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Mealtimes at Blackford Hall are as follows:
- Breakfast: 7:30 am-9:00 am
- Lunch: 11:30 am-1:30 pm
- Dinner: 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late
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PROGRAM

WEDNESDAY, November 16—7:30 PM

Welcome
Susan Swain and Robert Seder

OPENING KEYNOTE
Introduction by
Robert Seder

Michael Oldstone
Scripps Clinic & Research Foundation
“Endothelial cell mediated modulation of cytokine storm during influenza infection”

WEDNESDAY, November 16—8:30 PM

SESSION 1 DECISION MAKING IN MEMORY CELL DIFFERENTIATION

Chairperson: S. Swain, University of Massachusetts Medical School, Worcester

Fate decisions and transcriptional control of CD8 T cell differentiation
Weiguo Cui, Ying Liu, Susan M. Kaech.
Presenter affiliation: HHMI/Yale University, New Haven, Connecticut.

Transcription factors that program T and B cell memory
Rafick Sekaly.
Presenter affiliation: Vaccine and Gene Therapy Institute Florida, Port.
St. Lucie, Florida.
SESSION 2  INNATE PROGRAMMING OF ADAPTIVE IMMUNITY I

Chairpersons:  E. Pearce, Trudeau Institute, Saranac Lake, New York
D. Lewis, Stanford University School of Medicine, California

Expanding functions of CD4 memory T cells
Presenter affiliation: University of Massachusetts Medical School,
Worcester, Massachusetts.

Mitochondrial respiratory capacity is a critical regulator of CD8 T cell memory development
Presenter affiliation: Trudeau Institute, Saranac Lake, New York;
Washington University, St. Louis, Missouri.

Programming vaccine responses with innate immunity
Bali Pulendran.
Presenter affiliation: Emory University, Atlanta, Georgia.

Notch signaling regulates PD-1 expression during CD8⁺ T cell activation
Jean-François Daudelin, Natacha Cotta-Grand, Mélissa Mathieu, Paméla Thébault, Nathalie Labrecque.
Presenter affiliation: Maisonneuve-Rosemont Hospital, Montreal, Canada.

CD8α⁺ dendritic cells are an obligate cellular entry point for productive infection by *Listeria monocytogenes*
Brian T. Edelson, Kenneth M. Murphy.
Presenter affiliation: Washington University School of Medicine, St. Louis, Missouri.

The RNA-binding protein tristetraprolin controls the balance between Interleukin-12 family members
Céline Molle, Tong Zhang, Cyril Gueydan, Oberdan Leo, Stanislas Goriely.
Presenter affiliation: Université Libre de Bruxelles, Gosselies, Belgium.
Mechanisms underlying the potent activity of cationic lipid-DNA complexes (CLDC) as an immunostimulant and adjuvant
Presenter affiliation: Stanford University School of Medicine, Stanford, California.

THURSDAY, November 17—2:00 PM

SESSION 3 POSTER SESSION

Genetic heterogeneity in susceptibility to leprosy
Presenter affiliation: National Centre of Applied Human Genetics, New Delhi, India.

Recognition by the host innate immune system of the Yersinia pestis vaccine and pathogen is required for protection
Presenter affiliation: US Army Medical Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland.

Characterization of Latet, a novel gene for which allelic variation affects type 1 diabetes in mice
Michelle P. Ashton, Leanne Mackin, Iris Tan, Ashley Mansell, Meredith O'Keeffe, Thomas C. Brodnicki.
Presenter affiliation: St Vincent's Institute, Melbourne, Australia.

Dendritic cell selectively generate cytotoxic T lymphocyte epitopes
Caroline A. Bernhard, Thomas Brocker.
Presenter affiliation: LMU Munich, Munich, Germany.

Roles of TRAF5 in TLR signaling
Claire M. Buchta, Gail A. Bishop.
Presenter affiliation: University of Iowa, Iowa City, Iowa.
Dose-dependent breakdown of established inhalation tolerance by lipopolysaccharide—Implications for the induction of asthma
Timothy J. Chapman, Jason Emo, Nida Meednu, Fariba Rezaee, Tiru Rangasamy, Steve N. Georas.
Presenter affiliation: University of Rochester, Rochester, New York.

Role of IL-12 in programming human CD8+ T lymphocytes
Fatema Z. Chowdhury, Hilario J. Ramos, James Forman, David Farrar.
Presenter affiliation: UT Southwestern Medical Center, Dallas, Texas.

The fusion process modifies the function of lymphocyte-dendritic cell hybrids and decreases the production of IFN-γ and IL-10
Karen S. Cruz, Graziela G. Romagnoli, José Alexandre M. Barbuto.
Presenter affiliation: Institute of Biomedical Sciences, University of Sao Paulo, Brazil., Sao Paulo, Brazil.

Self-DNA release mediates the adjuvant effects of aluminum salts
Thomas Marichal, Keichi Ohata, Denis Bedoret, Claire Mesnil, Catherine Sabatel, Kouji Kobiyama, Pierre Lekeux, Cevayir Coban, Shizuo Akira, Ken J. Ishii, Fabrice Bureau, Christophe J. Desmet.
Presenter affiliation: GIGA-R, University of Liege, Liege, Belgium.

Type I interferon blocks Th2 development and function—A potential therapy for atopic diseases
J. David Farrar, Jonathan P. Huber, Gagan Bajwa, Nan Jiang, Sarah R. Gonzales, Michell A. Gill.
Presenter affiliation: UT Southwestern Medical Center, Dallas, Texas.

A bacterial virulence factor targets TRAF proteins to impair the host immune response
Xiaofei Gao, Philip Hardwidge.
Presenter affiliation: University of Kansas Medical Center, Kansas City, Kansas.

Type I interferon acutely regulates human memory T cell function
Sarah R. Gonzales, Jonathan P. Huber, J. David Farrar.
Presenter affiliation: UT Southwestern Medical Center, Dallas, Texas.

Regulation of effector T cell responses by the surface receptor Tim-3
Presenter affiliation: University of Iowa, Iowa City, Iowa.
Lymphocyte function antigen-1 and leukotoxin interaction
Anukriti Gupta, Scott C. Kachlany.
Presenter affiliation: UMDNJ, Newark, New Jersey.

Shiga toxin-producing *E. coli* prevent activation of the inhibitor of NFκB kinase (IKK) complex
Xiaofei Gao, Philip R. Hardwidge.
Presenter affiliation: University of Kansas Medical Center, Kansas City, Kansas.

*S. Typhi*-imprinted dendritic cells mediate Th1 education and enhanced responses to a foreign antigen
Shannon J. Heine, Karina Ramirez, Gabriela Mellado-Sanchez, Rosangela Salerno-Goncalves, Marcella F. Pasetti.
Presenter affiliation: University of Maryland, Baltimore, Baltimore, Maryland.

Antigen delivery by β-sheet aggregated protein potentiates efficient immune response
Julie H. Huang, Anton P. Bussink, Cornelis W. Seinen, Tomasz M. Poplonski, Martijn F. Gebbink, Tuna Mutis.
Presenter affiliation: University Medical Center Utrecht, Utrecht, the Netherlands.

Toll-like receptor (TLR) - mediated induction of cyclooxygenase (COX)-2 in human vaginal epithelial cells—Effect of seminal plasma on COX-2 expression
Theresa Joseph, Irina Zalenskaya, Gustavo F. Doncel.
Presenter affiliation: CONRAD, Eastern Virginia Medical School, Norfolk, Virginia.

Diacylglycerol kinase zeta but not diacylglycerol kinase alpha suppresses development of nTregs
Rohan P. Joshi, Tao Zou, Taku Kambayashi, Matthew Riese, Gary A. Koretzky.
Presenter affiliation: University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.

Binding of superantigen toxins into the CD28 homodimer interface is essential for induction of Th1 cytokine genes that mediate lethal shock
Presenter affiliation: Faculty of Medicine, The Hebrew University, Jerusalem, Israel.
Foxo1 control of memory CD8+ T cell differentiation
Myoungjoo Kim, Weiming Ouyang, Ming O. Li.
Presenter affiliation: Weill Cornell Graduate School of Medical Sciences, New York, New York; Memorial Sloan-Kettering Cancer Center, New York, New York. 28

APOBEC3A is a potent restrictor of foreign DNA in both the nucleus and the cytoplasm of human cells
Allison M. Land, Reuben S. Harris.
Presenter affiliation: University of Minnesota, Minneapolis, Minnesota. 29

Novel plant virus-like particle vaccine platform induces immune system activation through Toll-like receptor 7 signaling
Marie-Eve Lebel, Denis Leclerc, Alain Lamarre.
Presenter affiliation: INRS, Laval, Canada. 30

Transgenic Eimeria-based eukaryotic vaccine vector system for avian disease control in chickens
Xianyong Liu, Guangwen Yin, Qiyao Lv, Xiaoxi Huang, Jun Zou, Xun Suo.
Presenter affiliation: China Agricultural University, Beijing, China. 31

Profiling of microRNA during human monocyte differentiation into dendritic cells and its regulatory role in dendritic cell apoptosis and function
Changming Lu.
Presenter affiliation: University of Alabama, Birmingham, Alabama. 32

A potential approach toward immunoregulations against Alzheimer disease using a Drosophila model
Elie Maksoud, Jules A. Hoffmann, Hidehiro Fukuyama.
Presenter affiliation: CNRS, Strasbourg, France; Strasbourg University, Strasbourg, France. 33

Evolutionary genetics, systems biology, and host-pathogen interactions—The keys to genomic medicine and therapeutic intervention?
Karen P. Mooder.
Presenter affiliation: British Columbia Institute of Technology, Burnaby, Canada. 34
Infant immunity to measles—Immunogenetic predictors of vaccine non-responsiveness in Kenyan children
Ann Moormann, Caitlin Bonney, Hua Fang, Hughes Mulama, Inna Ovsyannikova, Gregory Poland.
Presenter affiliation: University of Massachusetts Medical School, Worcester, Massachusetts.

MHCII anchor residues do not affect TCR specificity
Ryan W. Nelson, Dan J. Beisang, Marc K. Jenkins.
Presenter affiliation: University of Minnesota, Minneapolis, Minnesota.

Innate immune responses during acute and chronic LCMV infection
Presenter affiliation: Emory University, Atlanta, Georgia.

Transcutaneous immunization induces rapid resolution of biofilms in the middle ear during experimental otitis media due to nontypeable Haemophilus influenzae
Laura A. Novotny, John D. Clements, Lauren O. Bakaletz.
Presenter affiliation: The Research Institute at Nationwide Children’s Hospital, Columbus, Ohio.

SEVI binds to and enhances phagocytosis of bacterial pathogens
Fernando Ontiveros, Lauren Brooks, David Easterhoff, Brittany Ross, Joanna Olsen, Stephen Dewhurst.
Presenter affiliation: University of Rochester School of Medicine, Rochester, New York.

Detection of immune response in tuberculous meningitis by multimodal approach—An update
Shripad A. Patil.
Presenter affiliation: National Institute of Mental Health and Neurosciences, Bangalore, India.

Is the phenotypic delay in differentiation of breast cancer patients’ monocytes into dendritic cells (MO-DCs) the source of functional bias by the induction of regulatory T cells?
Rodrigo N. Ramos, Cristiano J. de Moraes, Ana Paula S. dos Santos, Patrícia C. Bergami-Santos, Fabio M. Laginha, José A. Barbuto.
Presenter affiliation: Laboratory of Tumor Immunology, University of Sao Paulo, Sao Paulo - SP, Brazil.
Nucleoprotein nanostructures—A mucosal subunit vaccine candidate for neonates protective against the respiratory syncytial virus
Aude Remot, Catherine Dubuquoy, Julie Bernard, Jenna Fix, Mohammed Moudjou, Jean-François Eleouët, Agnès Petit-Camurdan, Sabine Riffault.
Presenter affiliation: INRA (French National Institute for Agronomical Research), Jouy-en-Josas, France.

Tumor cells incorporate exosome-carried molecules derived from mature dendritic cells and become susceptible to T cell cytotoxicity
Graziela G. Romagnoli, Patrícia A. Toniolo, Isabella K. Miguel, Patrícia C. Bergami-Santos, José Alexandre M. Barbuto.
Presenter affiliation: Institute of Biomedical Sciences - University of Sao Paulo - USP, Sao Paulo, Brazil.

Analysis of human antibody repertoires in healthy individuals
Florian Rubelt, Volker Sievert, Florian Knaust, Christian Diener, Svetlana Mollova, Werner Müller, Theam S. Lim, Karl Skriner, Hans Lehrach, Zoltán Konthur.
Presenter affiliation: Max Planck Institute for Molecular Genetics, Berlin, Germany.

Characterization of leukocyte inflammatory responses to Toll like receptor (TLR) 7, 8 and 9 ligands
Presenter affiliation: Novartis Institutes for Biomedical Research, Basel, Switzerland.

Autophosphorylation of interleukin-1 receptor associated kinase (IRAK)-1 leads to constitutive p38 phosphorylation and sustained inflammation in sarcoid BAL-cells
Ruchi Rastogi, Gabriel Nunez, Lobelia Samavati.
Presenter affiliation: Wayne State University School of Medicine, Detroit, Michigan.

Tissue-expressed B7-H1 critically controls intestinal inflammation independently of PD-1 and adaptive immunity
Lisa Scanduzzi, Xingxing Zang.
Presenter affiliation: Albert Einstein College of Medicine, New York, New York.
Human TOLLIP regulates TLR2 and TLR4 signalling and its polymorphisms are associated with susceptibility to tuberculosis

Allergen-associated danger signals and receptors—NADPH oxidase activity, Toll-like receptor 4 and its adaptor TRIF are not necessary for mucosal sensitization to pollen
Presenter affiliation: Meakins-Christie Laboratories, Department of Medicine, McGill University, Montréal, Canada.

TLR4-TRIF signaling promotes T-cell and ICOS-dependent inhibition of murine allergen-induced airway hyperresponsiveness
Presenter affiliation: Meakins-Christie Laboratories, McGill University, Montréal, Canada.

Immunological abnormalities in fatigue-related illness
Donald R. Staines, Ekua W. Brenu, Jana Batovska, Sharni Hardcastle, Mieke van Driel, Sonya Marshall Gradisnik.
Presenter affiliation: Queensland Health, Robina, Australia.

Semaphorin 7A mediates West Nile virus induced neuronal cell death and encephalitis
Hameeda Sultana, Girish Neelakanta, Harald Foellmer, Ruth R. Montegomery, Erol Fikrig.
Presenter affiliation: Section of Infectious Diseases, New Haven, Connecticut.

Distinct roles for CXCR6+ and CXCR6−CD4+ T cells in pathogenesis of chronic colitis
Daisuke Takahashi, Yasushi Mandai, Koji Hase, Hiroshi Ohno.
Presenter affiliation: RIKEN, Yokohama, Japan.
Differential expression of “suppressors of cytokine signaling” (SOCS) in monocyte-derived dendritic cells from breast cancer patients
Patricia A. Toniolo, Fábio Laginha, Niels O. Câmara, José Alexandre M. Barbuto.
Presenter affiliation: Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil.

Different gene expression signature and B cell memory induction in Ugandan, European and North American adults receiving the yellow fever vaccine for the first or second time
Presenter affiliation: Vaccine and Gene Therapy Institute Florida, Port St Lucie, Florida; University of Miami, Miami, Florida.

Enhanced immunogenicity of MHC class I-restricted tumor-associated altered peptide ligands
Hannes Uchtenhagen, Evi Stahl, Adil D. Duru, Patrick Celie, Ton N. Schumacher, Adnane Achour.
Presenter affiliation: Karolinska Institutet, Stockholm, Sweden.

TLR7 activating nanoparticles as effective immune adjuvants
Christina C. Wu, Michael Chan, Mojgan Sabet, Fitzgerald S. Lao, Rommel I. Tawatao, Howard B. Cottam, Donald G. Guiney, Dennis A. Carson.
Presenter affiliation: University of California San Diego, La Jolla, California.

THURSDAY, November 17—4:30 PM
Wine and Cheese Party
THURSDAY, November 17—7:30 PM

SESSION 4  INNATE PROGRAMMING OF ADAPTIVE IMMUNITY II

Chairpersons:  L. Haynes, Trudeau Institute, Saranac Lake, New York
              A. Hill, Wellcome Trust Centre for Human Genomics,
              Oxford, United Kingdom

Malaria vaccines
Adrian Hill.
Presenter affiliation: Wellcome Trust Centre for Human Genomics,
Oxford, United Kingdom.

The impact of aging on CD4 T cell function
Laura Haynes.
Presenter affiliation: Trudeau Institute, Saranac Lake, New York.

Host galectins influence immunity and immunopathology to virus infections
Barry T. Rouse.
Presenter affiliation: University of Tennessee, Knoxville, Tennessee.

Impairment of CD4+ T cell cognate helper functions in response to influenza immunization with aging
Julie S. Lefebvre, Ashlee H. Petell, Paula A. Lanthier, Sheri M. Eaton,
Laura Haynes.
Presenter affiliation: Trudeau Institute, Saranac Lake, New York.

The Blimp-1 homologue Hobit is an NKT-cell specific transcription factor that regulates the effector function of NKT cells
Klaas van Gisbergen, Kirsten Hertogs, Natasja Kragten, Felix
Wensveen, Stipan Jonjic, Jorg Hamann, Martijn Nolte, Rene van Lier.
Presenter affiliation: Academic Medical Center, Amsterdam,
Netherlands; Sanquin Research, Amsterdam, Netherlands.
SESSION 5  ORGAN SPECIFIC IMMUNITY

Chairpersons:  S. Turley, Dana-Farber Cancer Institute, Boston, Massachusetts
              Y. Belkaid, NIAID, National Institutes of Health, Bethesda, Maryland

Innate and adaptive responses to commensals during mucosal infections
Timothy Hand, Liliane Dos Santos, Antonio Pagan, Marion Pepper, Mark Jenkins, Charles Elson, Yasmine Belkaid.
Presenter affiliation: NIAID, National Institutes of Health, Bethesda, Maryland.

Regulation of T cell memory at mucosal sites
David Masopust, Kristin Anderson, Kerry Casey, Kathryn Fraser, Jason Schenkel.
Presenter affiliation: University of Minnesota, Minneapolis, Minnesota.

Regulation of dendritic cell and T cell function by lymphoid stroma
Shannon Turley.
Presenter affiliation: Dana-Farber Cancer Institute, Boston, Massachusetts.

Developmental and functional specialization of mononuclear phagocytes in the intestinal muscularis
Milena Bogunovic, Paul Muller, Marylene Leboeuf, Kara Margolis, Israel F. Charo, E. Richard Stanley, Miriam Merad.
Presenter affiliation: Mount Sinai School of Medicine, New York, New York.

Apoptotic cells suppress mast cell inflammatory responses via the CD300a immunoreceptor
Presenter affiliation: University of Tsukuba, Tsukuba, Japan.
Modulation of innate and adaptive immunity by repeated administration of a TLR7 ligand
Presenter affiliation: University of California San Diego, La Jolla, California.

Tissue-expressed B7x regulation of the T cell response in a pulmonary infection model
Kimberly A. Hofmeyer, Xingxing Zang.
Presenter affiliation: Albert Einstein College of Medicine, Bronx, New York.

Dendritic cell-derived Tim-3 is a universal repressor of nucleic acid-mediated innate antitumor immune responses
Masahisa Jinushi.
Presenter affiliation: Hokkaido University, Sapporo, Japan.

FRIDAY, November 18—2:00 PM
SESSION 6 MICROBE-IMMUNE INTERACTIONS

Chairpersons: L. Hooper, University of Texas Southwestern Medical Center, Dallas
D. Littman, Howard Hughes Medical Institute, New York University School of Medicine, New York

Commensals, transcription factors and intestinal immunity
Dan Littman.
Presenter affiliation: Howard Hughes Medical Institute, New York University School of Medicine, New York, New York.

Immune adaptations to the intestinal microbiota
Lora V. Hooper, Sohini Mukherjee, Shipra Vaishnava.
Presenter affiliation: The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas.
Innate immune regulation of gut microbiota and potential therapeutic opportunities to treat metabolic disease and chronic infections
Andrew Gewirtz.
Presenter affiliation: Georgia State University, Atlanta, Georgia.

TLR2 signaling contributes to rapid inflammasome activation during bacterial infection
Crystal L. Jones, David S. Weiss.
Presenter affiliation: Emory University, Atlanta, Georgia.

A temporal role of type I interferon signaling in CD8+ T cell maturation during acute West Nile virus infection
Presenter affiliation: Washington University School of Medicine, Saint Louis, Missouri.

FRIDAY, November 18—4:30 PM

KEYNOTE SPEAKER

Introduction by
Susan Swain

Craig Thompson
Memorial Sloan-Kettering Cancer Center

“How do cells remember?”

FRIDAY, November 18

BANQUET

Cocktails 6:00 PM       Dinner 6:45 PM
SESSION 7  
THE JOURNEY WITHIN: HUMAN IMMUNOLOGY

Chairpersons:  
A. Lanzavecchia, Institute for Research in Biomedicine, Bellinzona, Switzerland  
M. Davis, Howard Hughes Medical Institute, Stanford University School of Medicine, California

Dissecting the human T and B cell response to pathogens  
Antonio Lanzavecchia.  
Presenter affiliation: Institute for Research in Biomedicine, Bellinzona, Switzerland.  

Non furry immunology  
Mark Davis.  
Presenter affiliation: Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, California.

Development of a malaria vaccine  
Bob Seder.  
Presenter affiliation: NIAID, National Institutes of Health, Bethesda, Maryland.

A dynamical systems perspective of cytokine signaling responses by human T cells  
Neda Bagheri, Qing Han, Douglas A. Lauffenburger, J. Christopher Love.  
Presenter affiliation: Massachusetts Institute of Technology, Cambridge, Massachusetts.

Novel targets for broad spectrum vaccines and drugs against influenza  
Jaap Goudsmit.  
Presenter affiliation: University of Amsterdam, Amsterdam, Netherlands; Crucell, Amsterdam, Netherlands.

A systemic cytokine response defect stratifies older adults into distinct immune profiles  
Shai S. Shen-Orr, David Furman, Brian A. Kidd, Patricia Lovelace, Ying-Wen Huang, Yael Rosenberg-Hasson, Holden T. Maecker, Sally Mackey, Cornelia L. Dekker, Atul J. Butte, Mark M. Davis.  
Presenter affiliation: Stanford University School of Medicine, Stanford, California.
Memory T cell responses in acute influenza A infection in humans
Cecilia Chui, Chris Li, Tom Wilkinson, John Oxford, Andrew McMichael, Xiaoning Xu.
Presenter affiliation: MRC Human Immunology Unit, Oxford, United Kingdom.

Plasmodium falciparum-specific anti-inflammatory responses are upregulated after acute malaria but short-lived in the absence of ongoing P. falciparum exposure
Silvia Portugal, Jacqueline Moebius, Boubacar Traore, Kassoum Kayentao, Aissata Ongoiba, Ogobara K. Doumbo, Peter D. Crompton.
Presenter affiliation: NIAID, National Institutes of Health, Rockville, Maryland.

Intracellular targeting of antigen determines the capacity of human blood dendritic cell subsets to cross present on MHCI
Lillian Cohn, Bithi Chatterjee, Anna Smed Sörensen, Nohiro Nakamura, Cecile Chalouni, Richard Vandlen, Jenifer Widger, Tibor Keler, Ira Mellman, Lelia Delamarre.
Presenter affiliation: Genentech Inc., South San Francisco, California.
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FATE DECISIONS AND TRANSCRIPTIONAL CONTROL OF CD8 T CELL DIFFERENTIATION.

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Memory CD8 T cells arise following infection from a heterogenous population of effector T cells that contains cells of various differentiation states. Many of these effector CD8 T cells develop into end-stage terminal effector cells that die following infection and a smaller portion develops into cells with greater memory cell potential and longevity. Understanding how effector CD8 T cell differentiation is regulated to generate cells of diverse cell fates is important and much progress has been made in identifying several transcriptional factors that regulate effector and memory cell fates, function and phenotypes. In this talk we will discuss new factors that promote and sustain memory cell precursor identity and metabolic states.

We have studied the mechanisms by which memory CD4 T cells provide protection against infection with influenza A virus (IAV). We find that even a restricted Th1-polarized, in vitro generated, population of IAV-specific CD4 memory cells can act to combat infection at multiple stages of the response in different organs. First we find induction of innate immunity that is seen in the lung at 2-3 days. Before 5 days the memory cells provide powerful B cell help, mostly in the secondary lymphoid sites. After, peaking around 6 days, memory effectors migrate to the lung where they clear virus by both IFNγ dependent mechanisms and perforin-dependent cytolytic activity. These memory effectors are better able to provide protection than primary effectors derived from naïve cells when generated either in vitro or in vivo.

This extensive multifunctionality and organ-specificity is evident in both cytokine production patterns, in certain functionally-associated cell surface phenotypes and is easily seen in genome-wide analysis. With these tools, we have identified several critical functional differences between primary and secondary effectors that are likely to explain some of the superior properties of memory cells.
MITOCHONDRIAL RESPIRATORY CAPACITY IS A CRITICAL REGULATOR OF CD8 T CELL MEMORY DEVELOPMENT

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CD8 T cells undergo major metabolic changes upon activation, but how metabolism influences the establishment of long-lived memory T (T_M) cells after infection remains a key question. We found that CD8 T_M cells, but not effector CD8 (T_E) cells, possess substantial mitochondrial spare respiratory capacity (SRC). SRC is the extra capacity available in cells to produce energy in response to increased stress or work and as such is associated with cellular survival. We show that IL-15, a cytokine critical for CD8 T_M cells, regulates SRC and oxidative metabolism by promoting mitochondrial biogenesis and expression of carnitine palmitoyl transferase (CPT1a), a metabolic enzyme that controls the rate-limiting step to mitochondrial fatty acid oxidation (FAO). Our findings highlight an essential role for mitochondria in regulating the T cell survival and function after infection, and suggest that drugs that target mitochondrial SRC could hold promise as immunotherapeutics and might warrant further study for their ability to alter T cell responses.
NOTCH SIGNALING REGULATES PD-1 EXPRESSION DURING CD8⁺ T CELL ACTIVATION

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Following antigen encounter, naive CD8⁺ T lymphocytes upregulate the expression of PD-1 (Programmed cell Death 1), an inhibitory receptor involved in T cell activation and T cell exhaustion. However, the molecular events regulating PD-1 transcription are still poorly defined. Notch signaling is a key pathway controlling cell fate choice in a large number of cells, including immune cells. Furthermore, CD8⁺ T cells and antigen presenting cells express Notch receptors and their ligands, respectively. Recently, Notch has been implicated in the control of Eomes, Perforin and Granzyme B expression by CD8⁺ T cells. The aim of this study was to evaluate the possible role of Notch signaling on PD-1 expression during CD8⁺ T cell activation.

To evaluate the implication of Notch signaling in the regulation of PD-1 expression, we used artificial APCs (aAPCs; B6 MEC-1 cells line, transfected with B7.1 and the OVA257-264 peptide) expressing Notch ligands to activate OT-1 CD8⁺ T cells in vitro. In our OT-1/aAPC coculture, we observed a transcriptional induction of the classical effectors of this pathway, Hes-1 and Deltex. We then evaluated if inhibition of the Notch signaling pathway during the priming will affect CD8⁺ T cell activation. To do so, we used DAPT, a γ-secretase inhibitor that prevents the proteolytic cleavage of the Notch Intracellular Domain (NICD) responsible for the activation of target gene transcription. In this system, inhibition of Notch signaling using low dose of DAPT (20µM) led to a selective defect in the up-regulation of PD-1 expression by activated OT-1 CD8⁺ T cells. Moreover, the reduction of PD-1 expression was due to a transcriptional defect as shown using RT-qPCR experiments. Chromatin Immunoprecipitation (ChIP) was done using anti-RBP-Jk (a corepressor which form a complex with NICD to activate gene transcription) and anti-NICD antibodies. These ChIP experiments showed that RBP-Jk and NICD bind to the PD-1 promoter at two RBP-Jk consensus sites in the PD-1 promoter at the peak of the PD-1 mRNA expression but not in naive OT-1 CD8⁺ T cells.

Our results provide new insights into the role of Notch signaling in CD8⁺ T cell activation and identify the Notch signaling pathway as a regulator of PD-1 expression. Given that PD-1 is associated with T cell exhaustion in chronic infections, Notch may be a relevant therapeutic target to modulate CD8⁺ T cells responses in chronically infected individuals.
CD8α+ DENDRITIC CELLS ARE AN OBLIGATE CELLULAR ENTRY POINT FOR PRODUCTIVE INFECTION BY LISTERIA MONOCYTOGENES

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The intracellular bacterium Listeria monocytogenes infects macrophages, dendritic cells (DCs), monocytes, and neutrophils during early infection, although the roles played by each of these cell types in the initiation and control of bacterial spread remain unclear. Previously, a general requirement for CD11c⁺ cells in establishing splenic Listeria infection was shown, although the identity of the CD11c⁺ cell required to promote infection was unknown. Here, we examined Listeria infection in Batf3⁻/⁻ mice, which selectively lack CD8α⁺ DCs and peripheral tissue-resident CD103⁺ DCs. Batf3⁻/⁻ mice exhibited markedly enhanced resistance to splenic and hepatic Listeria infection. In wild-type (WT) mice, Listeria organisms were initially located in the splenic marginal zone, and migrated to the periarteriolar lymphoid sheath (PALS) where they grew exponentially. In situ infection of splenic CD8α⁺ DCs was visualized using anti-Langerin and anti-Listeria antibodies, and both infected and non-infected CD8α⁺ DCs migrated from the marginal zone to the PALS very early after infection. In Batf3⁻/⁻ mice, however, Listeria organisms remained trapped in the marginal zone, failed to traffic to the PALS, and were rapidly cleared by phagocytes. Nevertheless, infection of Batf3⁻/⁻ mice effectively primed both CD4 and CD8 T cell responses to Listeria antigens, although these mice required higher inocula to generate responses equivalent to those of WT mice. Overall, these results suggest that Batf3-dependent DCs provide initial cellular entry points within the reticuloendothelial system by which Listeria establishes productive infection, although these DCs are not absolutely required for T cell priming to this pathogen.
THE RNA-BINDING PROTEIN TRISTETRAPROLIN CONTROLS THE BALANCE BETWEEN INTERLEUKIN-12 FAMILY MEMBERS

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Interleukin(IL)-12 and the related heterodimeric cytokines IL-23 and IL-27 play crucial and distinct roles in shaping the innate and adaptive immune responses against invading pathogens. Imbalanced expression of these three cytokines can lead to auto-immune or inflammatory disorders. We previously demonstrated the critical role of Interferon Regulatory Factors (IRFs) in controlling their transcriptional activation and influencing the balance between these 3 cytokines. Herein, we focused on the post-transcriptional control of these cytokines. Adenine and uridine-rich elements (AREs) found in the 3' untranslated regions (UTRs) were shown to confer rapid decay of mRNA and to regulate the expression of many inflammatory mediators. We identified multiple ARE elements in the 3'UTR of each subunit that were able to destabilize an heterologous reporter transcript in transient transfection experiments. However, in absence of tristetraprolin (TTP), a key ARE-binding protein implicated in mRNA decay, LPS-stimulated bone marrow-derived dendritic cells (BMDCs) produced high levels of IL-23 but normal levels of IL-12/23p40 or IL-27. Production of IL-12p70 was modestly increased in these conditions. We observed a strong impact of TTP on the mRNA half-life of the IL-23p19 subunit. In vitro and in vivo experiments indicate that dysregulated production of IL-23 in absence of TTP strongly promoted IL-17 production by CD4 T cells. TTP-/- mice spontaneously develop cachexia, myeloid hyperplasia, dermatitis and erosive arthritis. All these symptoms were found to be completely dependent on IL-23. In conclusion, our results identify a novel molecular mechanism implicated in the control of IL-23/Th17-driven pathology.
MECHANISMS UNDERLYING THE POTENT ACTIVITY OF CATIONIC LIPID-DNA COMPLEXES (CLDC) AS AN IMMUNOSTIMULANT AND ADJUVANT

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CLDC, which consists of a non-coding 4.23 kb plasmid DNA containing >280 CpG motifs complexed with the cationic lipid DOTIM and neutral cholesterol, is an unusually potent stimulant of innate immunity and an adjuvant for adaptive immunity. Both the DNA and lipid moieties are essential for these immunostimulant activities. Prior work in rodent and non-human primate (NHP) models has demonstrated the ability of CLDC to protect from lethal and non-lethal challenges with viruses (e.g., viral hemorrhagic fever and H5N1 avian influenza) and highly pathogenic bacteria (e.g., the Schu S4 strain of Francisella tularensis). CLDC adjuvant in rodents, NHPs, and humans consistently enhances antigen-specific CD8 T-cell responses, suggesting that cross-presentation is effectively accessed. More recent results will be presented showing that CLDC adjuvant can protect elderly NHPs from influenza A virus and that CLDC immunostimulation may have substantial activity against acute myelogenous leukemia in humans. To understand the cellular and molecular mechanisms by which CLDC acts, we have used murine models to determine the interaction of CLDC with dendritic cell (DC) populations and the subsequent events following this interaction that lead to adaptive immunity to T-dependent antigens. CLDC stimulated bone marrow-derived DCs in vitro for type I interferon and cytokine production in a TLR9-dependent manner, consistent with confocal microscopy data showing that it rapidly entered into an early endosomal compartment. However, both immunogenicity and protection in vivo following immunization with CLDC-adjuvanted inactivated influenza vaccines were TLR9-independent. Interestingly, conditional knockout experiments revealed that MyD88 expression by CD11c+ DCs were important for CLDC to induce antigen-specific CD8 T-cell but not CD4 T-cell immunity. Experiments are in progress to define the role of receptors upstream of MyD88 other than TLR9 in CLDC adjuvant activity. A completely CpG-less plasmid used in the CLDC complex retained adjuvant activity in vivo, suggesting a role for cytoplasmic DNA sensors in CLDC immunostimulation. We have established an in vitro system for CLDC stimulated cross-presentation in CD11c+ DCs, and knockdown experiments are in progress to determine the role in CLDC activity of cytoplasmic DNA sensors/adapters, including DDX41 and STING. The role of individual DC subsets in cross-presentation in vivo is also being pursued, e.g., using BATF3-deficient mice, which lack CD8-alpha+ and CD103+ DCs. Together, our findings suggest that targeting cytoplasmic DNA sensors in DCs may be a particularly robust approach for inducing antimicrobial and anti-tumor immune responses and protection.
LEPROSY, a chronic mycobacterial infection characterized by clinically defined polar manifestations, involves both genetic and environmental components. Numerous variants such as the \(VDR\), \(CTLA4\), \(COL3A\), \(TLR1\), \(SLC11A1\), \(IL12RB2\), have been described as risk-providers to leprosy. The association of several genomic regions with susceptibility to leprosy or severity of the disease in different populations combined with the heterogeneity in genetic susceptibility to this multifactorial disease makes the disease more complex to understand. Although India carries the majority of the global burden of leprosy and is one of the most genetically diverse populations of the world, the role of host genetic components in controlling susceptibility to leprosy has not been investigated intensively. We adopted a pathway-based approach towards investigating a role of functional genetic variants alone or in combination in anti-inflammatory cytokine network in a case-control study in two geographically distinct and unrelated populations. Furthermore, we used an \textit{in vitro} system to functionally characterize associated polymorphisms. Since no copy number variation (CNV) studies have been performed in leprosy so far, we attempted to find CNV in a cytokine \(IL10\), for its association with leprosy. Our results indicated association of 8 polymorphisms rs1800871, rs1800872, and rs1554286 of \(IL10\); rs3171425, rs7281762 of \(IL10RB\); rs2228048, rs744751 of \(TGFBR2\) and rs1800797 of \(IL6\) with leprosy and its subtypes. This association was replicated for 4 SNPs: rs1554286 of \(IL10\), rs7281762 of \(IL10RB\), rs2228048 of \(TGFBR2\), rs1800797 of \(IL6\). Interestingly, we also observed the SNP rs7281762 of \(IL10RB\) and rs2228048 of \(TGFBR2\) in a significant association with tuberculosis, an observation made for the first time, which is of relevance especially when both the mycobacterial species have the overlapping antigenic repertoire and the diseases share several immunological features. Moreover, we observed alleles of significant polymorphisms were associated with expression level of concerned gene. Furthermore, an absence of alteration in gene copy number of \(IL10\) was found in patients and controls. In conclusion, the present findings provide an insight into leprosy pathogenesis and add to the information regarding the complex puzzle of genetic factors involved in infectious diseases, especially leprosy and tuberculosis. There is a need to look for other immune-regulatory genes to better understand the interactive role of host genetic factors in the etiology of leprosy for efficient interventions.
RECOGNITION BY THE HOST INNATE IMMUNE SYSTEM OF THE YERSINIA PESTIS VACCINE AND PATHOGEN IS REQUIRED FOR PROTECTION

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Although the Yersinia pestis F1-V subunit vaccine is in human clinical trials, little is known about the interaction of the vaccine with the host immune system, and particularly with the innate immune system. Previous studies found that the antibody response to the Y. pestis F1-V vaccine plays a large part in protecting the host from plague. It has been recently suggested that cell-mediated immunity plays a role in protection of the host from a lethal challenge. We wanted to further investigate the host’s innate immune system on the response to the vaccine, and its involvement in protection against Y. pestis in the vaccinated host. The interaction of Y. pestis and the F1-V vaccine with Toll-like receptors (TLRs) was investigated using HEK cells that express individual TLRs. TLR2, TLR4, and TLR2/4 knockout (KO)s in C3H/HeN mice were used to examine the host innate immune response to the vaccine. The contribution of the myeloid differentiation protein 88 (MyD88) to the innate immune response to the vaccine and pathogen was studied in C57Bl/6 KOs. The effect of a mutation in TLR4 on the antibody response to the vaccine and Y. pestis challenge was studied in C3H/HeJ mice. Vaccinated mice were challenged by aerosol with Y. pestis CO92. At 37 C Y. pestis activated both TLR2 and TLR4. The plague F1-V vaccine weakly activated both TLR2 and TLR4. In TLR2, 4, 2/4, and MyD88 KO mice, the antibody class response to the vaccine was moderately lower than in wild-type mice. The expression of TNF-α and IFN-γ was dependent on TLR4 and MyD88 but not TLR2 in restimulated splenocytes from vaccinated mice. Mice with an inactivated TLR4 were still protected from a lethal aerosol challenge of Y. pestis CO92 after vaccination. However, vaccinated MyD88 KO mice succumbed to a Y. pestis challenge in spite of a slightly lower antibody response to the vaccine. In conclusion, the innate immune system moderately affected the antibody response to the plague F1-V vaccine. Activation of TLR4 and MyD88 was essential for the cell-mediated expression of TNF-α and IFN-γ in vaccinated mice. MyD88 was absolutely required for survival in vaccinated mice but not TLR4. The results suggest that multiple MyD88-dependent signal transduction pathways may be involved in protection from plague.
CHARACTERIZATION OF *LATET*, A NOVEL GENE FOR WHICH ALLELIC VARIATION AFFECTS TYPE 1 DIABETES IN MICE.

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Type 1 Diabetes (T1D) is a multigenic autoimmune disease in which T cells mediate the specific destruction of insulin-producing beta cells in the pancreas. The events that trigger T1D pathogenesis remain unclear, but studies of the non-obese diabetic (NOD) mouse strain have provided key insights into the genetics and pathogenesis of this disease. As in humans, T1D is a multigenic disease in NOD mice and >25 loci, termed *Idd* loci, have been linked to T1D susceptibility. To identify the underlying gene for the *Idd11* locus on chromosome 4, we established a panel of congenic mouse strains that have different C57BL/6-derived intervals for chromosome 4 on the NOD genetic background. These congenic strains exhibited different levels of T1D incidence and localised *Idd11* to a ~6.9 kb interval encompassing a novel gene, we have termed *Latet*. Bioinformatics and RACE analyses of *Latet* have identified 6 exons and 2 splice variants. There are a number of open reading frames, but none demonstrate significant homology with known proteins or protein domains, nor does *Latet* encode an obvious microRNA, suggesting that it may encode a functional long non-coding RNA rather than a protein. Quantitative real-time PCR (qRT-PCR) indicated that *Latet* expression is decreased in immune tissues of NOD mice compared with diabetes-resistant mouse strains. Specifically, antigen-presenting cells (e.g. dendritic cells, macrophages) exhibited the highest level of expression, whereas lymphocytes exhibited little to no expression of *Latet*. As C57BL/6 mice expressed higher levels of *Latet* than NOD mice, we have established a *Latet* knockout mouse strain on the C57BL/6 genetic background. Ongoing studies are focused on determining the role of *Latet* in the immune system and autoimmune disease using our unique panel of congenic and knockout mice.
To develop cytotoxic effector functions, naive CD8\(^+\) T lymphocytes must recognize specific Ag/MHC class I complexes on antigen presenting cells in the context of costimulatory molecules and cytokines. Dendritic cells (DCs) can provide all of these required signals and are commonly thought to be the most important antigen presenting cells (APC) in the immune system. While it has been demonstrated previously, that DCs are crucial to mount immunity to certain pathogens, it is unclear if this is a general rule, if other antigen-presenting cells could be necessary for anti-viral immunity. Also the importance of specific DC subpopulations such as CD8\(^+\) and CD103\(^+\) DCs during infection with different pathogens is unclear. Using a set of DC-specific transgenic and DC-deficient mice in combination with Herpes-Simplex-Virus (HSV), murine Cytomegalovirus (MCMV), Adenovirus (AV) and Lymphocytic Choriomeningitis Virus (LCMV) infection, we analyzed the DC-dependency of virus-specific CD8\(^+\) T cell responses. While HSV- and MCMV-specific CTL-responses were completely dependent on the presence of CD8\(^+\) DCs and could not be mounted by other DCs or non-DCs, Adenovirus-specific CTL-responses were CD8\(^+\) DC-independent, as they were normal in Batf3-deficient mice, that lack CD8\(^+\) and CD103\(^+\) DCs. Consequently, adenovirus- as well as HSV- and MCMV-specific CTL were undetectable in constitutive and inducible DC-deficient mouse strains that lack distinct subtypes of DCs. In marked contrast, LCMV-specific CTL-responses were detected at normal levels in Batf3-ko mice as well as constitutive DC-deficient mice, while they lacked – as described previously – in inducible DC-deficient mice. Using additional DC-specific transgenic mice we set out to identify alternative APCs, responsible for priming of LCMV-specific CTL. Our data indicate that APC other than DC might be necessary to induce CTL responses during certain, but not all viral responses.
Roles of TRAF5 in TLR Signaling

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Dysregulation in Toll-like receptor (TLR) signaling has been implicated in many disease states, including chronic inflammatory conditions such as atherosclerosis. The cytoplasmic adaptor proteins TNF receptor associated factor (TRAF)3 and TRAF6 are known to mediate TLR signaling. Recent data from our lab suggest for the first time that TRAF5 also plays a significant role in immune cell TLR functions. B lymphocytes, macrophages, and dendritic cells from TRAF5−/− mice show increased cytokine production and enhanced early signaling pathways after stimulation through various TLRs. Interestingly, B cells are most greatly affected by TRAF5 deficiency. Our data show that TRAF5 is a negative regulator of TLR signaling and restrains proinflammatory cytokine production. It has been reported that TRAF5 deficiency accelerates the development of atherosclerosis in mice, while human patients with coronary heart disease express lower levels of TRAF5 mRNA in blood compared with healthy controls. Elucidating the role of TRAF5 in TLR signaling may provide a new target for therapies against a variety of inflammatory conditions, including those predisposing to atherogenesis. Our current studies focus on identifying the key molecular interactions by which TRAF5 mediates its effects.
DOSE-DEPENDENT BREAKDOWN OF ESTABLISHED INHALATION TOLERANCE BY LIPOPOLYSACCHARIDE: IMPLICATIONS FOR THE INDUCTION OF ASTHMA

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Many human subjects develop symptoms of asthma in late childhood and adulthood, years after initial sensitization to allergens occurs. In order to better study this phenomenon, we established inhalation tolerance to ovalbumin (OVA) in mice, and then attempted to break tolerance using different adjuvants during OVA sensitization. Two established models of Th2 allergen sensitization were tested: OVA/Alum immunization via intraperitoneal route, and low-dose lipopolysaccharide (LPS 100ng)/OVA immunization via oropharyngeal route. Interestingly, tolerized animals were completely protected from sensitization in both cases. Despite this active suppression, a ten-fold higher dose of LPS (1ug) given at sensitization broke tolerance and induced IL-17 dominant eosinophilic and neutrophilic pulmonary inflammation following OVA aerosol challenge. Preliminary data suggests that, during tolerance breakdown, regulatory T cells (Tregs) in the lung-draining lymph node are still functioning to suppress the emerging effector T cell response, but Tregs fail to accumulate in the lung. These data suggest that alteration of the Treg response, coupled with initiation of an effector T cell response, results in breakdown of inhalation tolerance. Furthermore, these data suggest the first encounter with allergen does not completely determine the outcome of future responses. Defining the mechanisms by which inhaled adjuvants break established tolerance will enhance our understanding of allergen-driven immune responses.
ROLE OF IL-12 IN PROGRAMMING HUMAN CD8+ T LYMPHOCYTES

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CD8+ cytotoxic T lymphocytes play a major role in defense against intracellular pathogens, and their functions are specified by antigen recognition and innate cytokines. IL-12 and IFN-α/β are potent "signal 3" cytokines that are involved in both effector and memory cell development. While the majority of effector cells are eliminated as inflammation resolves, some survive within the pool of memory cells and retain immediate effector function. In this study, we demonstrate that IL-12 instructs a unique program of effector cell differentiation that is distinct from IFN-α/β. Moreover, effector memory T_{EM} cells within peripheral blood display many common attributes of cells differentiated in vitro in response to IL-12, including pro-inflammatory cytokine secretion and lytic activity. A pattern of IL-12-induced genes was identified that demarcate T_{EM} from central memory T_{CM} cells, and the ontologies of these genes correlated precisely with their effector functions. Further, we uncovered a unique program of gene expression that was acutely regulated by IL-12 and reflected in stable gene expression patterns within T_{EM}, but not T_{CM} cells in vivo. Thus, this study directly links a selective set of IL-12-induced genes to the programming of effector functions within the stable population of human CD8+ T_{EM} cells in vivo.
THE FUSION PROCESS MODIFIES THE FUNCTION OF LYMPHOCYTE-DENDRITIC CELL HYBRIDS AND DECREASES THE PRODUCTION OF IFN-γ AND IL-10

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Dendritic cells (DCs) are the major antigen presenting cells and a potent tool for immunotherapy protocols. There are different strategies to generate DC-based antitumor vaccines, including the fusion of DCs and tumor cells. Here, we investigated the stimulatory function of hybrid cells generated by the fusion of monocyte-derived DCs with allogeneic lymphocytes. Hybrid cells were generated by fusion of DCs and allogeneic Ly (cultured at 37ºC or 40ºC) by electroporation and used as stimulators for lymphocytes obtained from the same donors as the DC precursors. The proliferative response of CD4+ and CD8+ was evaluated by flow cytometry. Cell mixtures were used as controls. Our results showed that the CD4+ proliferation was significantly (p<0.05) higher when stimulators were mixed cells instead of fused (M37: 32.2% +/- 6.0 vs F37: 12.2% +/- 0.5; and M40: 32.5% +/- 9.5 vs F40 8.6% +/- 1.6). Also the cytokine production pattern in these co-cultures was affected by the fusion: the production of IFN-γ was significantly (p<0.01) higher in the cultures with mixed cells (M37: 400.5 pg/mL +/- 74.1 vs. F37: 93.4 pg/mL +/- 21 and M40: 384.7 pg/mL +/- 82.3 vs. F40: 42.8 pg/mL +/- 47.8); and a similar phenomenon occurred with IL-10 (M37: 56.3 pg/mL +/- 9.5 vs F37: 16.4 pg/mL +/- 12.1 and M40: 51.6 pg/mL +/- 9.1 vs. F40: 14.5 pg/mL +/- 10.6). The fusion process seems to impair significantly the ability of allogeneic lymphocytes to stimulate the proliferative and cytokine response of CD4+ lymphocytes to these cells. Interestingly, the response of CD8+ cells seemed to remain unaffected. Financial Support: FAPESP (#2010/18139-7; 2009/54599-5); CNPq (#303731/2007-9).
SELF-DNA RELEASE MEDIATES THE ADJUVANT EFFECTS OF ALUMINUM SALTS

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Much remains to be elucidated of the immunological mechanisms that govern the adjuvant effects of aluminum salts (alum), to this day the most widely used type of vaccine adjuvant. Recently, it was suggested that, like any other efficient adjuvant, alum might boost adaptive immune responses through the activation of innate immune signaling pathways such as the NLRP3 inflammasome. We observed that, in mice, alum causes the release of host cell DNA at sites of injection, which acts as an endogenous innate immunostimulatory signal mediating the adjuvant activity of alum. Indeed, purified mouse DNA in quantities similar to those detected at alum injections sites was as potent as alum in boosting T and B cell responses. Furthermore, local digestion of extracellular DNA using DNase I decreased alum-induced cellular and humoral responses. Host DNA signaling appears to differentially regulate IgE and IgG1 production in alum immunization, independently of inflammasome signaling. Indeed, we observed that alum-induced host DNA release activated CD11c⁺ CD11b⁺ Ly6C⁻ Ly6G⁻ inflammatory dendritic cells (iDCs). Alum-induced iDC activation depends on TANK-binding kinase-1 and Interferon response factor (Irf)3-dependent, but is independent of the activity of known DNA sensor pathways. Activated iDCs stimulate ‘canonical’ T helper type 2 (Th2) responses, associated with peripheral effector responses such as experimental allergy, but unable to promote IgG1 responses. This canonical Th2 response nevertheless was able to promote IgE isotype switching from independently activated B cell responses. Irf3-dependent iDC activation was independent of type I interferon signaling, but implicated autocrine signaling by IL12p40 homodimers. Finally, we propose that the boosting of IgG1 production by host DNA release occurs through the induction of T follicular helper responses via iDC- and Irf3-independent mechanisms. The finding of a link between innate immune detection of extracellular host DNA and the boosting of adaptive responses in alum-adjuvanted immunization may increase our understanding of the mechanisms of action of current vaccines and help in the design of new adjuvants.
Type I interferon (IFN-α/β) is a potent natural anti-viral cytokine that has been used clinically for almost 3 decades to treat a variety of viral infections, some forms of cancer, as well as the autoimmune disease multiple sclerosis. While the innate role of IFN-α/β in establishing the anti-viral state is well established, our group has focused on exploring how IFN-α/β shapes the adaptive T cell response, particularly in human. Recently, we found that IFN-α/β blocks IL-4-driven Th2 development and represses IL-4, IL-5, and IL-13 secretion from fully differentiated Th2 cells. We have now identified the Th2-specific transcription factor GATA3 as the molecular target of this suppression. IFN-α/β inhibits GATA3 expression by repressing the GATA3 promoter of exon 1a that is selectively regulated by IL-4 signaling during Th2 development. IFN-α/β further restricts Th2 cytokine expression and GATA3 exon 1a utilization in memory Th2 cells isolated from human peripheral blood. Finally, IFN-α/β specifically blocks acute Th2 cytokine secretion from T cell receptor-activated memory cells, suggesting an additional mechanism of suppression not involving long-term developmental programs. These negative regulatory pathways seem paradoxical considering that seasonal upper respiratory viral infections, that presumably induce IFN-α/β, exacerbate the symptoms of allergic asthma. However, we have recently found that plasmacytoid dendritic cells (pDCs) from allergic asthma patients secrete significantly less IFN-α/β in response to either influenza infection or TLR7/9 agonists than pDCs from healthy cohorts. Further, pDCs express the high affinity IgE receptor (FcεR1), and activation of FcεR1, either by anti-IgE crosslinking or with specific allergen, blocks IFN-α/β secretion in response to influenza infection. Thus, IFN-α/β, the main negative regulatory cytokine that blocks Th2 development and memory function, is inherently low in asthma patients and inhibited by allergic stimulation. Therefore, we propose that supplementation with IFN-α/β may be a viable therapy for the treatment of allergic diseases such as asthma.
A BACTERIAL VIRULENCE FACTOR TARGETS TRAF PROTEINS TO IMPAIR THE HOST IMMUNE RESPONSE

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Pathogenic E. coli strains contribute greatly to the enormous economic and health burden of food borne disease, among which enterohemorrhagic E. coli (EHEC) has emerged as an important cause of hemorrhagic colitis in developed countries. EHEC virulence proteins (effectors) are translocated directly into intestinal epithelial cells through a type III secretion system (T3SS). The T3SS is a molecular syringe, widely conserved among animal and plant pathogens, that directs the active transport of effectors into host cells. Non-LEE-encoded secreted protein NleB, which is a T3SS effector, has been proposed to be essential for bacteria colonization and transmission. Previous studies suggested that NleB might suppress NF-κB pathway activation by TNF-α stimulation. Here we show that NleB can inhibit not only NF-κB but also JNK pathway activation. We also discovered NleB might prevents TNF receptor-associated factor 2 (TRAF2) ubiquitination. Bacteria lacking nleB expression induce TRAF2 ubiquitination and degradation, whereas wild-type strains stabilize TRAF2 during infection. Functional mapping of NleB indicates that there are multiple domains involved in inhibiting NF-κB activation. NleB also selectively targets TRAF3 protein ubiquitination; K63- but not K48-mediated TRAF3 ubiquitination is attenuated during bacterial infection. Overall, our data indicate that NleB targets TRAF proteins to impair the host immune system and to benefit bacterial colonization.
Type I Interferon (IFN-α/β) is an innate cytokine important for fighting infections. Further, this cytokine is used for the treatment of a variety of diseases, including hepatitis C infection, multiple sclerosis, and certain cancers. Although the innate role of this cytokine has been studied extensively, our group has shown that IFN-α/β also plays a role in shaping the adaptive immune response in humans. Recently, we demonstrated that IFN-α/β inhibits naïve human T cell development into Th2 cells by regulating both mRNA and protein levels of the master transcription factor GATA3. This regulation of GATA3 in turn suppresses Th2 cytokine production without effecting IFN-γ production. In addition to effecting naïve T cell development, we have also observed an acute regulation of human memory T cell function upon treatment with IFN-α/β. Human memory T cells (CD4+/CD45RO+) activated with anti-CD3 in the presence of IFN-α/β for 4hrs resulted in reduced mRNA expression levels of Th2 cytokine genes, IL5 and IL13, but did not effect the Th1 cytokine gene, IFNG. However, GATA3 levels were not affected, suggesting a GATA3-independent mechanism for regulating acute Th2 cytokine gene expression in response to TCR activation. This mechanism seems to be specific to IFN-α/β, as neither IFN-γ nor IFN-λ treatment were able to repress Th2 cytokine mRNA expression to the extent of IFN-α/β. Future studies aim to identify the exact mechanism by which IFN-α/β regulates immediate Th2 cytokine gene expression in human memory T cells. Identifying this novel pathway suggests that treatment of atopic asthmatic individuals with IFN-α/β can regulate both Th2 cell development and memory Th2 cell function.
An effective T cell response to infection requires mediating a balance between pathogen clearance and prevention of host immunopathology. This entails initiation of a T cell response through priming by antigen-presenting dendritic cells (DCs) and contraction of that response by both T cell intrinsic and extrinsic regulatory mechanisms. T cell immunoglobulin and mucin domain–3 (Tim-3) is a cell surface molecule that appears to modulate both the initiation and termination of immune responses involving T cells. However, the mechanisms by which Tim-3 functions during these processes are poorly understood. To define the role of Tim-3 during T cell responses in vivo, we utilized Listeria monocytogenes (LM) infection of wild-type and Tim-3-/- mice. LM induces a well-documented potent inflammatory response and several tools have been developed for dissecting responses by DCs and T cells to LM infection. Our work thus far has demonstrated that Tim-3 deficiency reduces the magnitude of antigen-specific responses by both CD4+ and CD8+ T cells, suggesting that Tim-3 expression on DCs is important for DC activation and subsequent T cell priming. Conversely, LM infection of mixed bone marrow chimeras in which are DC populations are normalized demonstrated that Tim-3-/- T cells generate significantly more memory cells due to less intracellular caspase activation, suggesting Tim-3 expression on T cells activates pathways that induce cell death. These data support the hypothesis that Tim-3 modulates CD4+ and CD8+ T cell responses by positively regulating APCs and negatively regulating T cells.

To define the roles of T cell and APC expressed Tim-3, we are employing LM infection after adoptive transfer of antigen-specific, Tim-3+/+ or Tim-3-/- T cells to Tim-3 sufficient or deficient hosts, respectively. Determining the role of Tim-3 expression on APCs or T cells will further contribute to our understanding of how T cell responses can be regulated.
Lymphocyte Function Antigen-1 (LFA-1) is a β−2 integrin expressed on the surface of human WBCs. LFA-1 undergoes an inducible conformational change from a non adhesive inactive to an adhesive active state, the latter mediating migration from blood vessels into tissues. Hematological malignancies are characterized by proliferation of WBCs that overexpress activated LFA-1. LFA-1 is known to be the specific target of Leukotoxin (LtxA), a major virulence factor of oral bacterium Aggregatibacter actinomycetemcomitans that causes aggressive periodontitis. LtxA targets primate WBCs migrated to the periodontium countering host response against the bacterium. Because of this natural specificity, LtxA was tested for its cytotoxic effect on THP-1 cells in vitro, which were found to be very sensitive to LtxA. Humanized mouse models of leukemia (HL-60) showed prolonged disease free survival upon LtxA injection. Previous studies indicate preference of LtxA to activated WBCs compared to unactivated ones. Therefore, we wished to determine if this preference was due to LtxA specifically binding to active or open conformation of LFA-1. mAB24 was used as marker for active conformation of LFA-1 while CD11a (clone: HI111) was used as a marker for inactive conformation of LFA-1. Preincubation of fixed THP-1 cells with LtxA led to decrease in mAB24 binding indicating that LtxA competes with mAB24 binding site on active conformation of LFA-1. In contrast, preincubation with LtxA had no effect on binding of CD11a mAB HI111 which recognizes only the inactive conformation of LFA-1. These results indicate specificity of LtxA for active LFA-1. The levels of active LFA-1 on healthy WBCs isolated from human blood were relatively low at baseline but increased upon stimulation with phorbol esters. This increase was more pronounced in granulocytes and monocytes. Treatment of human granulocytes and monocytes with LtxA ex vivo showed depletion of only active LFA-1 population. Establishing this specificity is important to provide maximum therapeutic benefit targeting abnormal WBCs with active LFA-1 and sparing normal WBCs expressing inactive LFA-1 thereby minimizing immunosuppression.
SHIGA TOXIN-PRODUCING E. COLI PREVENT ACTIVATION OF
THE INHIBITOR OF κB KINASE (IKK) COMPLEX

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Shiga toxin-producing E. coli (STEC) cause food borne diarrheal disease outbreaks. These pathogens use a type III secretion system (T3SS) to inject virulence proteins (effectors) into host cells. We have identified an STEC effector named NleH1 that inhibits the host innate immune response through a unique mechanism. NF-κB activity at key innate immune response genes is regulated by ribosomal protein S3 (RPS3), which possesses an accessory nuclear function as an NF-κB subunit. NleH1 inhibits the IKKβ kinase complex from phosphorylating RPS3, a critical requirement for its nuclear translocation, thus reducing NF-κB activity at specific promoters.

STEC serotypes commonly implicated in causing outbreaks of hemorrhagic colitis also encode a homologous effector named NleH2. Despite sharing 84% identity with NleH1, NleH2 stimulates, rather than inhibits, RPS3/NF-κB-dependent transcription. This represents a novel example of how bacterial pathogens fine-tune NF-κB target gene expression using a homologous, yet antagonistic pair of virulence proteins.

Our work has clarified how IKKβ normally functions in coordinating the nuclear import of multiple transcription factor subunits and has begun to define the mechanism of how NleH1 inhibits this process. Data obtained from ongoing research will clarify how pathogens have evolved to co-opt the accessory nuclear functions of ribosomal proteins and will elucidate how bacteria have integrated their virulence proteins into host signal transduction pathways to disrupt immune responses.
S. TYPHI-IMPRINTED DENDRITIC CELLS MEDIATE TH1 EDUCATION AND ENHANCED RESPONSES TO A FOREIGN ANTIGEN

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Dendritic cells (DC) are the principal antigen presenting cells (APCs) inducing T cell activation and proliferation during immune responses to foreign antigen. The success of DC in stimulating T cells and directing their responses depends on the surface expression of co-stimulatory molecules and secretion of cytokines. The neonatal immune system contains non-experienced cells capable of limited antigen presentation and T cell activation that are unable to confer lasting protection against pathogen infection. Due to the requirement of DC maturation to elicit Th1-type responses and develop mucosal and systemic immunity, developing neonatal vaccines that stimulate DC could provide protection to this critically effected population. In the current study, we examined the capacity of S. Typhi to enhance maturation of DC derived from human umbilical cord blood (CB). To this end, we generated immature DC from CB by magnetically separating CD14⁺ or CD34⁺ cells and treating them with IL-4 and GM-CSF. These immature DC were stimulated with S. Typhi and their maturation status was compared to adult-derived DC. S. Typhi stimulated neonatal DC displayed significantly enhanced expression of activation markers and co-stimulatory signals compared to mock-treated cells and comparable to stimulated adult DC. Neonatal DC stimulated with S. Typhi also displayed increased cytokine secretion and fold increases were comparable to stimulated adult DC. We also determined the capacity of S. Typhi to influence and modulate T cell responses by co-culturing neonatal DC with naïve neonatal T cells in the presence or absence of S. Typhi priming. The generation of effector memory (EM) and effector memory RA⁺ T cell subsets was established by expression of CD62L and CD45RA. We found that the percentages of both EM and EMRA CD4⁺ and CD8⁺ T cells were increased upon S. Typhi treatment and these cells showed enhanced cytokine secretion. In order to assess the ability of Salmonella to confer these markers of maturation to DC in vivo, we immunized neonatal mice with S. Typhi. *Salmonella* enhanced DC maturation as evidenced by increased expression of CD80 and MHC II surface molecules in cells derived from nasal associated lymphoid tissue (NALT) and lungs from immunized mice. The data illustrate the ability of *Salmonella* vaccines to promote maturation of neonatal DC, improving their APC function and promoting Th1-type adult-like immune responses that can provide enhanced immunogenicity against foreign antigen.
ANTIGEN DELIVERY BY β-SHEET AGGREGATED PROTEIN POTENTIATES EFFICIENT IMMUNE RESPONSE

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The accumulation of β-sheet aggregates, such as amyloids or amyloid-like proteins, is a hallmark of protein misfolding diseases as this structure element is absent in natively folded proteins. Although the role of the immune system in the progression or control of these diseases has been widely studied, the connection between β-aggregation and its immunological effects remains unclear. Here we demonstrate the β-sheet aggregates can act as potentiator of immune response. We show that the β-sheet aggregates of ovalbumin, generated by thermic misfolding are actively taken up by antigen presenting cells through a scavenger receptor-mediated mechanism. The subsequent rapid processing and antigen presentation, via both MHC class I and class II pathways, leads to profound stimulation of antigen-specific primary CD4+ and CD8+ T cells. Our findings establish β-sheet aggregation as a novel immunogenic motif intrinsic in the antigen itself, which has important implications for the development of novel vaccines.
TOLL-LIKE RECEPTOR (TLR) - MEDIATED INDUCTION OF CYCLOOXYGENASE (COX)-2 IN HUMAN VAGINAL EPITHELIAL CELLS. EFFECT OF SEMINAL PLASMA ON COX-2 EXPRESSION.

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Sexual transmission of HIV is one of the main routes of HIV-1 dissemination. Inflammation of cervicovaginal mucosa is considered a factor that increases the rate of acquisition of HIV-1 and sexually transmitted infections are among the major contributors to inflammation. Epithelial cells of the female lower genital tract express TLRs that are known to be activated by pathogen associated molecules, which trigger innate immune and inflammatory responses. Earlier we demonstrated that COX-2, a key inflammation related enzyme is induced in human vaginal cells exposed to proinflammatory stimulants, TNF alpha and nonoxynol-9. In the present study, human vaginal epithelial cells (VK2/E6E7) were exposed to TLR ligands: bacterial lipopeptide, Pam3CSK4, (TLR1/2), lipoteichoic acid (LTA) (TLR2/6), mycoplasmal lipopeptide (MALP2) (TLR2/6), proteoglycan from yeast cell wall (zymosan) (TLR2/6), synthetic analog of viral dsRNA (polydeoxyinosinic-polydeoxyctydyllic acid [poly dl:dC]) (TLR3), and imiquimod (IMQ) (TLR7). In addition, VK2/E6E7 cells were treated with seminal plasma (SP). We demonstrate using qRT-PCR and immunoblot analysis that TLR ligands, as well as SP induce COX-2 expression in human vaginal epithelium. Importantly, COX-2 induction by Pam3CSK4, LTA and IMQ was enhanced synergistically in the presence of SP.

This study provides insight into the mechanisms of innate immune and inflammatory responses in the cervicovaginal mucosa and the role of SP in exacerbating local inflammatory processes. The importance of these actions is highlighted in the context of sexually transmitted infections, especially HIV-1.
DIACYLGLYCEROL KINASE ZETA BUT NOT DIACYLGLYCEROL KINASE ALPHA SUPPRESSES DEVELOPMENT OF NTREGS

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Diacylglycerol (DAG) is a critical second messenger of T cell receptor (TCR) signaling. Its activity is negatively regulated through phosphorylation by a class of proteins known as diacylglycerol kinases (DGKs). Lack of DGKα and DGKζ, isoforms of DGK primarily expressed in T cells, results in enhanced activation and proliferation of T cells to TCR stimuli. In addition, DGKζ knockout (DGKζKO) mice have more robust responses to viral infection and tumors. Presumably due to excessively strong TCR signaling, deletion of both DGKα and DGKζ results in a severe block in thymocyte development that is not seen with deletion of either isoform alone. Strong TCR signals may also direct developing thymocytes to the natural T regulatory cell (nTreg) fate. Consistent with its effect on TCR signaling, we have found that DGKζ deficiency significantly increases percentages of nTreg cells. The key structural features of DGKζ that are important for nTreg development are unknown. In cell lines, the kinase domain and phosphorylation of the myristoylated alanine-rich C-kinase substrate (MARCKS) domain control the enzymatic activity and localization of DGKζ. To probe the function of these domains, we used retroviruses to transduce DGKζKO bone marrow and re-express mutant DGKζ proteins using bone marrow chimeras. Validating our system, expression of wild-type DGKζ restored suppression of nTreg development compared to empty vector. Expression of kinase dead DGKζ did not suppress development of nTregs compared to empty vector, suggesting that kinase activity is important for this function. To our surprise, expression of a non-phosphorylatable MARCKS domain DGKζ did not restore suppression of nTreg development, suggesting that localization of DGKζ may play a critical role in its function. Because DGKα and DGKζ have been shown to localize differently in cell lines, we investigated if DGKα and DGKζ have different roles in nTreg development. Surprisingly, we found that mice lacking DGKα do not have higher percentages of nTregs, even in the presence of DGKζ heterozygosity. Suppression of nTreg development may therefore be a function specific to DGKζ and not DGKα. Current investigations are probing the mechanism behind the differences observed between DGKα and DGKζ deficient mice. As inhibitors specific to DGKα and DGKζ exist, understanding the differential roles of DGKα and DGKζ could reveal a specific therapeutic target for modulating levels of nTregs, which are crucial for the progression of certain cancers and in resistance to autoimmunity.
BINDING OF SUPERANTIGEN TOXINS INTO THE CD28 HOMODIMER INTERFACE IS ESSENTIAL FOR INDUCTION OF TH1 CYTOKINE GENES THAT MEDIATE LETHAL SHOCK

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Hitherto, CD28 was not known to bind microbial components yet we show here that bacterial superantigens co-opt CD28 as their receptor and that to induce a cytokine storm comprised mainly of IL2, IFN-γ and TNF-α, the superantigen must bind directly into the homodimer interface of CD28. The interaction can be blocked with peptides that mimic the contact domains in superantigen or CD28. These peptides, we show, attenuate inflammatory cytokine gene induction and thus protect animals from lethal toxic shock.

Staphylococcal and streptococcal superantigens, a diverse family of toxins, induce a Th1 cytokine response, orders of magnitude higher in intensity than that elicited during regular immune responses, which can lead to lethal shock. CD28 is a homodimer expressed constitutively on T cells that functions as the principal costimulatory ligand in the immune response through an interaction with its B7 coligands, yet we show here that to elicit Th1 cytokine gene expression and toxicity, superantigens must bind directly not only to the major histocompatibility class II molecule on the antigen-presenting cell (MHC-II) and the T Cell Receptor (TCR) but also into the dimer interface of CD28. Preventing access of the superantigen to CD28 suffices to block its lethality. Mice were protected from lethal superantigen challenge by short peptide mimetics of the CD28 dimer interface and by phage-display peptides selected to compete with the superantigen for its binding site in CD28. Superantigens use a conserved β-strand/hinge/α-helix domain of hitherto unknown function to engage CD28. Mutation of this superantigen domain abolished inflammatory cytokine gene induction and lethality. Structural analysis showed that when a superantigen binds to the TCR on the T cell and the MHC-II on the antigen presenting cell, CD28 can be accommodated readily as a third superantigen receptor in the quaternary complex, with the CD28 dimer interface oriented towards the β-strand/hinge/α-helix domain in the superantigen.

We thus identify the CD28 homodimer interface as a critical receptor target for superantigens. Our finding that CD28 is a receptor for the superantigen toxins broadens the scope of microbial pathogen recognition mechanisms and provides a novel approach for designing therapeutics that protect against toxic shock induced by an inflammatory cytokine storm.

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FOXO1 CONTROL OF MEMORY CD8+ T CELL DIFFERENTIATION

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Immunological memory is a characteristic function of the adaptive immune system, which mediates protective immune responses against secondary infection. Upon pathogen encounter, antigen-specific T cells undergo clonal expansion followed by a contraction phase and the differentiation of approximately 5-10% T cells into memory cells. Although key properties of memory T cells have been revealed, it is essential to understand the molecular mechanisms controlling memory T cell differentiation to optimize vaccine efficacy. Our recent studies have revealed crucial roles for the Forkhead box O family of transcription factors (Foxos) in the control of naive T cell homeostasis. Foxo1 regulates naive T cell survival and trafficking by inducing CD127, CD62L and CCR7, molecules that are highly expressed in memory T cells as well. To investigate whether Foxo1 is required for the generation of memory T cells, we used an effector CD8+ T cell specific Foxo1-deficient mouse model. We infected these mice with Listeria monocytogenes expressing ovalbumin (Listeria-ova) to examine antigen-specific CD8+ T cell responses. We found that the number and function of effector CD8+ T cells at day 7 post infection (p.i.) were comparable between wild-type (WT) and Foxo1-deficient mice. However, antigen-specific T cells underwent precipitate contraction in the absence of Foxo1 at day 60 p.i. As a consequence, Foxo1-deficient mice showed compromised recall responses and failed to efficiently clear bacterial infection. Transfer experiments with WT and Foxo1-deficient OTI cells revealed that Foxo1 played a cell-intrinsic role in promoting memory T cell generation. These findings demonstrate a critical role for Foxo1 in control of memory CD8+ T cell differentiation.
APOBEC3A IS A POTENT RESTRICTOR OF FOREIGN DNA IN BOTH THE NUCLEUS AND THE CYTOPLASM OF HUMAN CELLS

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APOBEC3A (A3A) is part of the seven-member APOBEC3 family, which is composed of innate cytidine deaminases including the HIV restrictors APOBEC3F and APOBEC3G. A3A, however, does not display HIV restriction, and instead has a role in the restriction of foreign (non-self) DNA, similar to the bacterial restriction enzymes that are regularly exploited in molecular biology labs. We have previously shown that A3A indeed seems specific for foreign DNA, and does not target intact chromosomal DNA, leading to the important question: how does A3A distinguish foreign vs. self? We hypothesized that A3A, despite its cell-wide localization, may only be active in the cytoplasmic compartment, perhaps via interaction with a regulatory partner, and may not be an active deaminase in dangerously close proximity to the chromosomal DNA in the nucleus.

To address this question, we constructed a series of A3A mutants that localized to only the nucleus or the cytoplasm and tested their restriction ability by quantitative differential DNA denaturation PCR, which assesses mutated intermediates by progressively decreasing the PCR denaturation temperature. Additionally, we tested our localization constructs by a flow-cytometry based foreign DNA restriction assay that quantifies loss of a fluorescent foreign DNA plasmid.

Surprisingly, our results indicate that regardless of localization, A3A is a potent foreign DNA restrictor. This suggests that localization is not the main method cells use to protect their genome from potentially lethal mutagenesis by A3A. Our finding has important implications for applications such as gene therapy, as our results suggest that human cells are capable of mutating introduced genetic material even if it can be introduced directly to the nucleus. We will continue our studies to determine the regulation of A3A and how it determines which DNA is a target and which is self and therefore safe.

Selected reference:
Vaccination is the most effective method to control infectious diseases. However, most current vaccines do not generate an appropriate cellular immune response that is essential to protect against chronic infections or cancer. We have recently shown that Papaya Mosaic Virus-Like Particles (PapMV VLP) are highly immunogenic in mice. In addition, they can form an epitope presentation system that induces protective humoral and cellular immune responses against various viral infections. Our hypothesis is that PapMV VLP contain pathogen associated molecular patterns (PAMPs) that are recognized by antigen presenting cells (APCs) and induce their maturation, which is important for the development of an appropriate adaptive immune response. The aim of this study was to identify the APC receptors interacting with PapMV VLP, the signal that they generate and the PAMPs involved.

To identify the APC receptors that interact with PapMV VLP, the HEK293-Blue cell line expressing different Toll-like receptors (TLRs) and possessing a reporter gene for TLR activation was first used. In this cell line, we saw that PapMV VLP induced specific activation of cells that express mouse TLR7. We confirmed the implication of this receptor in the recognition of PapMV VLP by using TLR7 KO mice. In fact, immunization of wild type (WT) mice with PapMV VLP induced an increased expression of CD86 and MHC-I in APCs and CD69 in B and T lymphocytes, whereas no activation was observed in MYD88 KO or TLR7 KO mice. Furthermore, we show that ssRNA present in PapMV VLP was responsible for TLR7 activation since the protein alone did not activate HEK293-blue cells or immune cells from immunized mice. In addition, PapMV VLP immunization of WT mice leads to IFN-α production as it is normally observed following endosomal TLR stimulation such as TLR7. Moreover, we observed that signalization by type I IFN pathway was important for the immunomodulatory capacity of PapMV VLP since absence of this receptor in mice prevents activation of immune cells after immunization and delays antibody production. We also noticed that in vivo depletion of plasmacytoid dendritic cells, which strongly express TLR7 and produce high amount of IFN-α, diminished the activation of immune cell after immunization.

In conclusion, these results shed light on the mode of action of PapMV VLP and clarify the mechanisms by which this adjuvant promotes the generation of cellular immune responses. This knowledge will help us design effective vaccines against persistent viral infections.
TRANSGENIC EIMERIA-BASED EUKARYOTIC VACCINE VECTOR SYSTEM FOR AVIAN DISEASE CONTROL IN CHICKENS

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There are more than 1000 species of protozoan parasites belonging to the genus Eimeria, which infect and cause coccidiosis in many mammals and poultry, especially in domestic animals such as rabbits, pigs and chickens. To reduce the annual economic loss of coccidiosis in the poultry industry, live oocyst vaccines containing several of seven Eimeria species of chickens have been widely used for more than 50 years. When vaccinated with a live oocyst vaccine, chickens can develop full protection against infection of homologous parasites after 3-4 weeks. During this period, repeated invasion, proliferation and releasing of intracellular parasites within three or more times of their life cycle primes and multiply boosts the host immune system, establishing the protective immunity. By virtue of this mechanism, we are developing transgenic Eimeria-based vaccine vectors expressing antigens derived from virus or bacteria. Using Yellow fluorescent protein (YFP) as a selection marker, several transgenic E. tenella lines were obtained via plasmid-mediated electroporation of sporozoites and in vivo selection. Under the regulation of Eimeria-specific promoter and signal peptide elements, HA, M2e or NP from H5N1 avian influenza virus (AIV) or α-toxin or surface layer protein B (SLB) from Clostridium perfringens could be expressed continuously or stage-specifically in parasite cytoplasm, nucleus or secreted into the parasitophorous vacuole. The antibody and CD8+ T cell responses induced by the antigen(s) expressed and delivered by recombinant parasites can be detected through ELISA, ELISPOT and intracellular cytokine staining assays. However, vaccination with transgenic parasites expressing NP antigen could not protect chickens against lethal H9N2 AIV lethal challenge in one preliminary trail, demonstrating that optimization of antigen expression and delivery in this novel vaccine vector system is needed in further studies. Overall, this transgenic Eimeria-based vaccine vector system demonstrated a different path for the development vaccine vectors other than recombinant viruses and bacteria.

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Dendritic cells (DCs) derived from hematopoietic progenitor cells and circulating monocytes play critical role in immune response and tolerance. To investigate the role of microRNAs (miRNAs) during DC differentiation, apoptosis and function, we profiled miRNA expression in human monocytes, immature DCs (imDCs) and mature DCs (mDCs). Stage-specific, differential expression of 27 miRNAs was found during monocyte differentiation into imDCs and mDCs. Amongst them, decreased miR-221 and increased miR-155 expression correlated with p27kip1 accumulation in DCs. Silencing of miR-221 or over-expressing of miR-155 in DCs resulted in p27kip1 protein increase and DC apoptosis. Moreover, mDCs from miR-155-/- mice were less apoptotic than those from wild type mice. Silencing of miR-155 expression had little effect on DC maturation, but reduced IL-12p70 production, whereas miR-155 overexpression in mDCs enhanced IL-12p70 production. Kip1 ubiquitination-promoting complex 1 (KPC1), suppressor of cytokine signaling 1 (SOCS-1) and CD115 (M-CSFR) were functional targets of miR-155. Furthermore, we provide evidence that miR-155 indirectly regulated p27kip1 protein level by targeting KPC1. The expression of miR-146b was dramatically up-regulated upon monocyte differentiation, and stably expressed in imDCs and mDCs. Silence of miR-146b was found to increase imDC survival, suggesting that this miRNA is important for DC apoptosis. However, miR-146b expression has little effect on DC maturation and IL-12 production. In addition, decreasing miRNAs during dendritic cell differentiation were found to be negatively relative to ATG gene expression, which are critical in antigen processing and presentation. Take together, our study uncovered miRNA signatures during monocyte differentiation into DCs and the new regulatory role of miRNAs in DC apoptosis, IL-12p70 production, and antigen presentation.
A POTENTIAL APPROACH TOWARD IMMUNOREGULATIONS AGAINST ALZHEIMER DISEASE USING A DROSOPHILA MODEL

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Alzheimer’s disease (AD) is the most common form of dementia. In this progressive disease, amyloid β (Aβ) accumulates in the brain. This is followed by plaques and tangles formation, neurodegeneration, and eventually memory loss. Recent reports have indicated a strong link between chronic innate inflammation and AD: (1) Immunohistochemical studies revealed the induction of pro-inflammatory mediators and the activation of microglia or astrocytes both in human AD and in AD model mice. (2) CD14 and TLR2/4/9 facilitate clearance of Aβ by microglial cells in AD model mice. (3) Induction of EAE in AD model mice reduces amyloid beta accumulation. (4) The use of several anti-inflammatory drugs (NSAID) has been associated with decreased AD incidence. (5) AD genetic-association studies (including GWA) detected several genes linked to immunity, namely IL-8, CR1, TNF and CCR2. These observations raise important questions, such as whether inflammation in AD is beneficial or harmful, especially in the context of innate inflammation, and in either way, how could the immune response be regulated to modify the disease’s course. Since Drosophila has been an excellent model for research on innate immunity, we addressed these questions using this relatively simple and well-established model. We generated transgenic flies that express either human Aβ42 or Aβ40 polypeptides in the nervous system. Flies expressing Aβ42 showed a reduced climbing ability as compared to those expressing Aβ40 or wild type flies. Also, microarray gene expression profiling of Aβ42 transgenic flies (vs. Aβ40) revealed high levels of gene expression of antimicrobial peptides, a hallmark of the immune responses during pathogen infections. This indicates that one of the two major inflammatory signaling pathways in Drosophila, IMD, largely similar to the TNF/TNF-R pathway in mammals, is activated but not the TOLL pathway. Thus, we generated various Aβ42-expressing flies carrying loss-of-function mutations in genes involved in the IMD pathway and monitored their climbing ability. Inactivation of the IMD pathway in our Drosophila AD model induced more severe climbing defects. Furthermore, we observed that IMD (similar to mammalian RIP1)-deficient Aβ42 AD model flies accumulated more Aβ42 in the brain, which was confirmed by ELISA and Immunohistochemistry. Taken together, our results indicate that the activation of the IMD signaling pathway is required for clearance of Aβ42 in Drosophila. We propose that regulations of the TNF signaling pathway might be a key to novel therapeutic interventions against AD.
EVOLUTIONARY GENETICS, SYSTEMS BIOLOGY, AND HOST-PATHOGEN INTERACTIONS: THE KEYS TO GENOMIC MEDICINE AND THERAPEUTIC INTERVENTION?

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It is widely accepted that the human genome adapts to selective pressures in its environment, and that these adaptations often result in phenotypic trade-offs. A canonical illustration of such a trade-off are the beta- and alpha-globin polymorphisms found to render host resistance to infection with *plasmodium sp.*, while also invoking the phenotypic expression of beta or alpha-thalassemia and sickle cell anemia. Both this and countless other examples show that the war between human host and pathogen is an epic one, defined by the creative efforts of both host and pathogen to perturb each other’s activities at every turn. However, it is clear that the immunological tactics of both host and pathogen are counteracted by the tremendous redundancy known to exist in human biological pathways regulating inflammation and infection. Such redundancy allows pathogens multiple routes to launch an effective attack, while leaving the host with numerous alternative means to mount a defense. This research proposes a model that applies the foundations of human systems biology, genomics and host-defense mechanisms to bridge our understanding about the evolutionary relationship between infectious and inflammatory disease. The efficacy of this model will be illustrated by exploring examples of how genomic markers for susceptibility or resistance to infectious disease may also inform the propensity for the development of complex inflammatory disease as secondary sequelae. This model has potential value in developing appropriate therapeutic algorithms enabling both the targeted delivery of vaccination programs and other interventions to mitigate inflammatory disease risk. There also exists an opportunity to develop molecular diagnostic assays predicting one’s risk of expressing infectious or secondary inflammatory disease phenotypes. Both of these genomic medicine applications will be discussed using examples having both currency and potential clinical utility.
INFANT IMMUNITY TO MEASLES – IMMUNOGENETIC PREDICTORS OF VACCINE NON-RESPONSIVENESS IN KENYAN CHILDREN.

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Measles virus (MV) is a major cause of infant morbidity and mortality worldwide. Global MV vaccine coverage has reached 83%; yet measles outbreaks occur even in well-vaccinated populations. MV is extremely contagious and greater than 95% population immunity is necessary to interrupt transmission. MV-antibody are known to confer protection however the importance of cell-mediated immunity (CMI) is not as conclusive, even though CMI is clearly important for resolving disease. In areas where wild-type MV is endemic the first vaccination is given at 9-months of age resulting in 70-80% sero-conversion. Maternally transferred neutralizing antibodies have been implicated as the main contributor to MV vaccine interference in infants however vaccine failure can occur even in the absence of passive antibodies. MV vaccine responses have been shown to have high heritability, with genetic determinants including HLA, cytokine and cytokine receptor and MV cellular receptor genes, i.e. CD46, signaling lymphocyte activation molecule (SLAM/CD150) and dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN; CD209). To gain more insight into MV-immunity, we conducted a study to examine serologic trajectory patterns generated from monthly MV-antibody levels measured in Kenyan infants (n=162) from 2 to 24 months of age. This revealed four significantly distinct patterns prior to MV vaccination: two that waned but started from different ‘maternally-derived’ set points, a third that immediately increased within the first months of life, and a fourth that waned but then increased. Pre-MV vaccination serology patterns were used to predict MV-vaccine responses and of the 78% infants whose passive MV-antibodies had waned, 20% of them failed to sero-convert. MV-specific IFN-γ responses were present in 21% of these infants but they were evenly distributed across pre-vaccination trajectory patterns and did not correlate with post-vaccination antibody responses. Ongoing T-cell memory and candidate gene studies within our Kenya population will be factored into our multivariate model. Defining the combination of intrinsic and extrinsic factors underlying poor MV-vaccine immunogenicity and impairment of immunologic memory is required to ultimately succeed in global MV eradication.
A major limitation of studying pathogen-specific T cell responses and generating subunit vaccines is the identification of immunodominant epitopes. For CD4+ T cells, these epitopes are peptides bound to major histocompatibility complex class II proteins (pMHCII). Specific T cell receptor (TCR):pMHCII interactions are dependent upon recognition of four TCR contact residues within the peptide that are accessible to the TCR. The three dimensional orientation of TCR contact amino acids is dependent upon interspersed MHCII anchor residues that hold the peptide in the MHCII binding groove. We tested the hypothesis that TCR specificity for an immunodominant epitope was dependent upon both TCR contact and MHCII anchor residues using a pMHCII tetramer-based approach and a known immunodominant peptide in C57BL/6 mice, 2W. To our surprise, we found that approximately 80% of T cell clones primed in response to 2W peptide could not discriminate between 2W and an altered peptide with identical TCR contact amino acids but nonconserved mutations in MHC anchor residues. Thus, the four TCR contact amino acids in the two peptides functioned independently of the five MHC class II anchor residues. An implication of this finding is that the potential for cross-reactivity of CD4+ T cells with host self peptides is more dramatic than currently appreciated and holes in the T cell repertoire created by clonal deletion could account for the relative paucity of known immunodominant epitopes.
Innate immune responses during acute and chronic LCMV infection

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Infection of mice with LCMV Armstrong or Clone 13, results in acute and chronic infections respectively. However, the early cellular and molecular mechanisms that mediate these disparate outcomes are poorly understood. We have characterized the innate immune responses that occur during acute and chronic LCMV infection, with a view to identifying early correlates and mechanisms of the later immune responses and viral loads. Two temporally distinct phases of innate immune response were observed: early DC responses during the first 72 hours of infection, and later myeloid cell expansion peaking at day 14 post infection.

Surprisingly, during the first phase the kinetics of cytokine production by DC and myeloid cells was crucially dependent upon the dose of infection, rather than on the LCMV strain. During this phase of both ARM and C13 infection pDC were transiently depleted while conventional DC subsets (cDC) continued to decrease during this period, with CD8α+ DC nearly completely absent by 72h post infection. Within 24h, DC in mice infected with high doses of either ARM or C13 LCMV upregulated the expression of the activation markers CD80, CD86, and I-Ab, as well as inhibitory markers such as PD-L1. Lower doses of ARM required at least 12 hours more to reach similar activation levels. The kinetics of IL-12 and TNF production by DC and myeloid cells was dependent upon infectious dose, not LCMV strain. Finally, DC and myeloid cells from enriched during the first 3 days of ARM and C13 infection were similarly able to stimulate OT1 T cells in vitro.

In the second phase, by day 5, total myeloid cells were expanded in the spleen and the blood during both ARM and C13 infection. However, during C13 infection both populations of myeloid cell numbers continued to increase, peaking at day 14 with up to ~25-fold expansion over naïve mice. Monocytic cells from day 14 chronically infected mice showed increased expression of CD115 and CD80, characteristic of suppressor myeloid cells. C13-derived day 14 myeloid cells were able to suppress in vitro proliferation of splenocyte-stimulated OT1 T cells. Specific inhibition of iNOS abrogated the suppressive function of C13-derived myeloid cells in vitro. By administering anti-Gr-1 antibody to C13 infected mice we were able to temporarily deplete myeloid cells, leading to increased cytokine production by LCMV-specific CD8+ T cells. Early innate immune activation by LCMV is dependent upon the infectious dose, and both ARM and C13 activate DC to stimulate T cells. During chronic infection myeloid cells become massively expanded and are able to inhibit T cell proliferation and function, contributing to viral persistence.
TRANSCUTANEOUS IMMUNIZATION INDUCES RAPID RESOLUTION OF BIOFILMS IN THE MIDDLE EAR DURING EXPERIMENTAL OTITIS MEDIA DUE TO NONTYPEABLE HAEMOPHILUS INFLUENZAE

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Nontypeable Haemophilus influenzae (NTHI) is a causative agent of the pediatric disease otitis media (OM). As the chronicity of OM is attributed to the formation of biofilms within the middle ear, we evaluated the potential of transcutaneous immunization (TCI) to induce resolution of pre-existing NTHI biofilms during experimental OM. Moreover, we performed a kinetic analysis of biofilm eradication and assessed the mechanisms that contributed to the observed resolution.

NTHI biofilms were first established in the middle ears of chinchillas prior to TCI with a novel chimeric immunogen [chimV4] that targets both NTHI OMP P5 and Type IV pilin adhesins admixed with the adjuvant LT(R192G-L211A) [dmLT]. To promote induction of an immune response in proximity to the middle ear, formulations were applied to chinchilla pinnae and to analyze the kinetics of disease resolution, NTHI within middle ear biomasses or fluids were enumerated every 2-3 days after receipt of the first vaccine dose. Receipt of chimV4+dmLT resulted in a significant reduction in NTHI within middle ear biomasses and fluids beginning 5 days after the first immunization, compared to receipt of dmLT alone (p<0.05).

Mechanisms to promote biomass resolution included efflux of dermal dendritic cells (DCs) from the pinnae, with subsequent migration to the NALT after TCI with chimV4+dmLT. IFN-γ and IL-17A production by CD4+ T-cells indicated the induction of a Th1/Th17-type immune response. Whereas immunogen-specific antibody was detected in serum and middle ear fluids, antibody titers were modest and biomass resolution was detected prior to measurable antibody.

Moreover, as a greater relative quantity of IL-8, IL-1α, C5a, MIF and IL-16 was detected within middle ear fluids after TCI with chimV4+dmLT compared to animals that received dmLT alone, innate immune elements likely also contributed to the observed biomass resolution. Examination of cellular influx into the middle ear and contribution of host defense peptides is pending.

These data provided insight to the mechanisms by which TCI induced eradication of NTHI from the middle ear via activation of DCs, induction of a Th1/Th17 immune response and contribution of innate immune effectors. TCI could expand the use of traditional parenteral, preventative vaccines to also include treatment of active OM, in addition to other diseases of the respiratory tract due to NTHI.

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SEVI BINDS TO AND ENHANCES PHAGOCYTOSIS OF BACTERIAL PATHOGENS

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The semen-derived enhancer of viral infection (SEVI), is a positively charged amyloid fibril found in semen derived from a proteolytic cleavage fragment of prostatic acid phosphatase (PAP248-286). It has been shown to facilitate HIV-1 transmission in vitro by increasing the rate of virion sedimentation and decreasing electrostatic repulsion between virions and target cells. However, the normal physiologic role for SEVI remains unclear. We hypothesize that SEVI may function as an antimicrobial agent, preventing semen-mediated transmission of pathogenic microorganisms. We further hypothesize that SEVI's antimicrobial activity may leverage its unique fibrillar structure, thus allowing it to complement the action of traditional, short cationic antimicrobial peptides, which insert themselves into microbial membranes. To test whether SEVI has immuno-defensive properties, we investigated its ability to bind to bacteria found in the reproductive tract. SEVI was found to bind directly to Staphylococcus aureus, Escherichia coli and Neisseria gonorrhoeae, whereas non-cationic SEVI fibrils (in which positively charged lysine and arginine residues were replaced by alanines) were defective for bacterial binding. Consistent with our hypothesis, SEVI did not directly inhibit bacterial growth in vitro. Rather, it promoted bacterial aggregation and significantly enhanced the phagocytosis of bound bacteria by primary human macrophages. SEVI also increased pro-inflammatory cytokine (TNF alpha) release from primary human macrophages that were exposed to bound bacteria. Our results point to a possible role for SEVI in the control of semen-borne bacterial pathogens.
Tuberculous meningitis (TBM) is a serious form of chronic infection of the Central Nervous System caused by Mycobacterium tuberculosis. The severity of the condition is highlighted from the fact that untreated TBM for more than four weeks is fatal. Further, early diagnosis and effective therapy is of utmost importance to contain the disease and to reduce sequelae. However, mortality and morbidity in children is still a matter of concern in both developed and developing countries. The present study highlights different approaches adopted at National Institute of Mental Health and Neurosciences, a tertiary Neurosciences Center, over the period. As the pathogen takes up to eight weeks to grow on LJ media and the direct microscopy is usually inconclusive, the onus lies mainly on Immunodiagnosis to get a clue about the disease. The other clinical condition of chronic meningitis which closely mimic the TBM are: cysticercal meningitis, fungal meningitis, carcinomatous meningitis, partially treated pyogenic meningitis and at times viral meningitis. On an average we analyze 1500-2000 CSF samples suspected for chronic meningitis per year. The laboratory diagnosis of TBM is mainly by detecting the pathogen, pathogen product or anti-pathogen antibodies. Culture of the pathogen is done on the LJ media, BACTEC method and Bactalert method. Indirect method of detecting the antigen is done by Immune complex method and antibody response is detected using indirect ELISA. Dot blot and western blot is also performed to confirm the immune response in few cases. All the above tests are done in the CSF samples of patients who are clinically, bacteriologically and radiologically suspected/confirmed to be cases of TBM. Age wise and sex wise analysis of cases over the years reflected the pattern of infection in different age groups and both the sexes mainly in the productive age group. It is observed that the immune response (upto 80%) is higher than culture results obtained for the pathogen in definite cases of TBM. Immune complex assay is an important aid in the immunodiagnosis. Western blotting helps in confirmation of the immune response. Culture of the pathogen helps in instances where atypical presentation of cases is noticed and in some cases where there is lack of immune response. Rapid culture and multimodal immunodiagnosis would both be important for the early case detection. 

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IS THE PHENOTYPIC DELAY IN DIFFERENTIATION OF BREAST CANCER PATIENTS’ MONOCYTES INTO DENDRITIC CELLS (MDCs) THE SOURCE OF FUNCTIONAL BIAS BY THE INDUCTION OF REGULATORY T CELLS?

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Dendritic cells (DCs) are unique professional antigen-presenting cells (APCs) adapted to initiate and coordinate adaptive immune responses and also crucial for the induction/maintenance of T cell tolerance even in the subversion of anti-tumor immunity.

Thereby, in this study we evaluated the pattern of T cell stimulation by monocytes (Mo), monocyte-derived immature DCs (Mo-iDCs) and monocyte-derived mature DCs (Mo-mDCs) from cancer patients, investigating the possible roles of TGF-beta and CD86 co-stimulatory molecule and aspects of the differentiation process.

Mo-iDCs were differentiated from breast cancer patients’ blood monocytes in presence of GM-CSF and IL-4 for seven days, TNF-alfa addition (day 5), was used to obtain Mo-mDCs. We evaluated phenotypically, by flow cytometry, the Mo to Mo-DC during the differentiation process. Mo and Mo-DCs were also co-cultured with CD4+CD25neg lymphocytes. Cell activation and de novo Tregs (CD4+CD25+Foxp3+) generation were analyzed after 6 days. In Mo-iDCs-T lymphocyte co-cultures, we tested the effects of monoclonal anti-TGF-beta antibodies upon lymphocyte stimulation by Mo-iDCs (FACS-sorted in CD86Low and CD86High subpopulations).

Interestingly, patients’ monocytes induced a significantly higher frequency of CD25 expression and a lower Treg frequency, with higher TNF-alfa and IFN-gamma levels. Surprisingly, we found no differences between Mo-iDCs and Mo-mDCs in Tregs induction or lymphocyte activation. Mo-iDCs FACS-sorting showed that, when compared to CD86Low, CD86High Mo-iDCs induced a higher frequency of CD25 lymphocytes, but also a higher number of Tregs. Antibody blocking of TGF-beta in unsorted Mo-iDCs – T cells co-cultures, caused a 50% decrease in Treg frequency, an effect that was not noted in sorted CD86High Mo-iDCs-T cells co-cultures. Furthermore, during patient’s Mo to Mo-iDC differentiation, firstly, a lower number of gated cells (SSC x FSC) were observed. Secondly, our data suggested about 24hs of delay in CD14 loss of expression which could be reflected on the retardment of CD80, CD86 and PD-L1 molecules. Still, patient’s cells always expressed higher levels of HLA-DR and, also, different levels of CD86Low and CD86High on PD-L1+ cells. Those apparent bias needs to be considered on the effectiveness of cancer immunotherapy based on patients’ Mo-DCs.
NUCLEOPROTEIN NANOSTRUCTURES: A MUCOSAL SUBUNIT VACCINE CANDIDATE FOR NEONATES PROTECTIVE AGAINST THE RESPIRATORY SYNCYTIAL VIRUS.

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There is still no licensed vaccine against human respiratory syncytial virus (RSV) which causes severe bronchiolitis in young infants. The nucleoprotein N, a major component of the RSV nucleocapsid, is remarkably conserved among RSV subtypes and is recognized as a target of protective T cell responses. We reported a method to produce recombinant N assembling in homogenous rings composed of 10-11 N subunits\(^a\). Intranasal vaccination of adult BALB/c mice with N-rings and a detoxified E.coli enterotoxin\(^b\) as adjuvant proved protective against an RSV challenge, without causing adverse lung inflammatory reactions\(^c\).

In the present study, we evaluated the vaccine potential of N-rings in 5 to 7 day-old BALB/c pups: a single intranasal administration of N-rings with LT(R192G) provided a significant reduction of the viral load after an RSV challenge at five weeks of age. However, neonatal vaccination also generated an enhanced lung infiltration by polynuclear leucocytes following the RSV challenge. Analysis of antibody subclasses and cytokines produced after an RSV challenge or a boost administration of the vaccine suggested that neonatal vaccination induced a long lasting but Th2 biased local immune memory. Adding CpG-ODN to the vaccine formulation significantly reduced the Th2 bias, without impeding protection. In conclusion, protective vaccination against RSV can be achieve in neonates but requires an appropriate combination of adjuvants.

\(^a\) Tran et al., J. Gen. Virol 2007

\(^b\) LT(R192G), kindly provided by J. Clements (Tulane University, USA).

\(^c\) Roux et al., PLoS ONE 2008
TUMOR CELLS INCORPORATE EXOSOME-CARRIED MOLECULES DERIVED FROM MATURE DENDRITIC CELLS AND BECOME SUSCEPTIBLE TO T CELL CYTOTOXICITY

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Exosomes (Exo) are secreted nanovesicles that result from the fusion of multivesicular bodies with the plasma membrane. They contain membrane proteins and genetic material which can be transported to other cells, thus contributing to intercellular communication in the body. Exo originated from dendritic cells (DCs) are able to induce direct and indirect lymphocyte responses and can induce antitumor responses in mice. This work was designed to evaluate the possibility of using DC-derived Exo to convert tumor cells into immunogenic cells. Mature DCs (mDCs) were differentiated from healthy donors’ blood monocytes by culture, for seven days, in presence of GM-CSF, IL-4, and, in the last 2 days, TNF-\(\alpha\). mDCs culture supernatant was cleared from cells and was submitted to ultracentrifugation for the isolation of nanovesicles (dEx), which were characterized by flow cytometry for the expression of typical DC and dEx markers. dEx were added to cultures of the human breast adenocarcinoma cell line, SK-BR-3, which were evaluated by flow cytometry and used as target cells in a cell cytotoxicity assay. All different dEx preparations carried HLA-ABC, CD86, CD11c, CD81 and CD18. HLA-DR and CD54 were present in some preparations, but not in others. Exo treated (60-130 \(\mu\)g/106 cells) SK-BR-3 tumor cells expressed class I and class II HLA molecules, CD18, CD80, CD86 and CD83 (all molecules carried by the dEx and absent on non-treated SK-BR-3 cells). The highest level of dEx-carried molecules’ detection was observed at 6-8 hour after treatment with dEx, when up to 69% of the cells reacted with class II specific antibodies. Transfer of dEx-carried molecules to tumor cells was confirmed by fluorescence microscopy. In the cell cytotoxicity assay, treatment of tumor cells with dEx derived from BSA-treated DCs, increased their susceptibility to killing by in vitro BSA-primed CD3+ lymphocytes. These results are consistent with the potential of DC-derived Exo to affect tumor cells’ surface molecule expression, thus possibly transforming non-immunogenic cells into immunogenic tumor cells. Financial support: FAPESP #04/09956-0; 07/58597-1; 09/54599-5; CNPq #303731/2007-9
ANALYSIS OF HUMAN ANTIBODY REPERTOIRES IN HEALTHY INDIVIDUALS

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Antibodies are a major part of the immune system and consist of immunoglobulin heavy and light chains, which are encoded by rearranged genes assembled from subsets of V (variable), D (diversity, only heavy chains) and J (joining) gene segments. Antibody repertoire diversity, potentially of 108 or more unique molecules in a single individual, build the basis of antibody-based antigen recognition and protection. The diversity of antibody repertoires is further increased by somatic recombination and hypermutation to achieve high specificity and selectivity in the detection of foreign antigens.

To obtain a deeper insight into the nature of human antibody repertoires, we developed an unbiased pyrosequencing method on a Roche Genome Sequencer FLX System yielding and compare large sets of rearranged antibody sequences. We acquired V(D)J recombination patterns of heavy and light chains of all immunoglobulin classes and subclasses in 14 healthy individuals of different age and gender. To handle these datasets, engineering of a new database is ongoing, which will hold all collected sequence information as well as cover specific questions about antibody repertoires. We envisage that our study will conclude in new ways for sequencing and analysis of antibody repertoires in humans.
CHARACTERIZATION OF LEUKOCYTE INFLAMMATORY RESPONSES TO TOLL LIKE RECEPTOR (TLR) 7, 8 AND 9 LIGANDS

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TLRs are a major class of pattern recognition receptors (PRRs) that recognize conserved microbial structures, known as pathogen-associated molecular patterns (PAMPs). PAMPs include microbial derived proteins, lipids, as well as nucleic acids. For example, ligation of TLRs 7, 8 or 9 using ssRNA (TLR7/8) or ssDNA (TLR9) can induce cytokine production, co-stimulatory molecule upregulation and leukocyte differentiation. TLRs can also recognize endogenous ligands and there is evidence that signaling via these innate immune receptors contributes to autoimmune disease pathology. For instance, TLR7 and 9 pathways can be activated by endogenous nucleic acids (in the form of immune complexes) which are implicated in initiating and prolonging pathology in systemic lupus erythematosus (SLE). In murine models of SLE, both peritoneal macrophages and B-1 cells have been suggested to contribute to disease pathology; however there is little information on the outcome of TLR7/9 ligation on these cell types. Consequently we sought to characterize the responses of peritoneal leukocytes (and splenocytes) to TLR7 and 9 ligands. Using peritoneal leukocytes and splenocytes from naïve animals we observed that ssRNA and R848 (TLR7 pathway agonist) stimulated increased levels of pro-inflammatory cytokines such as IL-6, IL-8, IL-12p70 and TNF alpha relative to TLR9 (CpG1826 oligodinucleotide) stimulation. In addition, ssRNA induced expression of so-called IFN-inducible genes Mx-1, Ifi-1 and Oas-1 despite minimal induction of IFN-alpha. In vivo we could detect type I IFNs, IP-10 and IL-6 in sera following intravenous injection of TLR7 and 9 ligands. Curiously, there was minimal induction of type I IFN mRNA in ex vivo blood leukocytes. We are currently in the process of evaluating the TLR7/9 dependence of cytokine production and IFN-inducible genes as well as attempting to characterize the populations of cytokine producing cells in vivo. Collectively this data will contribute to our understanding of the effects of TLR7/9 ligation on leukocyte effector function.
Sarcoidosis is a chronic inflammatory disorder of unknown etiology affecting barrier organs like lung, skin, and eyes. Its similarities to infectious granulomatous diseases suggest a major role for inciting microbial triggers. The presence of activated macrophages and the expansion of oligoclonal T cells and B cells suggest a sustained activation of inflammatory signaling pathways or a lack of negative feedback regulation in this disease. Lungs are exposed to toxins and microbial products and are affected in sarcoidosis and lung involvement is the leading cause of morbidity and mortality in this disease.

We have shown that ex vivo cultured sarcoid bronchoalveolar cells (BAL cells) exhibit a sustained activation of p38 MAPK at basal level and in response to both nod like receptor 1 (NOD) -1 and toll like receptor (TLR)-4 ligands. Sustained p38 activation was associated with a significantly higher expression of inflammatory cytokines including IL-1β, TNF-α and IL-6 at baseline and in response to both ligands as compared to healthy controls.

Ligand mediated TLR activation, including TLR4, requires receptor dimerization and recruitment of several adaptor molecules leading to activation of a kinase cascade with subsequent activation of p38 and expression of proinflammatory cytokines. Interleukin-1 receptor associated kinase (IRAK)-1 interacts with MyD88 and is upstream of a kinase cascade whose activation subsequently leads to MAPK activation, including p38 and NF-κ B, followed by production of inflammatory mediators. We tested the hypothesis that aberrant regulation upstream to the p38 pathway plays a role in the pathogenesis of sarcoidosis. Using an ex vivo culture model of human BAL cells, we assessed involvement of IRAK-1 and TAK1-TAB1 in activation of p38 in BAL cells of patients with sarcoidosis.

Our data indicate that sustained p38 phosphorylation is associated with phosphorylation of IRAK-1 in the absence of ligands. BAL cells of sarcoid subjects responded to NOD-1 and TLR-4 stimulation with enhanced and prolonged IRAK-1 phosphorylation. Such activation was associated with MKK4 activation but not MKK3/6 activation. These results suggest that sarcoidosis is associated with constitutive activation of a IRAK1/MKK4/p38 signaling pathway, which may lead to aberrant production of pro-inflammatory molecules and disease.
The epithelial intestinal barrier is an essential boundary for the prevention of mucosal inflammation in response to luminal stimuli and for the maintenance of mucosal homeostasis. Dysregulation of intestinal symbiosis and concomitant aberrant activation of mucosal innate and adaptive immunity can result in gut injury, inflammation, and inflammatory bowel disease (IBD) including ulcerative colitis and Crohn’s disease. However, the fundamental pathophysiologic mechanisms underlying gut inflammation and IBD are still unclear. Interactions between B7 family members and their CD28 family receptors underlie the costimulatory and coinhibitory signals that critically regulate T cell function. In addition to the long-established pathways between the B7-1/B7-2 ligands and CD28/CTLA-4 receptors, the pathway of the B7 family member B7-H1 (PD-L1) and its receptor the CD28 family member PD-1 has a major role in inhibiting T cell responses as well as inducing CD8 T cell exhaustion in chronic viral infection. B7-H1 is mainly expressed on immune cells and PD-1 is expressed on activated T cells, therefore research on this pathway has focused on T cell coinhibition. The role of the B7-H1/PD-1 pathway in intestinal inflammation and IBD remains largely unknown. To a lesser extent, B7-H1 protein can be detected on some tissue cells, but the in vivo function of these tissue-expressed B7-H1 is largely unexplored.

Here we show that both human and murine intestinal epithelium express B7-H1. Mice deficient for B7-H1 were highly susceptible to dextran sodium sulfate (DSS)- or trinitrobenzenesulfonic acid (TNBS)- induced gut injury. B7-H1 deficiency led to high mortality and morbidity which were associated with severe pathological changes in the colon, loss of epithelial integrity, and systemic dissemination of commensal bacteria during intestinal inflammation. Furthermore, using bone marrow chimera mice, we found that B7-H1 expressed on intestinal epithelium, but not on hematopoietic cells, controlled intestinal inflammation. Finally, we revealed that the protective function of B7-H1 in intestinal inflammation and colitis was not dependent on PD-1, B7-1, or adaptive immunity. Thus, we have identified a novel pathway in which B7-H1 expressed on intestinal epithelial cells critically controls intestinal inflammation.
HUMAN TOLLIP REGULATES TLR2 AND TLR4 SIGNALLING AND
ITS POLYMORPHISMS ARE ASSOCIATED WITH SUSCEPTIBILITY
TO TUBERCULOSIS

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Introduction
Tuberculosis, one of the leading causes of death worldwide, stimulates
inflammatory responses with beneficial and pathologic consequences. The
regulation and nature of an optimal inflammatory response to Mycobacterium
tuberculosis (MTb) remain poorly understood in humans. Insight into
mechanisms of negative regulation of the Toll-like receptor (TLR)-mediated
innate immune response to MTb could provide significant breakthroughs in the
design of new vaccines and drugs for tuberculosis. We hypothesized that
TOLLIP and its common variants negatively regulate TLR signaling in human
monocytes and are associated with susceptibility to tuberculosis.

Methods and Results
To determine the function of TOLLIP in human peripheral blood monocytes,
we performed lentiviral-mediated knockdown of TOLLIP, then stimulated these
cells with TLR2 and TLR4 agonists. We found that TOLLIP negatively
regulated TNF and IL-6 production after stimulation with TLR2 and TLR4
ligands. In contrast, secretion of the anti-inflammatory cytokine IL-10 was
positively regulated by TOLLIP. In addition, we examined the TOLLIP mRNA
expression as well as cytokine responses of 54 healthy individuals to several
TLR ligands and stratified these results by TOLLIP single nucleotide
polymorphisms. We discovered 2 common polymorphisms that are associated
with either decreased levels of mRNA expression (rs3750920) or increased IL-6
production (rs5743899). Furthermore, in a case-population study in Vietnam
with 760 cord-blood samples and 671 TB patients with tuberculosis we found
that SNPs rs3750920 and rs5743899 were associated with susceptibility to
tuberculosis (p=7.32x10⁻⁵, 2.13x10⁻⁵, respectively).

Conclusions
Together, these data demonstrate that TOLLIP has an anti-inflammatory effect
on TLR signaling in humans and that TOLLIP deficiency is associated with an
increased risk of TB. To our knowledge, these data also show the first
associations of TOLLIP polymorphisms with any infectious disease. These
experiments also illustrate an unexpected mechanism of negative regulation of
TLR signaling in human TB pathogenesis.
ALLERGEN-ASSOCIATED DANGER SIGNALS AND RECEPTORS: NADPH OXIDASE ACTIVITY, TOLL-LIKE RECEPTOR 4 AND ITS ADAPTOR TRIF ARE NOT NECESSARY FOR MUCOSAL SENSITIZATION TO POLLEN

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Oxidative stress in allergic asthma may result from pollen-intrinsic oxidase activity or pro-inflammatory molecules and has been associated with activation of the toll-like receptor 4 (TLR4). Recently, signaling via TLR4 and its adaptor TRIF has been implicated in reactive oxygen species-mediated promotion of T helper 2 immune responses. We investigated whether oxidative stress and TLR4-TRIF signaling are critical in mediating experimental asthma induced by birch pollen exposure exclusively via the airways. Mice were sensitized by intratracheal administration of white birch pollen extract and challenged two weeks later in the same manner, or received heat-inactivated pollen extract only during sensitization or challenge. Mice were injected intraperitoneally prior to allergen exposure with the antioxidant N-acetyl-L-cysteine (NAC). Alternatively, TLR4 signaling was antagonized concomitantly with allergen exposure via the intratracheal route, or the development of allergic airway disease was evaluated in TLR4 or TRIF knockout mice. NAC inhibited inflammation and airway hyperresponsiveness (AHR) induced by airway exposure to birch pollen, even when administered only with the allergen challenge, but not when given exclusively during sensitization. Heat-inactivation of the pollen extract had no effect, either in sensitization or challenge, on the later development of allergic airway disease. TLR4 signaling substantially accounted for the birch pollen-induced airway inflammation and Th2 response, but oxidative stress-mediated AHR was TLR4- and TRIF-independent. Thus, oxidative stress does not critically impact allergic sensitization, but promotes airway disease to inhaled birch pollen, specifically AHR, independently of the pollen-intrinsic NADPH oxidase activity and TLR4-TRIF signaling. TLR4 is not necessary for sensitization and contributes only to pollen-induced airway inflammation. Overall, antioxidants may serve to counter pollen-induced disease-promoting mechanisms that cause airway dysfunction, but not the underlying disease-inducing factors, and TLR4 antagonism may be less effective as a therapeutic for pollen-induced airway disease.

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TLR4-TRIF SIGNALING PROMOTES T-CELL AND ICOS-DEPENDENT INHIBITION OF MURINE ALLERGEN-INDUCED AIRWAY HYPERRESPONSIVENESS

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Modulation of adaptive immune responses via the innate immune pattern recognition receptors, such as the Toll-like Receptors (TLRs), is an emerging strategy for vaccine development. Thus, defining the conditions and mechanisms by which these receptors can regulate disease in a safe manner is paramount. We have previously determined that nasal application prior to allergen challenge of Protollin, a mucosal adjuvant that is safe and well-tolerated in humans and composed of TLR2 and 4 ligands, is sufficient to elicit protective immunity in mice against allergic lower airway disease, characterized by reduced airway hyperresponsiveness (AHR), inflammation, T helper (Th)2 cell cytokines (interleukin (IL)-4 and IL-5) and serum IgE levels, with reduced inflammatory side effects compared to intrapulmonary administration. Protollin’s inhibition of experimental asthma is retained in Trlr2 -/- or MyD88 -/- mice, but not in Trlr4 -/- mice. It is not associated with Th1 or Treg responses in the respiratory mucosa, but rather an induction of ICOS expression in the nasal mucosa and on CD4+ T cells of the draining lymph nodes, and apparent recruitment of these cells to the lungs. We aimed to examine the role of TRIF signaling and the induction of ICOS in mediating this inhibition of allergic airway disease. Protollin failed to inhibit AHR and BAL eosinophilia in Trif -/- mice, and the percentage of CD4+ICOS+ cells induced by Protollin in lymph nodes and lungs of Trif -/- mice was significantly lower than in wild-type (WT) mice. In vivo neutralization of ICOS using monoclonal antibodies attenuated the inhibition of AHR by Protollin in WT mice and adoptive transfer of FACs-sorted CD4+ICOS+ or CD4+ICOS- cells from Protollin-treated WT mice to Trif -/- mice prior to allergen challenge, rescued the inhibition of AHR. The phenotype of these cells and its dependence on the TLR4-TRIF pathway is under investigation. We demonstrate a TLR4-TRIF-dependent inhibition of lower airway allergic disease mediated via the nasal mucosa that is at least partially dependent on the induction of ICOS and recruitment of CD4+ICOS+ cells to the lungs.

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IMMUNOLOGICAL ABNORMALITIES IN FATIGUE-RELATED ILLNESS.

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Background
Emerging infections and post-infection immune sequelae are of increasing importance in the practice of public health medicine as vaccination strategies need to consider novel immunological pathways. Seriously disabled patients suffering post-infection fatigue-related illness were examined for a range of immunological abnormalities possibly associated with vasoactive neuropeptide (VN) dysfunction. VNs control key neurotrophic and neuroprotective pathways associated with infection and immune responses and regulate adenylate cyclase (AC) action on ATP to produce cAMP. cAMP is regulated by phosphodiesterase enzymes (PDEs) as well as influencing the cyclic nucleotide response element modulator (CREM) family including the cyclic nucleotide response element binding protein (CREB) and inducible cAMP early repressor (ICER). ICER is a natural antagonist to CREB and has a fundamental role in cAMP-associated neuronal plasticity. While intended to function as a modifier of the ‘signal to noise ratio’ of cAMP, ICER may suppress weak signals thus exacerbating poor cAMP function. CREB signalling has been noted to inducing changes in synaptic plasticity that mediates the conversion of short-term memory to long-term memory. CREB signalling has been recently involved in several brain pathological conditions including cognitive and neurodegenerative disorders. PACAP/VIP are potent VNs and induce long-lasting neuroprotection through the induction of activity-dependent signalling via CREB supporting the critical role of CREB in neuroprotection.

Aim
This poster discusses dysregulation of some key metabolic and immunological pathways possibly implicated in the pathogenesis of fatigue-related illness. We investigated dysregulation of cAMP metabolism as indicated by disturbances of Foxp3, microRNA, adenylate cyclase (AC), CREB and ICER. As AC regulation is controlled in part by vasoactive neuropeptides of the PACAP/VIP family, the roles of these VNs in immune dysregulation strategies are discussed in further detail.

Results
Foxp3 and VPAC2R dysregulation and significant micro RNA anomalies appear evident in our study. Our results also suggest Th1 and Th2 dysregulation with impaired capacity to counter immunological activation.

Conclusions
Effects of this immunological dysregulation may be severe with significant impact on patients’ lives. The challenge is now to interpret these abnormalities in the context of clinically perplexing fatigue-related disorders. The role of infection in exacerbating adverse immune responses and clinical presentation in patients with fatigue-related illness requires further research.
West Nile Virus (WNV) is the most common cause of epidemic meningoencephalitis and overall case fatality rates in recent epidemics were highly increased. WNV has been categorized as an emerging pathogen due to its high prevalence, ability to cause severe disease in humans, and the absence of effective treatments and vaccines. Using mice model of WNV infection, we demonstrate that blocking the functions of a neuronal guidance factor Semaphorin 7A (Sema7A) by genetic manipulation or antibody blockade protected mice from lethal WNV infection. Sema7A deficient mice were more resistant to WNV infection and showed reduced viral loads and pathogenesis in comparison to the controls. Also, the mRNA levels of TNF-α, IL-6, IL-12, IFN-α and IFN-β cytokines were significantly decreased in brain tissue of Sema7A-deficient mice. Sema7A antibody-treated mice showed substantial resistance to the lethality caused by WNV than the IgG Isotype control antibody-treated mice. Mouse cortical neurons, human macrophages and mouse cerebrospinal microvascular endothelial in vitro cell line showed significant resistance to WNV infection upon treatment with Sema7A antibody suggesting that abrogating Sema7A function reduces WNV infection and pathogenesis. Consistent with these results, we also found that both the mRNA and proteins levels of Sema7A were dramatically increased during the course of WNV infection. Furthermore, we show that overexpression of Sema7A-Fc fusion protein increased viral burden in 293T cell line. Our studies suggest that Sema7A mediates viral infection and pathogenesis by modulation of the host signaling pathways that promote inflammatory or apoptosis signals and enhance neuronal cell death by hampering long-term immunity to West Nile pathogenesis. Overall, our studies not only lead to a greater understanding of the pathogenesis of WNV, but also show that abrogating the function of host molecules that connect both immune and nervous system such as Sema7A could be an effective therapeutic treatment against West Nile disease.
DISTINCT ROLES FOR CXCR6+ AND CXCR6- CD4+ T CELLS IN PATHOGENESIS OF CHRONIC COLITIS

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Inflammatory bowel diseases (IBDs), including Crohn’s disease (CD) and ulcerative colitis, are chronic and relapsing inflammatory disorder of the gastrointestinal tract. Studies of IBDs in mice and human implicate dysregulation of CD4+ T-cell subsets in the pathogenesis of IBDs. These colitogenic CD4+ T cells vigorously produce IFN-γ and IL-17A. We recently found that colitogenic CD4+ T cells are divided into two subgroups by expression of CXCR6, the sole receptor for CXCL16 that is upregulated in the inflamed colon of patients with CD and a mouse colitis model induced by adoptive transfer of CD45RBhiCD4+ T cells into Rag1-/- mice. In the mouse colitis model, 80% of colonic T cells expressed CXCR6. Although both CXCR6+ and CXCR6- populations exhibited an effector memory (TEM) phenotype (CD44hiCD62L-CD27-CD43+), only CXCR6+ population abundantly produced IFN-γ and IL-17A, suggesting a vital role of CXCR6+ population in induction of colonic inflammatory response. Nevertheless, retransfer of colonic CXCR6+ population from the colitic donors into Rag1-/- recipients failed to reproduce the disease. In contrast, the retransfer of CXCR6- population gave rise to colitis similar to the CD45RBhi-transferred model, with their phenotypic change into CXCR6+ Th1 and Th17 cells in colonic lamina propria. These data suggest that CXCR6+CD4+ T cells function as bona fide effector T cells, whereas the CXCR6- counterpart could rather possess the effector phenotype to be responsible for persistent and/or recurrent inflammatory response in the colon by supplying CXCR6+ population.
Differential expression of “Suppressors of Cytokine Signaling” (SOCS) in Monocyte-Derived Dendritic Cells from Breast Cancer Patients

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Dendritic cells (DCs) are professional antigen-presenting cells, crucial for the initiation and maintenance of immune response. Not surprisingly, they are important targets of tumor escape mechanisms from immune control. In cancer patients, both DCs and their precursors have phenotypic and functional defects. However, the mechanisms responsible for these deviations are still poorly understood. It’s known that DCs differentiation and maturation depend on cytokines, whose signaling pathways are critically regulated by the suppressors of cytokine signaling proteins (CisH, SOCS1 to SOCS7). This work, therefore, aimed to evaluate SOCS expression during the differentiation of monocyte-derived DCs (Mo-DCs) from breast cancer patients, comparing it to that of healthy donors. For DC differentiation, peripheral blood mononuclear cells CD14+ cells were isolated from healthy donors and from cancer patients by magnetic beads and cultured in the presence of IL-4 and GM-CSF for 7 days. The expression levels of SOCS were assessed by quantitative PCR. We, first, studied the gene expression level of various SOCS members during DC differentiation from healthy donors’ cells, at 30 minutes, 2 and 5 hours after IL-4 and GM-CSF treatment. For the SOCS2, SOCS3, SOCS4 and CisH we found increased expression levels of mRNA 2 hours after cytokines' treatment. Based on these results, we decided to study, initially, SOCS expression in patients’ cells, at that same moment. Our results showed that SOCS3 mRNA levels increased less (2-fold) in cancer patients’ cells than in healthy controls (8-fold). For SOCS2, we observed a 3-fold increase in mRNA expression in healthy donor cells but none in patients’ cells during DC differentiation. SOCS4 mRNA expression was low both in controls and in patients’ cells (with mRNA basal levels in cancer patients’ cells 2-fold lower than in controls). SOCS5 mRNA expression was 20 to 25-fold higher in patients’ cells (already in monocytes), whereas SOCS6, which had low mRNA baselines in controls' and patients' cells, increased 20-fold only in patients cell, after treatment with cytokines. These results confirm that SOCS' expression is altered in cancer patients’ Mo-DCs, suggesting that their kinetics of expression may contribute to the functional deficits of these cells.

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DIFFERENT GENE EXPRESSION SIGNATURE AND B CELL MEMORY INDUCTION IN UGANDAN, EUROPEAN AND NORTH AMERICAN ADULTS RECEIVING THE YELLOW FEVER VACCINE FOR THE FIRST OR SECOND TIME

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The yellow fever vaccine induces a very efficient immune protection to viral infection and has been recently used to determine in North American adults a gene expression signature that highlights the features of protective immune responses using a systems biology approach. This immune response involves the coordinated induction of several effector arms of innate immunity as well as adaptive immunity. Yellow fever vaccination induces highly variable titers of neutralizing antibodies (NAbs) that can persist for up to 35 years. However, it is still unknown if the same predictors of vaccine efficacy and memory induction would be applicable to different ethnic groups. We have analyzed the gene expression signatures and quantified NAb titers in individuals receiving a first or second dose of YF-17D vaccine in Uganda, Europe and North America. Even if we observed a common innate response profile in the three cohorts with highly similar top genes modulated 3 and 7 days after vaccination, we showed a reduced, delayed and qualitatively different innate immune response in Uganda compared to Europe and North America. In the European cohort, the NAb levels detected 10 years after vaccination were similar to the levels induced one year after a first vaccination. Strikingly, in the Ugandan cohort, low NAb titers were detectable 6 years after vaccination but not after longer period of time and the vaccine boost resulted in an increase in Nab titers. These differences in NAb responses in Ugandan adults were correlated with specific gene expression innate pathways. Our data suggest that the yellow fever vaccination induces a different innate immune response and generates a less persistent memory B cell response in Uganda. Understanding the mechanisms by which live attenuated vaccines induce an effective protection will provide a benchmark for the design of novel vaccines.
ENHANCED IMMUNOGENICITY OF MHC CLASS I-RESTRICTED TUMOR-ASSOCIATED ALTERED PEPTIDE LIGANDS

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We have recently defined a peptide modification that allows the design of highly immunogenic 'super-peptides' with an increased capacity to stabilize the murine major histocompatibility class I complexes (MHC-I) H-2Db and H-2Kb. Importantly, substitution of peptide position 3 to a proline (p3P) does not alter the peptide conformation when compared to their native infection-derived or non-immunogenic tumor-associated counterparts [1]. The increased immunogenicity therefore leads to enhanced T cell responses against native MHC-I/peptide complexes on target cells. We believe that the observed effects are based on enhanced interactions of the modified peptides with the heavy chain tyrosine residue Y159 that is conserved among most known MHC-I molecules. Stability measurements of H-2Db-Y159 mutants combined with crystallographic analysis demonstrated the importance of the interaction of p3P with the aromatic ring of Y159 for MHC-I stabilization and T cell activation. A soluble T cell receptor specific for H-2Db in complex with the melanoma-associated model peptide gp100 has been produced and Surface Plasmon Resonance measurements indicate a direct connection between the MHC-I/peptide complex stability and the in-vitro affinity of the T-cell receptor to the complex. The functional characterization of the capacity of H-2Db-Y159 mutants to activate T-cells has been initiated.

Importantly, our aim is to translate these promising results into several HLA-A2-restricted multiple myeloma and melanoma tumor associated antigens (TAA), in order to generate structurally conserved altered peptide ligands that enhance T cell responses against tumor cells. Using in-crystallo peptide exchange the three dimensional structures of HLA-A2 in complex with the multiple myeloma-associated peptide FR20 has been determined, providing a structural platform for the design of novel variants with increased stabilization capacity and higher immunogenicity. The characterization of the ability of HLA-A2/FR20 variants to expand tumor-reactive lymphocytes is ongoing.

A major goal of vaccine research is the development of adjuvants that induce rapid and sustained mucosal immunity without local or systemic "reactogenicity". Here we describe TLR7 activating nanoparticles with these properties. UC-1V270 consists of a purine-like TLR7 activating pharmacophore covalently linked to two C-12 phospholipid side chains. In aqueous solution, the drug self-assembled into ~120 nanometer particles, while in formulations containing lecithin and propylene glycol, it was incorporated into 140-180 nanometer particles that were stable for at least three weeks at room temperature. Intranasal administration of the TLR7 activating nanoparticles to mice induced intrapulmonary cytokine production, without apparent pneumonitis and with only minimal release of cytokines into the systemic circulation. The adjuvant effects of the nanoparticles were tested in a mouse model of pulmonary anthrax. Fifty percent of mice that received a single intranasal vaccination with irradiated spores (IRS), dispersed with the TLR7 activating nanoparticles, survived a normally lethal Bacillus anthracis challenge. Furthermore, complete protection from lethal anthrax infection was observed in mice that received 3 intranasal vaccinations with the TLR7 activating nanoparticles, at two-week intervals. In contrast, mice vaccinated with IRS alone all succumbed within three to six days of Bacillus anthracis exposure. High levels of antigen specific interferon gamma and interleukin 17 were released by IRS-pulsed splenocytes from the vaccinated mice, accompanied by expansion of a distinct population of T_h17 cells. The essential role of these two cytokines in the adjuvanted vaccine action was confirmed by in vivo depletion studies with interleukin 17 and interferon gamma specific antibodies. Collectively, these experiments indicate that the TLR7 nanoparticles applied to mucosal surfaces can potently enhance both T_h1 and T_h17 immune responses to antigens.

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The ability of the immune system to respond to vaccinations, such as that for influenza, declines with age. This is an important clinical problem since the elderly are often targeted for vaccination. While this decline is most likely due to a multitude of changes in the aging immune system, we have focused on age-related changes in naive CD4 T cell function and how these contribute to reductions in humoral responses following vaccination. To accomplish this, we have developed an adoptive transfer model system that permits us to explore specific questions that remain unanswered in this field. By transferring naive CD4 T cells from young or aged T cell receptor transgenic mice into hosts lacking endogenous CD4 T cells, we can easily assess the in vivo function of the donor cells following vaccination. Our studies have revealed three important points regarding CD4 T cells and humoral immune responses with aging: (1) There are intrinsic age-related defects in T cell function that lead to reduced B cell expansion, differentiation, IgG production and affinity maturation of antibodies; (2) These intrinsic defects in CD4 T cell function are the result of the persistence of the cells in the periphery as mice age, which is mainly the result of reduced thymic output; (3) These intrinsic defects can be overcome by the use of proinflammatory adjuvants which enhance the in vivo helper function of aged CD4 T cells and allow them to provide robust help for a humoral response following vaccination.
HOST GALECTINS INFLUENCE IMMUNITY AND IMMUNOPATHOLOGY TO VIRUS INFECTIONS

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It is characteristics of infections that the outcome is not uniform. Moreover, the execution of both host innate and adaptive responses to infection may cause notable tissue damage when acting to remove the infecting agent. The host has multiple events that function to minimize the extent of tissue damage but the downside of some of these counter-inflammatory effects can be impairment of protective immunity. We shall discuss the examples wherein the normal host galectin-9 response to both herpes simplex virus (HSV) and influenza can result in diminished immunity and impaired protection from challenge. These events are explained by a reduced acute and memory CD8 and CD4 T cell response in wild type animals compared to animals unable to produce galectin-9 because of gene knockout or given the sugar lactose which competes with gal-9 for binding to the TIM-3 receptor. The latter is upregulated on activated T cells with its binding to Gal-9 resulting in apoptosis of some, but not all, T cell subsets. With influenza, animals unable to produce gal-9 also had more rapid and elevated antibody responses. This was explained in part by shifting the kinetic pattern of the B cell response towards extrafollicular B cells in gal-9 knock out animals.

The normal galectin 9 response does benefit the host in situations where the immune responses to viruses results in tissue damage or is needed to help maintain homeostasis during viral infections. We shall describe the situation in the case of ocular infection with HSV where the virus induces a chronic blinding immune-inflammatory reaction orchestrated by CD4 T cells (stromal keratitis). We demonstrate in this situation gal-9/Tim-3 interaction plays a protective role and that inhibiting the interaction results in more severe disease. The mechanisms involved were multiple and included the apoptosis of proinflammatory effectors, the expansion of regulatory T cells as well as effects on VEGF production and the elicitation of corneal neovascularization. We shall also describe the relevance of the normal gal9/Tim3 reaction to the maintenance of the herpes viral latency and demonstrate that in the absence of gal-9 the stability of viral latency is increased.
Yearly immunization is the preferred method to protect against seasonal influenza, a major cause of death and hospitalization in the elderly population. The influenza vaccine, however, only provides 40-60% efficacy in people over 65 years old. This low efficacy results from low antibody titers with poor neutralizing activity. CD4+ T cells are critical for germinal center formation, which is required for the generation of high affinity antibodies by B cells. Using model antigens such as ovalbumin, our previous work showed that CD4+ T cells from old mice (>20 months) acquire age-associated defects that impair their cognate helper functions. In the present studies, we aimed to determine whether defects in the CD4+ T cell response to influenza immunization in aged mice is responsible for the poor vaccine efficacy. To do so, we vaccinated young (2-3 months) and aged (>20 months) Balb/c mice i.m. with 10^6 EID50 heat-inactivated influenza A/PR/8/34 (H1N1), a mouse adapted strain. The CD4+ T cell response in the draining lymph nodes (dLNs) was then evaluated by flow cytometry, while the non-dLNs were used as negative controls (baseline). Our data show that at least 2 times fewer CD4+ T cells express the T follicular helper (Tfh) markers CXCR5 and PD-1 in the dLNs of aged mice than in young mice at all time points assessed. The accumulation of these CXCR5+PD-1+CD4+ T cells was also delayed (starting about 2 days later) and more transient in the aged mice, with numbers back to baseline level by day 11 post-immunization while the number of CXCR5+PD-1+CD4+ was still ~5 times higher than baseline by day 15 post-immunization in young mice. Similar results were obtained when evaluating the generation of germinal center B cells, suggesting that CD4+ T cells also had impaired cognate helper functions in the aged animals. This resulted in lower PR8-specific antibody and lower neutralizing antibody titers in the aged mice compared to the titers measured in young mice. Following the addition of an adjuvant, such as the toll-like receptor 3 agonist poly(I:C), the proportion of CD4+ T cells expressing CXCR5 and PD-1, as well as the proportion of germinal center B cells, increased significantly in both young and aged mice dLNs. This leads to higher PR8-specific antibody titers in both young and aged mice.

In summary, our data strongly support our hypothesis that the impairment of CD4+ T cells functions with aging is a leading cause of the defective humoral response to influenza immunization in the elderly. The addition of adjuvants improves vaccine efficacy partly by improving CD4+ T cell functions.
The transcription factor Blimp-1 is widely expressed and mediates the terminal differentiation of many cell types including B and T cells. Recently, we identified a homologue of Blimp-1 in human effector CD8 T cells that we have named Hobit for Homologue of Blimp-1 in T cells. In contrast to humans, Hobit is specifically expressed at high levels in thymic and peripheral NKT cells in mice, but not in other T cell subsets or B cells under homeostatic conditions. To analyze the role of Hobit in NKT cell development and function, Hobit KO mice were generated. NKT cell development was not perturbed in Hobit KO mice except for a defect in a thymic population of mature NKT cells defined by expression of NK1.1, CD44 and Ly49C/I. Hobit regulated the terminal differentiation of peripheral NKT cells including the acquisition of NK1.1 expression and liver-directed migration. Glycolipids presented by CD1d molecules as well as inflammatory cytokines play a crucial role in the activation of NKT cells. We found that antigenic stimulation using α-GalCer resulted in rapid down-regulation of Hobit that acted as a repressor of IFN-γ expression and NKT cell expansion. In contrast, upon innate stimulation with the TLR3 stimulant poly IC or after mCMV infection Hobit induced expression of granzyme B. This demonstrates that the NKT cell-specific transcription factor Hobit enables NKT cells to mediate cytokine versus cytotoxic responses depending on antigenic and inflammatory stimuli.
INNATE AND ADAPTIVE RESPONSES TO COMMENSALS DURING MUCOSAL INFECTIONS

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Recent studies have highlighted the fundamental role of commensals in the maintenance of host homeostasis. In particular, maintenance of responsiveness against pathogenic microbes relies on the establishment of a dynamic equilibrium maintained in part by the stimulatory capacity of the flora. The gut also represents one of the primary sites of exposure to pathogenic microbes. In this environment, the pro-inflammatory properties of commensals can directly contribute to the pathogenesis of mucosal infection such as *Toxoplasma gondii*. This infection is also characterized by a reduction of the flora complexity and increase in gram-negative bacteria that in turn exacerbates the pathological process. Thus, at barrier sites, responses against pathogens are entwined with reactivity against the flora. How this milieu also leads to the priming of effector responses against innocuous microbes and how such responses could relate to the outcome of infections has not been addressed. Using various models of oral infections, we established that, surprisingly, priming of both humoral and long-lived CD4+T cell responses against commensal parallel pathogen exposure and that regardless to the virulence of the later. All together, our data shed a new light in our understanding of mucosal immune responses in which rather than been the exception, adaptive responses against commensals indiscriminately parallel response against pathogenic microbes and in a context dependent manner may contribute to the endogenous defense system of the host or pathogenic outcome.
The quantity, quality, and location of memory CD8 T cells relate to their capacity to protect against infection. This talk will illustrate the regulation of these parameters through our recent work on T cell differentiation in the mouse intestinal mucosa, female reproductive tract, and respiratory mucosa. Unpublished evidence will be presented indicating that resident memory CD8 T cells within small intestine epithelium maintain a recently activated phenotype. Antigen is not required for inducing or maintaining these stable effector-like memory CD8 T cells. Rather, local cytokine cues within the intestinal mucosa instruct resident T cells to adopt tissue-specific properties required for maintenance of local host protective immunity at this common site of infection. Unpublished data will be presented regarding the dynamics, differentiation, and anatomic compartmentalization of anti-viral T cell responses within the female reproductive tract. Lastly, new evidence will be discussed regarding the dynamics of anti-viral CD8 T cell responses in the lung. In vivo intravenous staining approaches reveal that the vast majority of memory T cells isolated from perfused lung represented a circulating populating localized within narrow capillary segments. Excluding this population from analysis revealed a starkly different model for the regulation of respiratory T cell trafficking, differentiation and maintenance of local immunity. Ramifications for vaccination and protection against mucosal infections will be discussed.
DEVELOPMENTAL AND FUNCTIONAL SPECIALIZATION OF MONONUCLEAR PHAGOCYTES IN THE INTESTINAL MUSCULARIS.

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Mononuclear phagocytes (MP) are formed by a heterogeneous population of hematopoietic professional antigen presenting cells called dendritic cells (DC) and macrophages that are found in most tissues. MP of the gut are strategically located below the surface epithelial layer in the intestinal mucosa allowing them to remain in close contact with luminal contents. We previously demonstrated that there are two types of MP in the intestinal lamina propria (LP) – a CD103+CX3CR1- DC population and a CD103-CX3CR1+ population that are likely macrophages. These populations arise from distinct precursor cells, require unique growth factor combinations for their development and acquire different abilities to migrate to the gut draining mesenteric lymph nodes (Bogunovic et al., Immunity 2009).

Intestinal muscularis is separated from the antigen-rich luminal environment by the mucosa and submucosa. In contrast to heterogeneous LP MP, MP in the intestinal muscularis represent a homogeneous population of cells with features of both DC and macrophages. In this study we compared developmental and functional properties of muscularis MP to better-characterized MP populations in the LP. Our initial phenotypical and morphological examination revealed resemblance of muscularis MP to the CD103-CX3CR1+ LP population. Similar to the CD103-CX3CR1+ LP cells, muscularis MP development relies on M-CSF and its receptor. However, in contrast to LP MP, muscularis MP dependency on M-CSFR is more severe and M-CSFR deficient mice completely lack muscularis MP. Furthermore, muscularis MP development in the steady state is controlled by CCR2 and CCL7 ligands. As a result, both CCR2- and CCL7-deficient mice have strongly reduced numbers of muscularis MP. High constitutive expression of CCR2 ligands by muscularis MP suggests that they regulate their own development through autocrine signaling.

Very little is known about the function of muscularis MP, especially in regards to their unique environment. Using a conditional muscularis MP depletion model that we recently established we show that selective depletion of muscularis MP induces intestinal paralysis suggesting that muscularis MP control intestinal motility in the steady state. To our knowledge, this is the first report demonstrating that macrophages control intestinal motility in the steady state.
When a cell undergoes apoptosis, phosphatidylserine is exposed on the outer leaflet of the plasma membrane and delivers “eat-me” signals to phagocytes to engulf the apoptotic cell. Here, we identified an immunoreceptor, CD300a, as a new phosphatidylserine receptor that is constitutively expressed on mast cells. Although CD300a did not mediate phagocytosis of apoptotic cells by macrophages, it delivered an inhibitory signal to mast cells to produce LPS-induced inflammatory cytokines and chemokines upon binding to phosphatidylserine. In the cecal ligation and puncture peritonitis model, where a large number of cells undergo apoptosis in the peritoneal cavity, CD300a-deficient mast cells produced more chemoattractants for neutrophils than did wild type mast cells, resulting in the increased neutrophil recruitment and bacterial clearance in the peritoneal cavity. We conclude that apoptotic cells are under survey by mast cells via the non-phagocytic phosphatidylserine receptor CD300a, which regulates inflammatory responses against microbial infections.
MODULATION OF INNATE AND ADAPTIVE IMMUNITY BY REPEATED ADMINISTRATION OF A TLR7 LIGAND

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Some autoimmune and inflammatory diseases have been linked to excessive stimulation of Toll-like receptors (TLRs). Accordingly, pharmacologic inhibition of TLR signaling has been proposed as a treatment strategy for these common maladies. TLR7 is the only TLR for which low molecular weight, orally active ligands have been synthesized. Hence, it is important to analyze the effects of TLR7 signal modulation on innate and adaptive immune responses. In the present experiments, the high affinity synthetic TLR7 ligand IV136 (chemical name 9-benzyl-8-hydroxy-2-(2-methoxyethoxy) adenine) was administered daily to SJL mice hyperimmunized with a proteolipid peptide (PLP) to induce inflammation of the central nervous system (CNS). The chronically treated mice displayed no untoward clinical effects, and the treated SJL mice had significantly less CNS inflammation than mock treated control animals. Continuous administration of low dosage IV136 had no cytokine inductive effects, consistent with diminished CNS inflammation. Splenocytes from treated EAE mice secreted lower interferon-gamma, IL-17, but similar IL-10. CNS microglial cells, from the treated animals had reduced levels of activation markers (CD80, CD86) compared to control animals, and diminished cytokine production after in vitro restimulation with a TLR7 agonist or with bacterial lipopolysaccharide. Moreover, antigen pulsed dendritic cells from WT mice did not efficiently stimulate primed CD4 T lymphocytes in the presence of IV136, as measured by either cell proliferation or interferon gamma release in vitro experiment. However, CD4 T cells from TLR7 null mice were fully capable of responding to unmanipulated bone marrow derived mononuclear cells pulsed with antigen. Cytofluorometric analyses did no reveal major drug induced changes in T lymphocyte subsets in spleen in the treated EAE mice. These results indicated that chronic administration of a TLR7 ligand can safely inactivate bone marrow derived cells expressing the receptor, which reside in the marrow, spleen, or the CNS. The diminished adaptive immune responses of CD4 T cells are secondary to the reduction in innate immunity.

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TISSUE-EXPRESSED B7X REGULATION OF THE T CELL RESPONSE IN A PULMONARY INFECTION MODEL

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The B7 family of proteins provides regulatory signals that can either costimulate or coinhibit T cells during both activation and effector phases. The most recently identified member of this family is B7x (B7-H4, B7S1), a coinhibitory molecule whose as yet unidentified receptor is expressed on activated T cells. Previous mRNA expression data showed that B7x is more highly expressed in peripheral non-lymphoid tissue than lymphoid tissue, with the highest levels seen in lung. This is in marked contrast to the classic B7 family members B7-1 and B7-2, which are most highly expressed on antigen presenting cells and in turn lymphoid tissues. Mirroring mRNA expression, we found that B7x protein is expressed in mouse lung and other non-lymphoid tissues, but not in lymphoid tissues. We hypothesized that B7x may act as a regulator of the T cell response to disease in the lung and addressed this by testing the outcome of a pulmonary infection model in mice lacking B7x (KO). The infection model we used was *Streptococcus pneumoniae*, an opportunistic pathogen whose clearance is typically attributed to the humoral immune response, although several studies in the past few years have revealed that T cells are also important for control. KO mice were significantly more resistant to intranasal challenge with a lethal dose of *S. pneumoniae* as compared to their wild-type (WT) counterparts. They also had less severe immunopathology that tended to localize in alveolar spaces, while inflammation in WT mice was perivascular. At 12 hours post-infection, KO mice had a lower bacterial burden in the lungs and later had reduced bacterial dissemination into the blood and spleen. Due to this decreased bacteremia, the KO mice had a less severe sepsis than WT mice as indicated by lower levels of inflammatory cytokines, such as IL-6 and TNF-α. Despite the early regulation of bacterial clearance we found no significant difference in various aspects of the innate immune response, including natural antibodies against *S. pneumoniae* and neutrophil or macrophage-mediated phagocytosis. However, analysis of the immune infiltrate in the lungs showed that KO mice had significantly higher numbers of both total and CD62L<sup>lo</sup> CD44<sup>hi</sup> CD4<sup>+</sup> and CD8<sup>+</sup> T cells, no difference was observed in the spleen. We also found that KO mice had significantly reduced numbers of neutrophils in the lungs as compared to WT mice. Not only does our work support a role for B7x in regulating lung immunity, but the control via enhanced T cell response supports emerging data from recent years showing that these cells are important for control of *S. pneumoniae* infection.
DENDRITIC CELL-DERIVED TIM-3 IS A UNIVERSAL REPRESSOR OF NUCLEIC ACID-MEDIATED INNATE ANTITUMOR IMMUNE RESPONSES.

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Although recent evidences have been verified the importance of nucleic acids-mediated pattern recognition pathways in boosting antitumor immune responses, the therapeutic efficacies of adjuvant containing nucleic acids remain insufficient in clinical settings. Therefore, the identification of the molecular machineries by which tumor microenvironments suppress nucleic acids-mediated innate immunity should improve therapeutic efficacies of cancer immunotherapy. Here, we identified TIM-3 as a key factor to circumvent antitumor immunogenicity mediated by nucleic acids. TIM-3 was highly expressed in tumor-associated dendritic cells (TADCs) of murine tumor models and advanced cancer patients, and coordinated actions of tumor-derived soluble factors were responsible for inducing TIM-3 in myeloid cell lineages. DC-derived TIM-3 suppressed innate immune responses mediated by TLR or cytosolic sensor recognition of nucleic acids by galectin-9-independent mechanisms. TIM-3 mainly acted as a putative receptor for HMGB-1, by which inhibited HMGB binding with nucleic acid in endosome. Importantly, DC-derived TIM-3 is responsible for suppressing therapeutic efficacies of nucleic acid-based adjuvants against established tumors. Moreover, DC-derived TIM-3 plays a critical role in attenuating antitumor effects of chemotherapeutic agents at least in part through impaired immunogenicity of nucleic acids released from dying tumor cells. Together, these findings define the novel mechanisms by which DCs suppress antitumor innate immune responses mediated by nucleic acids at tumor microenvironments.
Humans harbor 100 trillion intestinal bacteria that aid in the extraction of dietary nutrients. We are unraveling the immune mechanisms that promote symbiotic host-bacterial relationships by limiting the ability of resident bacteria to cross intestinal barriers. Using a germ-free mouse model, we have discovered that resident intestinal bacteria trigger epithelial cell expression of RegIIIγ, a member of the C-type lectin family of carbohydrate binding proteins. RegIIIγ directly kills Gram-positive bacteria and thus represents a novel class of antibacterial proteins. We have uncovered the molecular mechanism of RegIIIγ bactericidal activity, showing that it binds peptidoglycan on the surfaces of Gram-positive bacteria and carries out bacterial killing by forming a hexameric pore in the bacterial inner membrane. We have found that RegIIIγ expression is governed by epithelial Toll-like receptors and that expression is triggered when bacteria colonize intestinal surface tissues. Mice engineered to lack RegIIIγ show severe defects in the ability to limit bacterial colonization of the intestinal mucosal surface, and exhibit increased activation of intestinal adaptive immune responses. Thus, RegIIIγ represents a novel mechanism of lectin-mediated mucosal defense that is critical for maintaining homeostasis with the intestinal microbiota.
INNATE IMMUNE REGULATION OF GUT MICROBIOTA AND POTENTIAL THERAPEUTIC OPPORTUNITIES TO TREAT METABOLIC DISEASE AND CHRONIC INFECTIONS

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The human intestine contains approximately $10^{14}$ (1-2 kg) of bacteria, collectively referred to as the gut microbiota, which is comprised of about 5000 distinct species. While the majority of these bacteria are not a threat to their host, the gut microbiota nonetheless contains numerous bacteria that can be viewed as opportunistic pathogens and often sporadically includes bacteria viewed as traditional pathogens in that they are capable of causing disease in healthy hosts. The ability of the intestine to keep the microbiota in-check is essential for human survival. We have observed that bacterial flagellin, which allows bacteria to achieve locomotion, is a central target by which the intestinal immune system protects itself from both pathogens and commensal microbes of the gut microbiota. Consequently, deletion of the flagellin receptor, TLR5, a component of the innate immune system that recognizes the bacterial protein flagellin impairs the ability of the intestine to rapidly squelch pathogenic challenges resulting in prolonged inflammation. Moreover, mice lacking TLR5 were prone to developing spontaneous colitis. However, altering the microbiotas of TLR5 mice in a manner designed to reduce their colitis caused TLR5-deficient mice to develop hallmark features of metabolic syndrome including hyperlipidemia, hypertension, insulin resistance, and increased adiposity. These metabolic changes correlated with alterations in the composition of the gut microbiota and, importantly, transfer of the gut microbiota from TLR5-deficient mice to wild-type germ-free mice conferred many features of metabolic syndrome to the recipients. These results support the emerging view that the gut microbiota plays a role in multiple chronic inflammatory diseases including both IBD and metabolic disease and suggest that manipulation of the microbiota may be one approach to combat these disorders. Lastly, recent work in our lab indicates that exogenous administration of TLR ligands may be an effective means of eliminating select members of the microbiota and thus, may be used as a novel approach to treat chronic infections.
TLR2 SIGNALING CONTRIBUTES TO RAPID INFLAMMASOME ACTIVATION DURING BACTERIAL INFECTION

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Early detection of microorganisms is provided by surface-expressed and endosomal Toll-like receptors (TLRs). Upon detection of microbial components, TLRs initiate a signaling cascade leading to the expression of proinflammatory cytokines including IL-6 and IL-1β. Some pathogenic bacteria subvert the TLR response by rapidly escaping endosomes and entering the cytosol. However, these bacteria may be recognized by the inflammasome, which leads to release of the proinflammatory cytokines IL-1β and IL-18 and death of the infected cell, an important host defense that eliminates the pathogen’s replicative niche. While TLR signaling is required for expression of the inflammasome substrate pro-IL-1β, it has not been shown to directly contribute to inflammasome activation during infection. Therefore, we investigated whether TLR2 and the inflammasome cooperate during infection using the model pathogen Francisella novicida. We show that infected TLR2−/− macrophages exhibited delayed inflammasome activation compared to wild-type macrophages as measured by cell death, caspase-1 activation, and IL-18 release. TLR signaling molecules, MyD88 and NF-κB, were also required for rapid inflammasome activation. Interestingly, TLR2−/− macrophages also exhibited delayed inflammasome activation in response to infection by pathogenic Listeria monocytogenes but not to infection by commensal E. coli. Furthermore, TLR2−/− mice exhibited lower levels of cell death, caspase-1 activation, and IL-18 production than wild-type mice upon infection. These results show that TLR2 contributes to rapid inflammasome activation in response to infection by pathogenic bacteria in vitro and in vivo. In addition to further characterizing the role of TLR2, we have shown that TLR2 and the inflammasome provide an integrated, multi-tiered recognition and defense system capable of eliciting an immune response tailored to the level of threat imposed by invading bacteria.
A TEMPORAL ROLE OF TYPE I INTERFERON SIGNALING IN CD8+ T CELL MATURATION DURING ACUTE WEST NILE VIRUS INFECTION

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A genetic absence of the common IFN-α/β signaling receptor (IFNAR) in mice is associated with diminished innate immunity, enhanced viral replication and altered adaptive immune responses. However, analysis of IFNAR-/- mice is limited to studying the functions of type I IFN at all stages of viral infection. To define the temporal functions of type I IFN signaling in the context of infection by West Nile virus (WNV), we treated mice with MAR1-5A3, a neutralizing, non cell-depleting anti-IFNAR antibody at later stages of infection. Inhibition of type I IFN signaling at or before day 2 after infection was associated with markedly enhanced viral burden, whereas treatment at day 4 altered WNV dissemination less dramatically. Blockade of type I IFN signaling starting at day 4 induced dysfunctional CD8+ T cells with depressed cytokine responses and expression of phenotypic markers suggesting exhaustion. The later maturation phase (days 4-8) of anti-WNV CD8+ T cell development requires type I IFN signaling. Collectively, our results suggest that cell non-autonomous type I IFN signaling shapes maturation of antiviral CD8+ T cell response at a stage distinct from the initial priming event.
DISSECTING THE HUMAN T AND B CELL RESPONSE TO PATHOGENS

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Memory T and B lymphocytes and long lived plasma cells represent a repository of the antigenic experience of an individual. By analyzing the specificity and function of these cells we can gain insights into the human immune response and identify correlates of protection. We have developed methods to dissect the functional heterogeneity and antigenic repertoire of human T, B and plasma cells. These methods are used: i) to identify subsets of effector and memory T cells with distinct role in immune surveillance and protection in different tissues against different classes of pathogens, and ii) to dissect the relative role of plasma cells and memory B cells in the humoral response to pathogens and to isolate broadly neutralizing antibodies. A better understanding of the class and specificity of the human immune response will be instrumental to guide the design of effective vaccines.
A DYNAMICAL SYSTEMS PERSPECTIVE OF CYTOKINE SIGNALING RESPONSES BY HUMAN T CELLS.

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An immune response involves the release of many cytokines with various immunomodulatory functions. Its efficacy depends, in part, on the types of cytokines secreted by activated T cells and the timing of their release. Through serial microengraving [1], the Love Lab quantifies single- and multiple-cell cytokine secretion dynamics, offering a unique strategy to investigate time-dependent functional differences specific to immunophenotypes. Based on these quantitative experimental measurements, new computational tools for analyzing dynamic cellular systems reveal the temporal evolution of specific cytokine responses important for immune function with single-cell resolution. Experiments using defined stimulatory perturbations provide a unique approach to investigate the regulatory mechanisms involved in T cell function. As a result, we are able to better resolve and predict the nonlinear functional dynamics governing qualitatively different T-cell responses. This systems-level methodology should enable the identification of unique time-dependent functional signatures indicative of productive immune responses.

Every year influenza epidemics return, dominated depending on the continent, by either an influenza A virus of group 1 or group 2 or an influenza B virus (FluView [Internet], a weekly influenza Surveillance Report prepared by the influenza division at Center for Disease Control and Prevention. Update: August 20, 2011, Available from: http://www.cdc.gov/flu/weekly and Flunet [Internet], a global tool for influenza virological surveillance, GISRS WHO, Update: August 23, 2011, Available from http://www.who.int/influenza/gisrs_laboratory/flunet/en). Severity of the disease depends more on the vulnerability of risk groups than on the virus group. The number of drugs for treating severe influenza is limited and the efficacy is highly dependent on the moment of diagnosis related to the moment of infection. Vaccines are made anew every year based on the prediction of causative dominating strains for the southern and northern hemisphere. Clearly, there is a need for better drugs that are effective up to late in the disease course and vaccines that induce broad and long lasting protection. Recently, we described a series of antibodies that are able to broadly neutralize influenza A group 1 and group 2 viruses. All of these antibodies bind to the hemagglutinin stem region as has been shown by crystallography (Throsby et al, PLoS ONE, 2008; Ekiert et al., Science, 2009; Ekiert et al, Science 2011). New results will be presented on the further characterization of these antibodies and data on advances made in the use of such antibodies for treatment of severe influenza as well as for design of a universal influenza A vaccine.
A SYSTEMIC CYTOKINE RESPONSE DEFECT STRATIFIES OLDER ADULTS INTO DISTINCT IMMUNE PROFILES

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While it is known that immune responsiveness declines with age, we lack a systematic picture of immune aging and diagnostics for those at risk. Here we analyze different components of peripheral blood—the pipeline of the immune system—including: gene expression, frequencies of various cell subsets, cytokine and chemokine levels and signaling responses to several cytokines by measuring phosphorylation of STATs proteins, in 29 young and older individuals who were vaccinated with the seasonal Influenza vaccine. We identify a novel and systemic cytokine response defect in many of the older adults that involves multiple cytokines and cell types. Stratifying older adults by their cytokine responses, we identify two distinct phenotypes: cytokine non-responders (CNR) who are concomitant for the majority of known immunosenescence markers and reduced influenza antibody pre-titers, and cytokine responders (CR) who are not. The cytokine response deficiency is generally stable or worsens over a period of three years and is primarily due to increased basal levels of phosphorylated STAT proteins. Integrative analysis of our data and mathematical modeling suggest that rising levels of pro-inflammation in older adults mediate downregulation of JAK/STAT pathway phosphatases and an increase in baseline state. These findings suggest a new mechanism for cellular unresponsiveness and could be useful for screening and treatment of elderly individuals for immune health and senescence.
MEMORY T CELL RESPONSES IN ACUTE INFLUENZA A INFECTION IN HUMANS

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Influenza virus causes morbidity and mortality worldwide. Neutralizing antibodies are the main correlates of protection against influenza infection. Although effective in protecting an individual upon exposures to a homologous virus, such protection will be ineffective against variants or a new subtype in which HA and NA that are antigenically distinct. Despite substantial evidence in animal models which suggests the critical roles of T cells in the control of infection, the precise role of cellular immunity in humans remains unclear. To address this, we conducted influenza challenge studies in humans and studied the role of pre-existing and developing immunity in limiting the illness. Healthy adult volunteers with no detectable antibodies to the challenge viruses were infected with live seasonal H3N2 or H1N1 viruses. A matrix of overlapping peptides covering the whole influenza viral proteome and \textit{ex vivo} IFN-gamma ELISPOT assays are used to measure the kinetics and quantities of influenza-specific memory T cell responses circulating in the peripheral blood prior to or during infection. We found that a large increase, in both magnitude and breadth, of influenza-specific T cell responses by day 7 post-challenge, at which point the viruses were completely cleared and the virus-specific antibodies were still undetectable in the serum. T cell responses returned to baseline by day 28 post infection. These T cells induced are highly activated (CD38\textsuperscript{+}) and proliferative (Ki-67\textsuperscript{+}). Moreover, pre-existing memory T cells persist in most individuals and predominantly are CD4\textsuperscript{+}, but not CD8\textsuperscript{+} T cells, specific against internal proteins (nucleoprotein and matrix proteins). Most importantly, these CD4\textsuperscript{+} cells were associated with both lower virus shedding and less severe illness. Further functional analyses revealed that these CD4\textsuperscript{+} T cells are polyfunctional in terms of cytokine production and cytotoxicity, capable of killing antigen-loaded autologous B cell lines in vitro by chromium release assay. With a strong degree of cross-reactivity against the current pandemic H1N1 strain, it suggests that these CD4\textsuperscript{+} T cells may have an important role in limiting severity of an infection by new strains in the absence of any pre-existing antibody response. Our study therefore provide an insight on how cellular immunity can be targeted in conferring broad protection against emerging subtypes of influenza A viruses.
**Plasmodium falciparum**-specific anti-inflammatory responses are upregulated after acute malaria but short-lived in the absence of ongoing **P. falciparum** exposure

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**Plasmodium falciparum** malaria remains a major public health threat. Optimism that an effective malaria vaccine can be developed comes from the observation that malaria immunity can be acquired through natural infection; however, this goal has been hindered in part by a poor understanding of the interaction between **P. falciparum** and the human immune system. Immunity to mild malaria is acquired after years of repeated infections in endemic areas, whereas children become ‘tolerant’ to the immunopathology of severe malaria after few **P. falciparum** exposures. The mechanisms that underlie the relatively rapid acquisition of immunity to severe, **P. falciparum**-induced inflammation remain poorly understood. To address this, we conducted a 1-year longitudinal study in Mali where the malaria season is intense over a 6-month period; this is followed by a 6-month dry season when little to no **P. falciparum** transmission occurs. The objective was to determine how **P. falciparum**-specific inflammatory responses are modulated 7 days after treatment of febrile malaria and whether this modulation persists in the absence of ongoing parasite exposure. PBMCs from children aged 2-10 years were collected before the malaria season (uninfected), 7 days after treatment of the first febrile malaria episode, and after the following 6-month dry season (uninfected). Genome-wide expression analysis of thawed PBMCs revealed that recent febrile malaria primarily affected pattern recognition receptor signaling pathways, in particular, NALP3 and IL1-β were significantly down-regulated. **P. falciparum**-specific IL-10 responses in the supernatants of stimulated PBMCs were markedly upregulated 7 days after treatment of febrile malaria but this response was short-lived in the absence of ongoing **P. falciparum** exposure (end of dry season). The majority of IL-10 was produced by T cells which expanded after febrile malaria and then contracted to pre-infection levels after the dry season. Despite a marked increase in the **P. falciparum**-specific IL-10 response following febrile malaria, the pro-inflammatory cytokine response remained relatively constant before and after infection. Taken together, these data suggest a possible mechanism by which immunity to the inflammation-driven clinical manifestations of malaria are rapidly acquired during the malaria season and then lost in the absence of ongoing parasite exposure.
Targeting antigens to dendritic cells (DCs) is an effective strategy to elicit cellular and humoral immunity. The DC network consists of multiple subsets with distinct antigen processing and presenting capabilities. In mice, the CD8+ DC subset preferentially induces CD8+ T cell responses against pathogens and tumor cells, and is thus an excellent target in vaccination strategies. In humans, the role of various DC subsets is not as well characterized. The BDCA3+ DC subset, which appears to cross present antigens more efficiently to CD8 T cells in vitro than other DC populations, has been proposed to be the human counterpart of murine CD8+ DCs. We compared cross presentation quantitatively using receptor-mediated antigen internalization in the different blood DC subsets. We found that BDCA3 DCs are in fact more adept at cross presentation when antigens are targeted to lysosomes via DEC205. Enhanced cross presentation in BDCA3 DCs does not result from differential antigen uptake, nor from a higher ability to present antigen on MHCI in general, whether through surface loading of peptide or direct presentation of endogenously synthesized antigens. We next examined the contribution of the intracellular trafficking pathway of antigens. We found that cross presentation is more robust when antigens are targeted to early endosomes as compared to lysosomes, and this is associated with reduced antigen degradation. Interestingly, antigen targeting to early endosomes results in similar levels of cross presentation by all blood DC subsets. Our results suggest a specialization of lysosomes in BDCA3+ DCs, perhaps by allowing higher antigen translocation to the cytosol. Our data also show that all blood DC subsets can cross present antigens efficiently, and this capacity is controlled by intracellular trafficking of the antigen. Targeting antigens to early endosomes appears to be a good strategy to induce global cross-presentation by all DC subsets and to elicit more potent T cell responses in vivo.
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<th>Participant List</th>
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**VISITOR INFORMATION**

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<tr>
<th>EMERGENCY</th>
<th>CSHL</th>
<th>BANBURY</th>
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<tbody>
<tr>
<td>Fire</td>
<td>(9) 742-3300</td>
<td>(9) 692-4747</td>
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<tr>
<td>Ambulance</td>
<td>(9) 742-3300</td>
<td>(9) 692-4747</td>
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<tr>
<td>Poison</td>
<td>(9) 542-2323</td>
<td>(9) 542-2323</td>
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<tr>
<td>Police</td>
<td>(9) 911</td>
<td>(9) 549-8800</td>
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<td>Safety-Security</td>
<td>Extension 8870</td>
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**Emergency Room**

**Huntington Hospital**

270 Park Avenue, Huntington

631-351-2300 (1037)

**Dentists**

Dr. William Berg

631-271-2310

Dr. Robert Zeman

631-271-8090

**Doctor**

MediCenter

234 W. Jericho Tpke., Huntington Station

631-423-5400 (1034)

**Drugs - 24 hours, 7 days**

Rite-Aid

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631-549-9400 (1039)

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Dial the four numbers (****) from any tan house phone to place a free call.

**GENERAL INFORMATION**

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Located in Grace Auditorium, lower level.

**Photocopying, Journals, Periodicals, Books, Newspapers**

Photocopying – Main Library

Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri

10:00 a.m. – 6:00 p.m. Saturday

Helpful tips - Obtain PIN from Meetings & Courses Office to enter Library after hours. See Library staff for photocopier code.

**Computers, E-mail, Internet access**

Grace Auditorium

Upper level: E-mail only

Lower level: Word processing and printing.

SMTP server address: mail.optonline.net

To access your E-mail, you must know the name of your home server.

**Dining, Bar**

Blackford Hall

Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00

Bar 5:00 p.m. until late

Helpful tip - If there is a line at the upper dining area, try the lower dining room
Messages, Mail, Faxes
Message Board, Grace, lower level

Swimming, Tennis, Jogging, Hiking
June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m.
Two tennis courts open daily.

Russell Fitness Center
Dolan Hall, east wing, lower level
PIN#: Press 61380 (then enter #)

Concierge
On duty daily at Meetings & Courses Office.
After hours – From tan house phones, dial x8870 for assistance

Pay Phones, House Phones
Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

CSHL’s Green Campus
Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a “single stream waste management” system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory’s efforts can be improved. This book, for example, is printed on recycled paper.
1-800 Access Numbers

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MCI 9-1-800-674-7000

Local Interest
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Sagamore Hill 516-922-4447
Whaling Museum 631-367-3418
Heckscher Museum 631-351-3250
CSHL DNA Learning x 5170

New York City

Helpful tip -
Take Syosset Taxi to Syosset Train Station
($9.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue).
Train ride about one hour.

TRANSPORTATION

Limo, Taxi
Syosset Limousine 516-364-9681 (1031)
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Syosset Taxi 516-921-2141 (1030)
To head east of CSHL - Huntington Village
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Orient Point/ New London 631-323-2525 (1038)

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Enterprise 631-424-8300
Hertz 631-427-6106

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America West 800-237-9292
British Airways 800-247-9297
Continental 800-525-0280
Delta 800-221-1212
Japan Airlines 800-525-3663
Jet Blue 800-538-2583
KLM 800-374-7747
Lufthansa 800-645-3880
Northwest 800-225-2525
United 800-241-6522
US Airways 800-428-4322