peptides promoted cytokine production from injury-elicited CD8 T cells, confirming the presence of ERV-reactive CD8 T cells within the skin. Ongoing work involves generating tetramers and TCR transgenic mice to track ERV-specific T cells and define their role in barrier protection. We expect this work will uncover how the immune system recognizes ancient viruses through a novel class of T cells, called to the skin by LCs, to contribute to tissue physiology.

Poster #137

West, Erin
NHLBI

CD4 T cell intrinsic arginase 1 controls the kinetics of Th1 induction and contraction

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Arginase-1 (Arg1), the enzyme catalyzing the conversion of arginine to ornithine and urea, is a hallmark of IL-10-producing immunoregulatory M2-macrophages. However, its expression in T cells is disputed. Here we demonstrate that induction of Arg1 expression is a key feature of lung CD4 T cells during mouse in vivo influenza infection. Conditional ablation of Arg1 in CD4 T cells accelerated both virus-specific T-helper (Th)1 effector responses and its resolution, resulting in efficient viral clearance and significantly reduced lung pathology. Using unbiased transcriptomics and metabolomics, we found that Arg1 deficiency was distinct from arginase 2 deficiency and causes altered glutamine metabolism. Rebalancing this perturbed glutamine flux normalized the cellular Th1 response. CD4 T cells from rare ARG1 deficient patients or CRISPR-Cas9-mediated ARG1 deletion in healthy donor cells phenocopied the murine cellular phenotype. Collectively, CD4 T cell-intrinsic Arg1 functions as an unexpected rheostat regulating the kinetics of the mammalian Th1 lifecycle with unexpected implications for Th1 associated tissue pathologies.

Poster #138

Williamson, Kim

Uniformed Services University of the Health Sciences

Plasmablast Ig repertoire dynamics through repeat Plasmodium falciparum challenges

Patricia Ferrer, Andrew Frank, Andrea A. Berry, Kirsten E. Lyke, Kim C. Williamson

Patricia Ferrer, Andrew Frank, & Kim C. Williamson: Microbiology and Immunology Department, Uniformed Services University of the Health Sciences
Andrea A. Berry & Kirsten E. Lyke: Center for Vaccine Development and Global Health, University of Maryland School of Medicine

The development of natural immunity against malaria requires multiple exposures and even after protection develops against clinical disease individuals continue to be susceptible to infections. To study the dynamics of the immune response to repetitive P. falciparum infected mosquito exposures, we used controlled human malaria challenges with the same strain (homologous) of P. falciparum. The homologous strain was used to eliminate differences in immune responses due to strain polymorphisms and focus on the maturation of the human immune response to repeat exposures. Consistent with the gradual immune response observed in the field, after 4 sequential parasite exposures over the course of 2 years, symptoms decreased and the time to parasite detection on a blood smear increased significantly, but all the volunteers continued to be susceptible to infections. Anti-parasite antibody levels increased over time and were able to inhibit liver cell invasion in an in vitro assay. The Ig repertoire of plasmablasts collected 7 days after peak parasitemia demonstrated large clonal expansions in one or two sequential infections that decreased after subsequent exposures. This pattern suggests a marked difference in the lineage of plasmablast and memory cells populations. Naïve B cells stimulated by the parasite to proliferate and differentiate into plasmablasts may fail to differentiate into memory cells that can produce both plasmablasts and memory cells following subsequent exposures. This differentiation pattern would lead to new sets of expanded clones at each parasite exposure, instead of further maturation of the same antibodies generated during the first response. Such a response could enhance the diversity of the antibody pool, but would delay the generation of a strong, durable memory response.

Poster #139

Yamada, Eiko
NIDCR

Exploring cGAS-STING Pathway in Sjögren's Disease: Driver of IFN production and Potential Therapeutic Target

Eiko Yamada, D.D.S., Ph.D., Mehdi Abed, Shyh-Ing Jang, Ph.D., Paola Perez, Ph.D., Kalie Dominick and Blake Warner, D.D.S., M.P.H., Ph.D.

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Sjögren's Disease (SjD) is a common systemic autoimmune disease affecting predominantly women with a heterogenous clinical presentation and no effective therapy or cure. Although the pathogenic mechanisms of SjD are unknown, overexpression of interferons (IFN) are found in SjD patients and recent studies offered evidence of activated cGAS-STING pathway in systemic lupus patients. We aim to better understand the pathogenetic drivers of SjD focusing on cGAS-STING pathway and to explore potential inhibitors. We hypothesize that cGAS-STING pathway is activated in SjD patients and contributing to both local/systemic symptoms and dysregulation of IFNs. Our preliminary RNA sequencing (RNAseq) demonstrated that minor salivary gland (MSG) from SjD patients exhibited significantly increased IFNs scores and correlated with lymphocytic inflammation in the glands. Also, our single cell (sc) RNAseq of SjD MSG showed higher IFN related genes expression and composite IFN scores in epithelial, inflammatory, and mesenchymal cells. Immunofluorescence confirmed these results showing increased nuclear phosphorylated interferon regulatory factor 3, a downstream target of STING, in STING-expressing SjD acini, ducts, and inflammatory infiltrates, showing utilization and activation of cGAS-STING pathway in the principal target organ of SjD. scRNAseq of SjD PBMC indicated that IFN expression correlates with presence of cGAS-STING pathway utilization in specific cell types. To confirm these findings, we used flow cytometry to measure activation of phosphorylated STING-pathway proteins. Basal phosphorylated protein levels were higher in SjD. To test if this pathway could be targeted therapeutically, we used a STING antagonist and this treatment demonstrated significant downregulated phosphorylated protein levels on all cell subsets without cytotoxicity. Taken together, these exciting preliminary findings support our central hypothesis. Furthermore, targeting cGAS-STING pathway has the potential to mitigate drivers of systemic and salivary gland inflammation and prevent damaging secondary effects of chronic inflammation including exocrine hypofunction and tissue destruction.

Poster #140

Yang, Neil
NIAID

The functional role of Helios in Foxp3- T conventional cells

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The transcription factor Helios is expressed in the majority of Foxp3+ Regulatory T cells (Treg), where it plays an essential role in Treg function. Loss of Helios in the Treg population induces systemic autoimmunity, marked by CD4 and CD8 hyperproliferation, enhanced germinal center formation, and systemic immune activation. Helios is also expressed in a small subset of conventional CD4+ T cells. However, when Helios is deleted in all CD4 cells, conventional and Treg, by crossing the mice to CD4Cre, this phenotype is abrogated, indicating that Helios must also play a significant role in the function of conventional CD4+ T cells.

Transcriptome analysis of the Foxp3-CD4+ memory population revealed distinct differences between CD44hiHelios+ and CD44hiHelios- populations but did not allow us to identify a specific subset of the memory population responsible for phenotypic loss observed in the CD4Cre mouse model. In order to examine specific subsets of CD4+ memory cells, we performed single cell transcriptome analysis of CD44hi memory cells from WT mice and mice with the CD4 specific deletion of Helios. Characterization of the CD44hiHelios+ population showed that Helios expression changed dramatically with age, so cells from young (5-6 weeks) and old (5-6 months) mice were included in the analysis. Both longitudinal (young vs old) and cross-sectional (WT vs KO) comparisons were made, with emphasis placed on cross-sectional comparisons at old age. The sequencing results indicated significant transcriptomic and numeric shifts in multiple subpopulations of the analyzed splenocytes, including the almost complete loss of one cluster in both the young and old Helios deficient mice. Taken together, the observed transcriptomic and clustering changes may explain the role Helios plays in the abrogation of autoimmunity in our model and may provide a starting point for us to analyze its function in other biological pathways.

Poster #141

Yoon, Sung Hwan
NIAID

A Proteomic Investigation of Antibiotic Resistance and Susceptibility in Mycobacterium abscessus and Mycobacterium massiliense in response to Clarithromycin.

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Mycobacterium abscessus is a rapidly growing respiratory pathogen, most commonly seen in patients with chronic lung infection like cystic fibrosis. Clarithromycin is the drug of choice to treat M. abscessus infection but in many cases the intrinsic and acquired antibiotic resistance (AR) leads to unsuccessful outcomes. Although mechanisms of AR like decreased bacterial membrane permeability, increased expression of antibiotic inactivating enzymes have been recognized; it is not completely elucidated and remains unclear. There are reports on resistance inducing genes however there is dearth of information on the proteins that actually confer this resistant phenotype. Proteomics studies were carried out to identify bacterial proteins that are informative of AR mechanism in M. abscessus and compared with susceptible strain, M. massiliense.

To observe changes in protein expression in response to antimicrobial treatment, M. abscessus and M. massiliense type strain and culture from patient isolates were treated with CLA for 3 days. Antimicrobial susceptibility test was carried out and 0.06, 0.25, 1, and 4ug/mL concentrations of CLA were selected for treatments. Patient isolates were chosen to help identify proteome changes that can differentiate inducible versus acquired CLA resistance. Label-free quantitation of proteins at each CLA concentrations have detected few hundreds log2 fold down-regulated proteins and up-regulated proteins in each samples. Several ribosomal proteins including GNAT family N-acetyltransferase that have been implicated in antibiotic resistance were among the up-regulated proteins in DT10. Stress response protein, csbd family protein is up-regulated for all samples. Partial least squares discriminant analysis (PLS-DA) method sorted out highly variant proteins among M. abscess and M. massiliense respectably.

Poster #142

Zhang, Amy
NEI

Human Gut Commensals Support Development of Spontaneous Ocular Autoimmunity in Genetically Predisposed Mice

Amy Zhang1, Reiko Horai1, Yingyos Jittayasothorn1, Jonathan Badger2, Vijayaraj Nagarajan1, Caitlin Murphy1, Akriti Gupta1, Wuxing Yuan2, Colm O'hUigin2, Rachel R. Caspi1

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Autoimmune uveitis is a T cell driven, intraocular inflammation that targets the neuroretina and is a major cause of blindness. In a mouse model of spontaneous autoimmune uveitis (R161H) that expresses a transgenic T cell receptor (TCR) specific to a retinal protein, depletion of gut microbiota attenuates disease, and retina-specific uveitogenic T cells are found to signal in the gut through their clonotypic TCR. These findings suggest that gut microbiota and/or their metabolites may provide innate and adaptive immune signals that trigger ocular autoimmunity.

Specifically, we examined the development of uveitis and its association with gut microbiota in gnotobiotic R161H mice colonized with healthy human flora, using the rationale that uveitis-triggering microbes must be present before the onset of disease. We reconstituted germ-free R161H mice with fecal samples from three healthy human donors, and performed fecal metagenomic sequencing, gut immunophenotyping and disease scoring.

Our results indicate that human-derived gut commensal microbes support autoimmunity in the spontaneous uveitis model. Human flora mice compared to SPF mice with normal mouse flora display an altered intestinal CD4 T cell effector and regulatory T cell profiles, and altered fecal metabolites. Microbiome analyses show that human flora mice retained a distinct but simplified gut microbial community compared to their original donor sample. Mice with high disease scores appeared to harbor more diverse gut flora than those with low scores. Furthermore, Verrucomicrobia, Actinobacteria, and Fusobacteria were enriched in mice with high disease scores, whereas Firmicutes appeared enriched in mice with low disease scores. Mono-association studies are underway to examine the ability of candidate microbes to modulate spontaneous autoimmune uveitis.

Poster #143

Zhang, Hongwei
NIAID

Leukocyte Trafficking in Severe COVID-19

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GWAS have identified an association between sequences at the chemokine receptor locus at 3p21 and severe COVID-19. Using flow cytometry, we have analyzed 14 chemokine receptors on NK cells, T cell subsets, monocytes and dendritic cells in 30 PBMC samples from 19 hospitalized COVID-19 patients together with samples from age- and sex-matched healthy donors. Expression of multiple inflammation-associated chemokine receptors was decreased across various lymphocyte subsets in COVID-19. For CXCR3 and CCR2, expression was significantly diminished on mucosal-associated invariant (MAIT) and other T cells, and CCR2 was also decreased on monocytes and pDCs. In addition, mRNAs for CXCR3 and CCR2 were less abundant in RNA isolated from the patients' T cells. We have demonstrated previously that CXCR3 and CCR2 are critical for firm arrest on activated endothelial cells and transendothelial migration, respectively, of human effector-capable, memory-phenotype T cells. Numbers of MAIT cells and pDCs were depressed in the patients' blood. We hypothesize that trafficking of these and perhaps other leukocytes into tissue in COVID-19 is mediated by CXCR3 and/or CCR2, leading to selective depletion of circulating CXCR3high and CCR2high cells - a hypothesis supported by experiments showing the preferential chemotaxis of CXCR3high and CCR2high cells in gradients of their ligands, CXCL10 and CCL2, respectively. Ultimately, identifying chemokines/receptors mediating leukocyte trafficking in COVID-19 will reveal roles for the chemokine system and specific leukocyte subsets in this disease and lead, potentially, to developing agents to reduce inflammatory damage.

Poster #144

Zhu, Xiaoliang
NIAID

Optimal CXCR5 Expression during Tfh Maturation Involves Bhlhe40-Pou2af1 Axis Downstream of Bcl6-Blimp1

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Tfh cells play a critical role during humoral immune response by helping B cells in antibody production. CXCR5 expression is critical for Tfh cell development and their migration into germinal center (GC). However, the regulation of CXCR5 expression during Tfh cell differentiation is still unclear. In this study, we found that the transcription factor Pou2af1/Bob1, which was reported to be essential for GC formation and B cell development, was also important for Tfh cell differentiation in a T cell intrinsic manner. On the other hand, Bhlhe40 inhibited Tfh cell generation partially by suppressing the expression of Bob1. Bhlhe40 expression gradually decreased from CXCR5-Bcl6-, to CXCR5-Bcl6+, to CXCR5+Bcl6+, which was accompanied by increased expression of Bob1. By using Bhlhe40 and Bob1 double knockout mice, we found that Tfh cell differentiation was blocked at the pre-Tfh (CXCR5int Bcl6int) stage indicating that Bob1 works at the downstream of Bhlhe40. In the mixed bone marrow model, we further verified, through confocal imaging, that Bob1-deficient CD4 T cells largely failed to migrate into GC in competition with WT counterparts. By contrast, Bhlhe40 deficient T cells were more efficient than wide type cells in becoming GC Tfh cells. Such migration behavior change can be explained by an alteration in the MFI of CXCR5. Genome-wide RNA-Seq analysis also confirmed an important role of Bhlhe40 in suppressing Bob1 and the requirement of Bob1 upregulation in optimal CXCR5 expression. Thus, our study revealed a critical transcriptional regulatory circuit involving Bhlhe40/Pou2af1 in regulating optimal CXCR5 expression and Tfh cell migration during the transition from pre-Tfh to GC-Tfh cells, which operates downstream of the Bcl6/Blimp-1 circuit that determines Tfh cell fate.

Poster #145

Ahmad, Javeed
NIAID

Bivalent molecules from structure-guided design effectively neutralize SARS-CoV-2 and variants

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The development of therapeutic antibodies/nanobodies has received extraordinary attention due to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. Synthetic nanobodies (sybodies), which have many advantages compared to conventional antibodies, are prime candidates for studies of their capacity to prevent viral entry. By X-ray crystallography, we identified the structural underpinnings of the interactions between a panel of sybodies (Sb14, Sb16, Sb45, and Sb68) and the SARS-CoV-2 receptor binding domain (RBD): binary complexes of Sb16-RBD and Sb45-RBD; and ternary complexes of Sb45-RBD-Sb68 and Sb14-RBD-Sb16. Sb45 and Sb16 bind the RBD at its interface with the RBD cellular receptor ACE2 with comparable footprints. However, the complementarity determining regions (CDR2 and CDR3) of Sb45 are in diametrically opposed positions compared to Sb16. The Sb45-RBD-Sb68 and Sb14-RBD-Sb16 complex ternary structures indicate that whereas Sb14 binds directly at the ACE2 interface, Sb68 binds near its perimeter. The ternary arrangement demonstrates that multiple sybodies may capture a significant portion of the RBD's surface. Cryo-EM structures of Sb45 bound to the SARS-CoV-2 spike protein were also determined. These sybody structures provided structural insights that were utilized to design and engineer extremely powerful biparatopic sybodies as well as bivalent molecules with ACE2-mimicking peptides to enhance the binding and neutralization potential against a range of SARS-CoV-2 variants.

Poster #146

Basu, Rahul
NIAID

A focused genetic screen uncovers genes which contribute to increased La Crosse Virus susceptibility in children

Presenter: Rahul Basu Authors: Rahul Basu1,2, Sundar Ganesan2, Clayton Winkler1, Karin E. Peterson1 and, Iain D.C. Fraser2

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La Crosse virus (LACV), a mosquito-borne orthobunyavirus, is one of the primary causes of pediatric viral encephalitis in the USA. Mice provide a useful experimental model for age-dependent LACV susceptibility as weanling mice will contract LACV-induced encephalitis, while adult mice are resistant. Encephalitis in weanling mice is associated with virus-mediated damage of the blood brain barrier (BBB), resulting in vascular leakage in the olfactory bulb/tract (OB) region of the brain. To examine mechanisms of LACV-induced BBB breakdown and infection of the CNS, we analyzed brain capillary endothelial cells (BCECs) isolated from weanling and adult mice to identify age-dependent response(s) of BCECs to LACV infection. Ex vivo cultured BCECs from weanling, but not adult mice, had detectable infected cells after several days. Further analysis of BCECs infected in vitro showed that weanling BCECs were more susceptible to virus infection than adult BCECs, with higher levels of infected cells and released virus, as well as cytopathic effects (CPE) and cell death. Using RNA-seq, we identified 35 genes as candidate regulators of age-dependent susceptibility of BCEC to LACV. We have conducted a focused siRNA screen of these genes in mouse endothelial cell line bEnd.3 and identified a subset of genes that specifically regulate viral infection. The top hits of these gene perturbation effects are being validated in primary BCECs. Follow-up studies are ongoing to establish which phase of viral infection (attachment or entry/ replication/ viral trafficking/ export and egress) is impacted, and preliminary data suggests that 7 hits from the screen exert specific regulatory effects on different phases of the viral life cycle. Importantly, two of the gene products, ephrin A2 and Connexin43 can restrict viral infection in weanling BCECs when added exogenously to host cells. Taken together, these genes may provide potential therapeutic targets for regulating LACV infection in pediatric encephalitis patients.

Poster #147

Berkson, Julia
FDA-CBER

Immunological and Microbial Responses to Bacteriophage Therapy Targeting Vancomycin-Resistant Enterococcus colonization

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Multidrug resistant bacteria are an emerging global threat with an urgent need for alternative therapies. Bacteriophage (phage) therapy is a promising treatment with several clinical trials currently underway. Yet, the overall effects of administration of this biologic on the mammalian immune system remain to be elucidated. While previous studies have shown production of antibodies against phage treatment, questions remain regarding the role of the innate immune response and any consequences of anti-phage immunity on efficacy especially when administered as a series of treatments. To investigate this question, we the developed a phage cocktail targeting vancomycin-resistant enterococcus (VRE) that significantly reduces the bacterial burden in a mouse model of intestinal VRE colonization. This cocktail was used to define changes in the innate and adaptive splenic cell compartments, cytokine expression, and antibody production during phage therapy compared to a vehicle control group. Phage cocktail exposure stimulated macrophage activation and induction of proinflammatory cytokines important for macrophage function. In addition, phage cocktail treatment elicited production of anti-phage neutralizing antibodies in the serum, especially after secondary exposure. Unexpectedly, we observed that different phage families in our cocktail, siphophage and myophage, have distinct sensitivities to antibody neutralization. We further investigated the mechanism underlying these responses to neutralization by defining the different proteins targeted by anti-phage antibodies. Finally, we showed that induction of anti-phage immunity reduces efficacy of phage therapy in our mouse model of VRE decolonization. This work contributes a better understanding of the immune responses that occur during bacteriophage treatment and could aid future advances and clinical trial strategies of this promising therapeutic strategy.

Poster #148

Bettencourt, Ian
NCI-Frederick

The peritoneal tissue resident macrophage niche is dynamic and is supported by both stromal and circulating immune cells.

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Tissue resident macrophages (TRM) are present throughout the body and are responsible for maintaining tissue homeostasis and protecting against pathogens. Each tissue has its own population of TRM, with distinct patterns of gene expression that are maintained in specific tissue niches comprised of stromal cells and soluble factors. One distinctly expressed gene in peritoneal TRM, aspartoacylase (Aspa), has primarily been studied in the nervous system. Aspa hydrolyses the nervous system-enriched metabolite N-acetyl-aspartate (NAA). Our previous work identified NAA as enriched in the peritoneal lavage fluid as compared to the serum. Moreover, perturbing the peritoneal cavity revealed that NAA levels are dynamic and react to immune stimuli, indicating the possible role of NAA as a regulator of the peritoneal TRM niche as well as influencing the peritoneal cavity environment across health and disease. To dissect the possible impact of the NAA/Aspa axis in the peritoneal niche we sought to assess the heterogenous cell populations of the peritoneal cavity niche simultaneously. We performed an in situ enzymatic digestion designed to liberate stromal cells from the peritoneal cavity in concert with the peritoneal TRM present in the lavage fluid. Surprisingly, our data showed that the stromal cell population also expresses Aspa, meaning it may play a more active role in NAA metabolism than previously appreciated. Furthermore, we were able to identify novel expression of N-acetylaspartate synthetase (Nat8l), the enzyme that catalyzes NAA production. In addition to unexpected Nat8l expression in the peritoneal TRM, it was also found in monocyte and neutrophil populations within the digested peritoneal cavity. Our work suggests that leukocyte trafficking supports NAA levels in the peritoneum to maintain the unique TRM populations. Future work will further dissect the role of NAA within the cavity niche and seek to understand its effects on the function of peritoneal TRM is underway.

Poster #149

Bing, Sojin
FDA-CBER

Differential T Cell Immune Responses to Deamidated Adeno-associated Virus Vector

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Objective: The immune response to recombinant adeno-associated virus (rAAV)-mediated gene therapy remains a major limiting factor in its successful implementation and is closely correlated to treatment outcomes. Prior studies have shown that rAAV vectors undergo a high degree of spontaneous deamidation, with the highest levels occurring at NG pairs on the AAV vector capsid. In this study, we investigated the cellular immune responses to peptides derived from spontaneously deamidated AAV.

Methods: We carried out predictive modeling of MHC-restricted potential epitopes for AAV9 capsid protein using the IEDB database. Then we investigated the binding affinity and immune responses of deamidated peptides, to study the effects of deamidation on the immunogenicity of rAAV.

Results: Some HLA molecules were predicted to bind with higher or lower affinity to deamidated (aspartic acid) peptides than WT peptides. To validate the binding predictions, 24 HLA molecules were tested for their binding to the various peptides. Some correlations between the predicted binders and the experimental binders were observed, confirming that some HLA molecules bind to WT and deamidated peptides differentially. Finally, the T cell immune response was evaluated using expanded PBMCs from untreated donors. Importantly, one deamidated site (N512) had a tendency to induce T cell activation in some samples that share HLA-DRB1*03:01. The immune response to the deamidated peptides was characterized as CD4+, effector memory with a Th1 cytokine signature. This indicates that the spontaneously occurring deamidation in AAV may increase the immunogenicity of AAV in some individuals.

Conclusion: Our study evidenced the difficulty of predicting the impact of rAAV capsid-specific cellular immunity on the safety of rAAV-based gene therapy products, as subtle deamidations may significantly enhance or reduce their immunogenicity.

Poster #150

Costa-da-Silva, Ana Caroline
NIDCR

What are Exhausted Effector T CD8 cells, revealed by Single-cell RNAseq, doing in human mucosal chronic GVHD?

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Chronic graft-versus-host disease (cGVHD), an autoimmune-like disease that occurs following allogeneic hematopoietic stem cell transplantation, is driven by pathogenic T cells that trigger host tissue damage. Despite advances in the identification of different T cell subsets involved in the initiation and persistence of murine cGVHD, availability of similarly detailed patient data remains scarce. Using single-cell RNA sequencing (scRNAseq) analysis of human oral mucosa (OM), a cGVHD effector site, we examined T cell populations and tissue-specific signals that regulate their differentiation and functionality. Through analysis of approximately 40,000 OM cells from 4 non-transplanted healthy volunteers, 4 transplanted patients without oral cGVHD and 5 patients with oral cGVHD, we observed enrichment of immune cells in oral cGVHD patients, particularly in the T CD8 cell compartment. T CD8 cells in different states of differentiation were assessed, however, we identified a unique population in the OM of cGVHD patients characterized by the expression exhaustion makers. This population had low expression of CD69 but expressed other markers of tissue residency (Trm), including CD103, BLIMP1 and FABP4. Additionally, this unique population of Trm in oral cGVHD patients was enriched for genes associated with exhaustion (TIM3, LAG3 and TIGIT). Interestingly, this population showed high expression of BATF, IRF4 and RUNX3, transcription factors that induce sustained effector T CD8 cell programs. Consistently, we also observed higher expression of effector molecules (IFNG, GZMB and PRF1) in this cell population. Within the population of TIM3+effectorhiT CD8 cells, there was elevated gene expression of STAT3 and BATF, which could mark a population of T CD8 cells consistent with persistent effector function in cGVHD. This specific population present within cGVHD-affected tissue, could contribute to ongoing tissue damage and pathogenesis during chronic phases of GVHD and may represent a novel target for cGVHD therapy.

Poster #151

Fisher, Megan
NIAID

TCR Signal Strength Indirectly Regulates Complex N-Glycosylation of Recently Activated CD4+ T Cells Via a Soluble Factor

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TCR signaling strength regulates CD4+ T cell differentiation by mechanisms that are poorly understood. TCR stimulation of CD4+ T cells causes rapid changes in glycosylation, including a dramatic increase in highly branched complex N-glycans. N-glycosylation regulates many cell-surface molecules involved in T cell activation and differentiation, including key cytokine receptors and the TCR itself. Glycosylation pathways and the nutrients that support them can also influence CD4+ T cell development. Therefore, we hypothesized that TCR signal strength directs CD4+ differentiation by regulating complex N-glycosylation. Using lectin-based staining and cell trace co-staining of human PBMCs, we found that the persistence of high levels on complex N-glycosylation as cells divide depends on TCR signal strength in cells stimulated with anti-CD3 and anti-CD28 for four days. Following initial increases of complex N-glycosylation occurring in all CD4+ cells with TCR/CD28-stimulation, strongly stimulated CD4+ T cells demonstrate successive decreases in complex N-glycosylation with greater numbers of divisions, while weakly stimulated cells sustain high levels of complex N-glycosylation comparable to peak levels. Whereas supplemental nutrients increase overall complex N-glycosylation in the population, they do not prevent the decrease in strongly stimulated cells as they divide. However, providing weakly stimulated cells with conditioned media from strongly stimulated cells recapitulates the strong-stimulus phenotype, suggesting that a strong TCR stimulus leads to production of a soluble factor that reduces cellular N-glycan complexity as they divide. Indeed, treatment with a variety of cytokines suppresses complex N-glycosylation as cells divide, including IL-12 and IL-4. These results suggest TCR signal strength regulates the production of secondary factors that affect early glycosylation events. This differential glycosylation may play a role in CD4+ T cell subset differentiation. Ongoing experiments will attempt to identify the factor(s) required for this effect and the signaling pathways they employ and the role of N-glycosylation in CD4+ T cell differentiation.

Poster #152

Hilligan, Kerry
NIAID

Pre-existing interferon gamma responses condition the lung to mediate early control of SARS-CoV-2 infection

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Interferons (IFNs) are critical for anti-viral host defence. Type-I IFNs are typically associated with early control of viral replication and promotion of inflammatory immune responses; however, less is known about the role of IFN-gamma in anti-viral immunity, particularly in the context of SARS-CoV-2. We have previously observed that lung infection with attenuated bacteria M. bovis BCG achieved though intravenous (iv) administration provides strong protection against SARS-CoV-2 infection and disease in two mouse models. Assessment of the pulmonary cytokine milieu revealed that iv BCG induces a robust IFN-gamma response, but low levels of type-I IFN. Neutralization of IFN-gamma in BCG-infected mice abrogated BCG afforded protection against SARS-CoV-2, indicating that the pre-existing IFN-gamma response within the lung may be involved in limiting viral replication. Indeed, intranasal administration of recombinant (r)IFN-gamma on two consecutive days prior to SARS-CoV-2 challenge recapitulated our observations in BCG-infected mice, with animals protected from weight loss and lower viral loads recovered from the lungs. Given the short timeframe with which rIFN-gamma confers protection against SARS-CoV-2, IFN-gamma was likely acting on a cell type directly targeted for infection by this virus, such as type-2 pneumocytes (AT2). Flow cytometry analysis of iv BCG-infected and rIFN-gamma-treated mice showed strong up-regulation of interferon-stimulated markers associated with anti-SARS-CoV-2 activity by AT2 that could indicate an "anti-viral" state prior to SARS-CoV-2 exposure. Importantly, bone marrow chimera experiments revealed that iv BCG-induced protection was lost if the non-hematopoietic compartment could not respond to IFN-gamma, providing further evidence that an epithelial cell type (such as AT2) is the target for IFN-gamma-mediated protection. Together, our data show that a pre-established IFN-gamma response within the lung is protective against SARS-CoV-2 infection, suggesting that concurrent or recent infections that drive IFN-gamma may limit the pathogenesis of this virus.

This work was supported by the Intramural Program of NIAID.

Poster #153

Islam, Zohirul
NIAID

The role of Matrin 3 (MATR3) in innate immune response

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MATR3 is one of the most abundant inner nuclear matrix proteins and is implicated in amyotrophic lateral sclerosis (ALS) and distal myopathy. MATR3 has been reported to have both DNA- and RNA-binding abilities. However, it is not understood mechanistically how mutation of MATR3 leads to ALS pathogenesis. To identify its RNA-binding roles, we did photoactivatable ribonucleoside enhanced crosslinking and immunoprecipitation (PAR-CLIP) in the human HAP1 cell line. To our surprise, we identified MATR3 binds to a set of interferon-stimulated genes (ISGs). Furthermore, loss of function mutations in MATR3 results in the upregulation of these ISGs. By using two different RNA sequencing methods Oxford Nanopore Technologies and Illumina, we demonstrated the upregulation of many ISGs in MATR3-deficient HAP1 cells. Furthermore, we have validated these results by Nanostring technology. Next, we hypothesized that HAP1 cells may be responding to endogenous double-stranded RNAs. Interestingly, we observed similar amounts of dsRNA in both MATR3 WT and KO cells which suggests that indeed intracellular dsRNAs are present and could be stimulating HAP1 cells; however, it is not the case that MATR3 KO cells are stimulated because they contain more dsRNAs. Currently, we are investigating which RNA sensors are activated in MATR3 KO cells. To further strengthen our findings, we are using DEGRON technology to inducibly degrade MATR3 protein in a reversible manner. Altogether, our results illustrate that MATR3 is a novel negative regulator of the innate immune response.

Poster #154

Jessop, Forrest
NIAID

Prolyl Hydroxylase Inhibition and HIF-dependent Metabolic Reprogramming Protects Against Lethal SARS-CoV-2 Infection in Mice

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Severe SARS-CoV-2 infection has been associated with dysregulated immune and metabolic responses. Correction of these defective host responses may provide a therapeutic avenue to limit disease severity. The transcription factor HIF-1α governs pro-survival metabolic adaptations and its stabilization has been positively correlated with decreased disease severity. However, the role of HIF-1α in severe SARS-CoV-2 infection remains poorly understood. In the current study, we established that SARS-CoV-2 infection impaired HIF-1α stabilization and associated metabolic reprogramming. Prolyl hydroxylases (PHD) directly regulate HIF-1α stabilization. Therefore, we evaluated the therapeutic potential of the α-ketoglutarate derivative dimethyloxalylglycine (DMOG), that targets these enzymes, following SARS-CoV-2 infection in vitro and in vivo. DMOG treatment of A549 cells infected with SARS-CoV-2 resulted in stabilization of HIF-1α and a shift toward glycolysis. Importantly, treatment of k18-hACE2 mice infected with SARS-CoV-2 with DMOG significantly increased survival. Similar to our in vitro findings, survival was attributed to DMOG-induced stabilization of HIF-1α and increase glycolysis. Further, we also observed reduction in amino acid pools, increased hypoxanthine, and enhanced pro-inflammatory and anti-viral cytokine/chemokine production among DMOG treated mice. Together these data demonstrate the critical role modulation of host metabolism plays in SARS-CoV-2 infection and support early targeting of PHD/HIF-1α signaling as a viable therapeutic strategy to limit severe disease.

Poster #155

Kim, Tae Sung
NIDCR

Neutrophil extracellular traps and extracellular histones mediate IL-17 inflammation and bone destruction in periodontitis

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Neutrophil infiltration is a hallmark of the inflammatory disease periodontitis, a prevalent oral condition in which inflammation leads to destruction of tooth-supporting bone. Herein, we document that in periodontitis, neutrophils expel neutrophil extracellular traps (NETs) to trigger disease immunopathology. In an established animal model of periodontitis, we demonstrate that genetic or pharmacologic inhibition of NET formation, or removal of NETs by DNase-Ⅰ, significantly prevents inflammation and bone destruction in vivo. Investigating the mechanisms by which NETs drive periodontal inflammation, we find that extracellular histones, a major component of NETs, trigger IL-17-mediated inflammation and bone loss. Importantly, human findings corroborate our experimental work. We document, in periodontitis patients, significantly elevated levels of NET complexes and of extracellular histones bearing classic NET-associated post-translational modifications. Strikingly, concentrations of NET components in disease lesions and in circulation, significantly correlate with the severity of bone destruction in patients, even in the absence of known confounding systemic disease. Collectively, our work reveals NET-associated components as pathogenic mediators, potential biomarkers, and plausible therapeutic targets for a very common inflammatory disease.

Poster #156

Krishnan, Anagha
NCI-Bethesda

Bottoms Up!: Inferring Relevant Immune Phenotypes from Bulk Cytokine Kinetics via Semi-Supervised Regression

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Immunologists often collect data on continuous multi-parametric single cell characteristics: flow cytometry, mass cytometry, single cell RNA-sequencing, etc. Immunologists then classify cells based on these characteristics via sequential hand-gating or high-dimensional clustering algorithms. Often, relevant cellular phenotypes (clusters) are determined by an iterative, intuition-driven process that requires significant biological validation, which begs the question: can we efficiently predict immunologically-relevant phenotypes?

To that end, we developed a semi-supervised regression algorithm that directly linked multi-parametric single cell data to bulk cytokine kinetics. Using cytokine production by T cells following priming by antigen-presenting tumor cells as a biological model system, we measured bulk interferon-gamma (IFNg) levels in tumor-T cell co-cultures using a robotic platform that collected and stored samples every six hours for 72 hours. From these samples, we constructed detailed fitted time kinetics of IFNg.

Using the same robotic platform, we measured thirty-two single-cell markers at each timepoint using a spectral flow cytometer. We then fed the single-cell data into a regression algorithm that clustered the data at various clustering depths (number of clusters) and assigned weights to each cluster representing the average amount of IFNg produced in that cluster. From this model, we recapitulated the bulk IFNg kinetics from the frequencies of IFNg-producing clusters without directly staining for intracellular IFNg. We biologically validated the weights of the model and determined that the weights correctly predicted the relative IFNg made by each cluster. Furthermore, by studying cluster interactions via pseudo-time trajectory analysis, we constructed a compartment model to describe the priming of T cells.

Our regression model serves as a broadly applicable method to directly link bulk cytokine kinetics to single-cell phenotypic data. We aim to use this method to infer bulk population characteristics (such as cytokine levels or killing) from multi-parametric single cell data to answer various new immunology questions.

Poster #157

Lopez-Munoz, Alberto D
NIAID

Cell Surface Nucleocapsid Protein: An Evolutionary Conserved Immunomodulatory Strategy of Betacoronaviruses?

Alberto D. Lopez-Munoz, Jonathan W. Yewdell

Cellular Biology Section, Laboratory of Viral Diseases, NIAID, NIH

Human Coronaviruses (HCoV) have been historically experienced as common cold viruses. However, in the last twenty years, highly pathogenic HCoV have emerged causing epidemic and pandemic severe acute respiratory syndromes (SARS) worldwide. Despite the unprecedent global response to COVID-19, which has generated over 300,000 publications in nearly three years, critical aspects of HCoV biology, pathogenesis and immunomodulation remain uncertain, including mechanisms underlying long COVID-19, the cytokine storm and coagulation dyscrasias.

Nucleocapsid (N), the most abundant viral protein expressed during HCoV infections, induces strong antibody and T cell responses. Recently, higher levels of free SARS-CoV-2 N in the blood have been correlated with more severe disease. N was considered to be strictly localized intracellularly. Yet, cell surface expression of N proteins is the rule among RNA viruses, inducing immunosuppression and serving as antibody targets.

We reported that extracellular SARS-CoV-2 N binds to infected and non-infected neighboring cells, inhibits in vitro chemotaxis of leukocytes and activates Fc receptor-expressing cells. We find that N from HCoV-OC43 infected cells, an endemic seasonal HCoV, is also expressed on the cell surface of live cells in high copy numbers, binding to donor and recipient neighboring cells by electrostatically associating with heparan sulfate/heparin, ubiquitous surface glycosaminoglycans found in all mammalian cells. HCoV-OC43 N binds with high affinity to the same set of 11 human chemokines as SARS-CoV-2 N, but also to an exclusive set of 6 additional cytokines. As reported for SARS-CoV-2 N, HCoV-OC43 N also inhibits CXCL12b-mediated leukocyte migration in chemotaxis assays, as did other highly pathogenic and endemic HCoV N proteins. Anti- HCoV-OC43 N antibodies bound to the surface of infected cells also activate Fc receptor expressing cells.

These data indicate that cell surface HCoV N may play an important evolutionary conserved role in manipulating host innate and adaptive immunity, beyond sequence and structural divergence.

Poster #158

O'Connell, Michael
NIAID

Enzymatically inactive tryptases function as partial agonists of LPS-mediated TLR4 activation

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During anaphylaxis tissue resident granulocytes called mast cells degranulate releasing pre-formed mediators, such as histamine and enzymatically active mature tryptases, into the local environment where they mediate symptoms of immediate hypersensitivity that in severe instances can result in hypotensive syncope and even death. Following degranulation mature tryptases quickly disassociate into inert monomeric tryptases and persist in the local environment and blood stream for several hours. In addition, in their zymogen form called pro-tryptases, enzymatically inactive tryptase precursors are constitutively secreted from mast cells into tissues.

Here, we demonstrate that monomeric tryptases - both in their zymogen forms and inactive mature monomers (inactive-tryptase) - can be taken up into fibroblasts when in the presence of LPS. In primary dermal fibroblasts and bone marrow stromal cell cultures stimulated with physiologic levels of inactive-tryptases and LPS, we see an increase in NF-kappaB target gene and protein generation including IL-1beta, IL-8, GM-CSF, and CCL2. We also demonstrate inactive-tryptases can enhance LPS-induced endothelial cell permeability, via physically engaging the TLR4-LPS complex in the absence of co-receptors LBP or CD14. Using immunoprecipitation and surface plasmon resonance we show that inactive-tryptases enhance the physical binding of LPS to TLR4. Further, we demonstrate that inactive-tryptases enhance CD14/LBP complex formation with TLR4, but the presence of inactive-tryptases in this complex results in reduced permeability, suggesting a role for inactive-tryptases as partial agonists/antagonists of LPS-induced TLR4 signaling. Indeed, we demonstrate that inactive-tryptases have a protective effect from LPS-induced cell death in an endotoxemic shock mouse model. Taken together, these data suggest that inactive-tryptases may be important modulators of TLR4-mediated innate inflammation and may help explain how a genetic trait associated with baseline over-expression of inactive-tryptases was selected for in humans on an evolutionary timescale.

Poster #159

Sharma, Rubina
NIDCR

Cytotoxic role of γδ T-cells in oral Chronic Graft-Versus-Host Disease (cGVHD)

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Chronic Graft-Versus-Host-Disease (cGVHD) an autoimmune-like disease following allogeneic hematopoietic stem cell transplantation (HSCT) targets multiple organs, including the oral cavity oral mucosa (OM) and minor salivary glands are frequently affected. Gamma-delta T cells (γδ T) are a unique conserved population of lymphocytes serving as a first line of innate immune defense against pathogens. This unique role in immune surveillance and tissue homeostasis is critical at the interface between OM and the oral cavity which is replete with microbial and allergic challenges to the immune system. Single-cell RNA sequencing analysis of human OM identified an increase in IFNγ and IL17 producing γδ T-cells in cGVHD versus unaffected post-transplant patients. These cells had upregulated expression of transcription factor EOMES playing crucial role in cytotoxicity related genes (GZMA, GZMB and PRF1) in cGVHD patients. To localize gene expression signatures, spatial transcriptomic analysis was done on human OM illustrating an increase in γδ T-cells in OM of cGVHD patients. γδ T-cells were frequently co-localized with GZMA, GZMB and PRF1 clusters at the basement membrane (frequent site of tissue damage) in cGVHD. Ligand receptor analysis revealed the expression of CXCR4 was significantly elevated in γδ T-cells in cGVHD OM interacting with CXCL12 ligand from fibroblast playing direct role in the recruitment of γδ T-cells into the OM through CXCR4-CXCL12 axis promoting the development of cGVHD. Flow cytometric analysis of OM biopsies indicated an elevated number of γδ T-cells in cGVHD than unaffected.

This data suggests a direct cytotoxic role of γδ T-cells in local production of IFNγ and IL17 cytokines contributing to the chronicity of GVHD. In future, use of genetic mouse models with γδ T-cell alterations will help clarify the role of γδ T-cells in cGVHD pathogenesis.

Poster #160

Singh, Satya
NIAID

Human CCR6+ Th memory cells form opposing extended gradients of Th17 and multilineage character with position-dependent mechanisms of plasticity

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Th17 and related (type 17) cells have been investigated in mice for their contributions to autoimmune diseases and have demonstrated roles in immune-mediated disorders and host defense in humans. However, the pathways of differentiation of type 17 cells and the structure of the type 17 memory population in humans are not well understood; such understanding is critical for manipulating these cells in vivo. By exploiting differences in levels of surface CCR6 and analyzing cells in bulk and as single cells, we found that human type 17 memory cells, including individual T cell clonotypes, form an elongated continuum of type 17 character along which cells can be driven by increasing the signature transcription factor, ROR□t. Throughout this continuum, cells are marked by extensive but progressively diminishing co-expression of genes of alternative lineages. The cells' phenotypes and epigenomes are stable across cell divisions under homeostatic-like conditions. Nonetheless, activation in non-polarizing and polarizing environments can induce additional functionalities by both previously imprinted and environmental mechanisms that contribute differentially across the continuum to yield the unusual plasticity ascribed to type 17 cells.

Poster #161

Xuan, Xie
NIAID

Eos is a critical transcription factor for T regulatory cell (Treg) function

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Eos is a member of the Ikaros Zinc Finger transcription factor family and was previously reported to be highly expressed in Tregs. Mice with a global deficiency of Eos or a selective deficiency of Eos in Treg develop systemic or organ-specific autoimmune disease at different ages. We have generated a specific anti-Eos monoclonal antibody that binds to wild type Tregs, but not Tregs from Eos deficient mice. We find that the percentage of Tregs expressing Eos rapidly increases with aging from almost 0% to 30~50% of thymic Tregs and 35~60% of splenic Tregs at 3 weeks of life and remains stable after that. In the thymus, Eos is only expressed on CD73+ Tregs, which suggests that Eos+ thymic Tregs represent peripheral Tregs that have recirculated to the thymus. We also have observed that Eos is co-expressed with CCR6 and CXCR4 in the thymus, which can be used as sorting markers for Eos+ Tregs. Eos is expressed at very low levels on induced Tregs, conventional CD4+ T cells and CD8+ T cells in vivo or in vitro, regardless of their activation status. Using immunofluorescence microscopy, we have found that Eos is highly co-localized with Helios, another Ikaros family member and HDAC1, but not other Ikaros family members such as Ikaros or Aiolos, suggesting that Eos and Helios may form heterodimers and together with HDAC1 collaborate in Treg function. Nevertheless, expression of Eos is independent of Helios and vice versa, as Treg from Helios deficient mice express normal or elevated levels of Eos, and Treg from Eos deficient mice express normal levels of Helios. Taken together, these results support the view that Eos plays an important role in Treg function in general and a unique role in trafficking of Tregs.

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