Deoxycytidine kinase (dCK) regulates a rate-limiting step in nucleoside metabolism. Specifically, dCK functions in deoxyribonucleoside salvage. dCK is able to provide cells with the precursors to generate all 4 dNTPs that are needed for DNA synthesis. The enzyme is preferentially expressed in hematopoietic cells and tissues and is further up-regulated in activated T and B lymphocytes. This preferential expression pattern indicates that dCK plays an important role in the development and functioning of the adaptive immune system. To further elucidate dCKs biological function in hemato- and lymphopoiesis, we created global and tamoxifen-inducible conditional dCK knockout (KO) mice. Global inactivation of dCK selectively and profoundly affected the development of T and B cells. The blocks in development were traced back to stages of lymphocyte differentiation where VDJ recombination occurs prior to massive clonal proliferation of T and B cell progenitors. Other phenotypically observations of the global KO mice include; severe decrease in thymic cellularity and overall lymphocyte numbers, secondary lymphoid organs had structural abnormalities, and mature KO T cells failed to proliferate in response to TCR cross-linking. Major hematopoietic lineages in the bone marrow of the global KO mice showed a greater than 10 fold decrease in deoxycytidine triphosphate (dCTP) pools compared to wild-type (WT) littermates. Our studies have demonstrated that the developmental defect is cell autonomous to hematopoietic stem and progenitor cells. Functional studies of mature T cells the conditional knockout mice have demonstrated that loss of dCK activity in mature T cells results in a severe defect in proliferative response to in vitro mitogenic stimulation. These findings provided the rationale for us to develop a novel small molecule inhibitor of dCK. We validated the ability of the inhibitor to recapitulate in WT mature T cells, the loss of proliferative response to mitogenic activation seen in the DCK global and conditional KO T cells. We are currently evaluating this inhibitor in murine disease models to evaluate the utility of this compound as a treatment for autoimmune disorders, transplant rejection, and potentially, lymphoid malignancies.
Presenter: Bajpai, Urmila

**Fc Receptor-Like 3 expression on Regulatory T cells in Rheumatoid Arthritis**

Urmila Bajpai, Louise A. Swainson, Jeff E. Mold, Jonathan D. Graf, John B. Imboden, and Joseph M. McCune

University of California at San Francisco, Division of Rheumatology and Division of Experimental Medicine

BACKGROUND: CD4+ regulatory T cells (Treg) suppress effector T cells and limit autoimmune disease. The suppressive activity of Treg is deficient in active rheumatoid arthritis (RA), a loss which may play a role in the pathogenesis of this disease. A single nucleotide polymorphism (SNP) in transmembrane Fc receptor-like 3 (FcRL3) has been associated with an increased risk of RA and in vitro studies show the FCRL3 SNP, -169C, leads to increased FcRL3 promoter activity (Kochi, et al). FcRL3 is highly expressed on Treg and FcRL3+ Treg are functionally less suppressive (Swainson, et al). We hypothesized that those RA patients harboring the FcRL3 RA-associated allele overexpress FcRL3 on Treg leading to increased immune activation in RA, as assessed by RA disease activity. METHODS: We performed a cross-sectional analysis of FcRL3 expression on Treg from RA patients in the UCSF RA Cohort utilizing flow cytometry and FCRL3 -169 SNP analysis. RESULTS: 1. RA patients with the RA-associated FCRL3 -169 SNP (C/C or C/T) express higher FcRL3 levels on Treg in comparison to Treg from RA patients with the T/T genotype. 2. FcRL3 expression on Treg correlates with RA disease activity (measured by disease activity score (DAS) and erythrocyte sedimentation rate (ESR)). 3. The significant correlation found between FcRL3 expression on Treg and disease activity is only seen in RA patients who harbor the FCRL3 RA-associated SNP (genotypes -169 C/C or C/T). CONCLUSION: In rheumatoid arthritis patients, FcRL3 expression on Treg correlates with rheumatoid arthritis disease activity in those patients with the FCRL3 RA-associated SNP (genotypes -169 C/C or C/T).
Presenter: Bando, Jennifer

Arginase-I is expressed by ckit+IL-7Ra+ populations in cryptopatches

Jennifer Bando, Hong-Erh Liang, Richard Locksley
UCSF

Abstract not to be posted.
Shaping the Immune Response to Francisella tularensis by the First Cell Type Infected

Lydia Barrigan, Lydia Barrigan, Shaun Steele, Shraddha Tuladhar, Matthew Woolard, Tom Kawula, and Jeffrey Frelinger

University of North Carolina - Chapel Hill

Shaping the Immune Response to Francisella tularensis by the First Cell Type Infected Lydia Barrigan 1,2, Shaun Steele 1, Shraddha Tuladhar 2, Matthew Woolard 3, Tom Kawula 1, and Jeffrey Frelinger 2. 1 Department of Microbiology and Immunology, University of North Carolina at Chapel Hill 2 Department of Immunobiology, University of Arizona 3 Department of Microbiology and Immunology, Louisiana State University Health Sciences Center

Francisella tularensis is a facultative, intracellular coccobacillus and the causative agent of tularemia. F. tularensis induces a host response that is dependent on the route of infection. Intranasal (i.n.) inoculations are more virulent and require fewer bacteria to produce a lethal infection than an intradermal (i.d.) inoculation (103 organisms i.n. versus 106 organisms i.d.). Interestingly, at one day post infection, the bacterial loads are similar in the spleen and lung regardless of the route of infection. We also found that i.d. inoculation resulted in IFN-Γ+ T cells in the lung whereas i.n. inoculation produced very few IFN-Γ+ T cells and instead many IL-17+ T cells in the lung. Due to the similar bacterial loads systemically after 1 day, but very different host responses, we hypothesize that the adaptive immune response is influenced by local events at the site of infection immediately following inoculation. To test this hypothesis, we identified the first cell type infected in the lungs of 6-10 week old C57BL/6 mice given intranasal inoculations of F. novicida U112, the live vaccine strain (LVS), or the highly virulent SchuS4 strain of F. tularensis using flow cytometry. At four hours post-infection, we found that for all three subspecies of F. tularensis, alveolar macrophages are the primary cell type infected, to the exclusion of other myeloid cells and lung parenchyma. Furthermore, we have identified cytokines produced by alveolar macrophages when cultured ex vivo and infected with F. tularensis. These data will help us further understand how the adaptive immune response to F. tularensis is shaped early after infection.
Presenter: Behnsen, Judith

Salmonella enterica serovar Typhimurium induces expression of IL-23 and IL-1[b] in human dendritic cells and monocytes by activating TLR signaling.

Judith Behnsen, Judith Behnsen, Christoph Blaschitz, Anshu Agrawal and Manuela Raffatellu
University of California, Irvine

Abstract not to be posted.
Presenter: Biethahn, Katharina

Regulation of Fc(e)RI-Mediated Mast Cell Degranulation by miRNA-155

Katharina Biethahn, Niko Föger, Silvia Bulfone-Paus
Research Center Borstel, Borstel, Germany

Abstract not to be posted.
Presenter: BOU GHANEM, ELSA

Differential ability of CD8+ T cells to rapidly secrete IFN[?] during L. monocytogenes infection

ELSA BOU GHANEM, Christina C. Nelson, Sarah E. F. DOrazio
University of Kentucky

A subset of CD44hi CD8+ T cells in some, but not all, strains of mice can rapidly secrete IFNγ during infection with Listeria monocytogenes (Lm) and other cytosolic intracellular pathogens that induce robust production of both IL-12 and IL-18. Previous studies showed that C57BL/6 mice produced more IL-12 during Lm infection than BALB/c mice. In this report, we show that T cell intrinsic factors also contribute significantly to the magnitude of the rapid IFNγ response. The CD8+ T cells that rapidly secreted IFNγ were not restricted to any particular memory T cell subset, however, we found that BALB/c mice had the greatest level of impairment in virtual memory cells, rather than true antigen-experienced cells. Adoptive transfer of IFNγ-secreting CD8+ T cells resulted in reduced bacterial burdens in low-responder mice challenged with Lm. To assess whether differential bystander activation also occurs in humans, we tested CD8+ T cells from 20 different human blood donors. Interestingly, CD8+ T cells from some donors had a robust IFNγ response (similar to C57BL/6 mice), while a few were unable to rapidly secrete IFNγ (like BALB/c mice). The magnitude of the rapid IFNγ response correlated more closely with the ability of T cells to efficiently respond to the presence of IL-12 plus IL-18 than with the level of IL-12 produced. Together, these results suggest that cytokine-driven bystander activation of CD8+ T cells can vary among individuals and may be a critical component of the innate immune responses that define overall host resistance to infection with intracellular pathogens.
Presenter: Brunette, Rebecca

The Role of HIN-200 Proteins in DNA Sensing

Rebecca Brunette, Rebecca L. Brunette, Debbie G. Whitley, Daniel B. Stetson
University of Washington

Abstract not to be posted.
Presenter: Buechler, Matthew B

Toll-like Receptor 7 promotes myeloid lineage development

Matthew B Buechler, Nikita Kolhatkar, Keith B Elkon, Jessica A Hamerman
University of Washington, Benaroya Research Institute

Abstract not to be posted.
Presenter: Buntzman, Adam

T Cell Receptor Repertoire Sharing

Adam Buntzman, Adam S Buntzman, Benjamin G Vincent, Harsha Krovi, Shaun P Steele, Jesse Walsh, Thomas B Kepler, and Jeffrey A Frelinger

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The potential for AB T cell receptor (TCR) diversity is massive, with each individual mouse having the theoretical capacity to generate over 1015 A B TCR variants. However, at any given time each mouse contains on the order of 108 T cells with 106 TCR variants. Thus, each mouse contains one billionth of the total theoretical repertoire. The probability that any two individual mice would stochastically generate the same TCR is extraordinarily low. However, the occurrence of shared TCR clones between individuals has been well documented. Various models have been postulated to ascribe causation for this TCR clonotype sharing phenomenon including: the simple CDR3 model (less junctional n nucleotides); the "convergent recombination model"; differential precursor frequency model; and the antigenic peptide shape model (peptide shape in the MHC cleft, e.g. "spicy/vanilla epitopes"). These models were generated based upon estimates of global TCR sharing using extrapolations from T cell subpopulations that were antigen selected or V B family selected subsets of cells. However, a global analysis of TCR repertoire sharing in mice has not yet been done. Herein, we describe the sequencing of the actual CD8+ TCR mouse repertoire from multiple mice using large scale pyrosequencing on the Roche 454 platform. Our data provide the first glimpse of the extent of TCR sharing between mice based on analysis of the whole repertoire, allowing us to generate a more general model of TCR sharing.
A Mouse Model for Corynebacterium pseudotuberculosis Infection

Elise Burger, Elise Burger, David Whorton, Eliseo Barajas, Roberta Pollock
Occidental College, Department of Biology

Pigeon Fever is an equine disease caused by the gram positive bacteria Corynebacterium pseudotuberculosis. The majority of infected horses develop large pus-filled external abscesses which resolve with a low mortality rate. Others develop internal abscesses which are difficult to detect and have a 40% mortality rate. We have implemented a mouse model to study the effectiveness of various vaccines and to gain more insight to the type of antibody and cytokine response. Four potential vaccine components were tested: (1) inactivated phospholipase D (PLD), which is the major exotoxin of this bacteria, (2) whole bacterial cell lysate, (3) the insoluble cell debris remaining after lysis, and (4) concentrated bacterial culture supernatant. BALB/c mice were vaccinated, boosted, and then challenged with 3x10^5 live bacteria. Preliminary ELISA data suggests that mice made a poor antibody response to PLD, but very strong responses to vaccines comprised of whole cell lysate and culture supernatant. Overall ELISA results of IgG and IgM levels over the course of the experiment will help us refine our model and create more efficacious vaccines. A better understanding of the immune response to C. pseudotuberculosis will greatly facilitate the development of a successful equine vaccine.
Type 1 Diabetes (T1D) is a polygenic autoimmune disease characterized by specific destruction of the insulin-producing β-cells of the pancreatic islets. The central role of autoreactive T cells in T1D implies that individuals with this disease harbor some kind of defect in the induction of T cell tolerance to self-antigens. It has been demonstrated that NOD mice, an animal model of T1D, have a genetic deficiency in thymocyte selection, and that this defect is T-cell intrinsic. In order to translate these observations to the human disease, we are developing an experimental method that will allow us to assess human thymocyte clonal deletion, and ultimately to compare the ability of T cell precursors from healthy and T1D individuals to go through the process of negative selection. We differentiated and expanded human thymocyte precursors from CD34+ hematopoietic progenitors by culture on an OP9 stromal cell line expressing the Notch ligand Delta-like-1. The T cell precursors were then transferred to fetal thymic organ cultures (FTOCs) to study their capacity to undergo clonal deletion. This was assessed by two different methods that induce the strong TCR signaling required to provoke apoptosis of self-reactive thymocytes. In the first one, the precursors were transduced with the AI4 TCR, and the repopulated FTOCs were treated with an AI4 agonist peptide to induce deletion of the monoclonal population by recognition of the self-antigen. In the second method, an anti-CD3 antibody was added to the FTOCs to stimulate cell death by TCR crosslinking. After the treatments, clonal deletion was evaluated by flow cytometric analysis. We show that our protocol efficiently generates human thymocytes and that both methods can induce clonal deletion of these cells. Thus, the proposed experimental strategy will be applied to compare cells from healthy and diabetic donors. The successful completion of these experiments should provide novel information on the pathophysiology of human T1D. (Supported by Juvenile Diabetes Research Foundation Grant 4-007-1057 to D.M. and C.B. and ADA fellowship 7-04-MI-03 to D.B.)
Mast cells are required to control of insulitis and autoimmune type 1 diabetes development
Daniela Carlosa, Fredy R. Gutierrezb, Juliana N. U. Yaochiteb, Kelen C. M. de Fariasb, Carlos R. Zarate-Bladesb, Simone G. Ramosc, Marcus V. Andraded, João Santana da Silvab, Fernando Q. Cunhaa* aDepartments of Pharmacology and bBiochemistry and Immunology and cPathology, School of Medicine of Ribeirão Preto, University of São Paulo, São Paulo, Brazil. dDepartment of Internal Medicine, School of Medicine, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
 Daniela Carlos, Daniela Carlos, Fredy R. Gutierrez, Juliana N. U. Yaochite, Kelen C. M. de Farias, Carlos R. Zarate-Blades, Simone G. Ramos, Marcus V. Andrade, João Santana da Silva, Fernando Q. Cunha
 University of São Paulo

Abstract not to be posted.
Presenter: Catlett, Ian M.

Performance Characteristics of an Assay for IL-28B Genotype as a Diagnostic Device to Improve Therapy of Chronic Hepatitis C Virus Infection

Ian M. Catlett, Sheila Seepersaud, Kevin Kelliher, Katherine Sussky, Meryll Corbin, Catherine Phillips, Benjamin Shames, Ravi Ramachandran, Yeelan Wang, Ann Marie Dunne, Desiree Yagovane, Leif Bengtsson, Jose Trevejo, Martyn Botfield

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Abstract not to be posted.
PU.1 regulates lineage progression during the early double negative (DN) stages of T-cell development

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PU.1 is an Ets-family transcription factor that plays an important role in the development of several blood lineages. Deletion of PU.1 in multi-potent precursors has a severe negative impact on T-cell development but its function in the intra-thymic stages of T-cell development remains undetermined. In the thymus, PU.1 is expressed in early CD4- CD8- double negative (DN) stages and is silenced as these cells commit to the T-lineage at the DN3 stage. Here we used a number of complimentary methods to disrupt PU.1 function in cells at the DN1, DN2a and DN2b stages of development and asked how this affected a) the expression of genes which constitute the core T-cell developmental program and b) development along the T-cell pathway. We used a construct expressing the PU.1 DNA-binding ETS domain fused to an Engrailed repressor domain to show that PU.1 represses several genes that normally initiate expression or attain peak levels only at the T-lineage-committed DN3 stage of development. PU.1 seems to exert this effect by positively regulating the expression of an important and as yet unidentified repressor of the T-lineage program since a construct lacking the PU.1 transactivation domain fails to produce the same effect. As predicted by the above results, we also show that early T-cells lacking PU.1 expression show faster DN progression as compared to wild-type cells. Thus, our results indicate an important role for PU.1 in coordinating the timing of expression of key T-lineage genes and hence also in the regulation of T-cell developmental progression.
Presenter: Chan, William

Microchimerism: a clue to the pathogenesis of the rheumatoid nodule

William Chan, Christopher Atkins, David Naysmith, Nicholas van der Westhuizen, Janet Woo, Valerie Cortez, J. Lee Nelson

Fred Hutchinson Cancer Research Center, University of Victoria, Vancouver Island Health Authority, University of British Columbia, University of Washington

Small amounts of foreign DNA (and cells) traffic bi-directionally during pregnancy and persist long-term in both the mother and her offspring, generating microchimerism (Mc). A significant increase of Mc was recently reported in the peripheral blood of rheumatoid arthritis (RA) patients compared to healthy individuals. Immunity to the Mc could occur upon immune system activation due to tissue damage. We hypothesize that this sequence of events is responsible for forming the rheumatoid nodule (RN), a lesion that occurs in areas prone to mild, repetitive traumas and is pathognomonic of RA when present in the setting of an inflammatory polyarthritis. To begin to test this hypothesis, we used quantitative PCR to detect foreign DNA in the RNs of women with RA, and determine the source. We tested 17 female RA patients, all of whom have had at least one son, for male DNA in their RNs (i.e. Mc contributed by the male fetus). We amplified the DYS14 gene located on the Y chromosome as a marker for male DNA. Fourteen of 17 patients were positive for male Mc, 3 with a concentration greater than the DNA equivalent of 1 cell per 100,000 cells tested (range 2.26-10.34). All but one of these 15 patients had a son before disease. To trace the origin of male DNA, we HLA-genotyped the patients and their sons and performed HLA-specific quantitative PCR to identify son-specific HLA sequences in the RNs. Of 7 patients studied with this approach, 5 were positive. An older male sibling or a miscarriage of a male fetus could be the source of male DNA in the patients who were negative for son-specific DNA. In summary, we provide evidence for Mc in the RN that was primarily of fetal origin, consistent with our hypothesis for RN formation.
Presenter: Chao, Cheng-Chi

**Anti-IL-1RAcP Therapy in Collagen-induced Arthritis**

Cheng-Chi Chao, Smiley Chen, Stefan Pflanz, and Eddie Bowman, Merck

IL-1 receptor accessory protein (IL-1RAcP) is an essential signal transducing component of the IL-1 receptor type I (IL-1R1) for the physiological activities of IL-1A or IL-1B. It is also used by at least six different IL 1-like cytokines. For example, IL-1RAcP and ST2 comprise the IL-33 receptor complex to bind IL-33 and signal its action. The signaling pathway for IL-1AcP involve the transcription factor NF-kB and the MAPKs p38 and JNK. Current clinical strategies are based on antagonizing IL-1B biology by blocking the ligand IL-1B or its specific receptor (IL-1R1). Targeting multiple pathways via IL-1RAcP may provide additional protective effects by neutralizing IL-1 and other IL-1 family members' pathobiologies. We assessed the in vivo efficacy of therapeutic anti-IL-1RAcP neutralization in collagen-induced arthritis. By dosing mice with early onset or therapeutic schemes, IL-1RAcP antagonism inhibited disease progression as assessed by visual disease scores. Histological data showed that anti-IL-1RAcP diminished the associated joint pathological changes such as inflammation, fibrosis, cartilage destruction, and bone erosion. The modulation of bone erosion was independently confirmed using micro-CT analysis, which demonstrated that anti-IL-1RAcP treatment protects against structural damage to the joint. In addition, anti-IL-1RAcP treatment lowers elevated serum RANKL and COMP in arthritic mice. These results indicate that IL-1RAcP may play a major role in driving joint destruction and that anti-IL-1RAcP neutralization could provide an additional therapeutic strategy for the treatment of rheumatoid arthritis.
Presenter: CHOI, YOUN SOO

Tfh differentiation is instructed by ICOS induction of Bcl6 during priming and competes with IL-2 signaling

YOUN SOO CHOI, Robin Kageyama, Danelle Eto, Tania C. Escobar, Robert J. Johnston, Laurel Monticelli, Christopher Lao, and Shane Crotty
La Jolla Institute for Allergy and Immunology

Germinal center formation and the development of most B cell memory and long term antibody responses requires a highly specialized helper CD4 T cell subset called follicular helper CD4 T cells (TFH). Even though Bcl6 is known as a master regulator for TFH differentiation, the nature of TFH cell differentiation remains controversial, because of a lack of information for when and how Bcl6 is induced for TFH differentiation commitment. Here we determine that TFH differentiation occurs immediately during priming in vivo. We demonstrate that ICOS is a critical early signal to induce Bcl6, and Bcl6 then induces CXCR5 expression, the canonical feature of TFH. Strikingly, a bifurcation between TFH and non-TFH is measurable by the second cell division of CD4 T cells, at day 2 after an acute viral infection: IL-2RAint cells express Bcl6 and CXCR5 (TFH program), whereas IL-2RAhi cells exhibit strong Blimp1 expression that represses Bcl6 (non-TFH program). Virtually complete polarization between Bcl6+ TFH and Blimp1+ non-TFH populations develops by 72 hours, all of which occurs in the absence of B cells. TFH are subsequently lost in the absence of B cells, demonstrating a B cell requirement for maintenance of Bcl6 and TFH commitment. High affinity neutralizing antibodies are generated from germinal center reaction by B cells and are the source of protective immunity for the vast majority of licensed human vaccines (mostly likely 24 out of 26). Therefore, understanding the essential role of TFH in driving the generation of protective antibody responses by B cells is a key area of investigation for rational vaccine design.
Presenter: Corse, Emily

Attenuated T Cell Responses to a High-Potency Ligand In Vivo

Emily Corse, Emily Corse, Rachel A. Gottschalk, Michelle Krogsgaard, James P. Allison
Memorial Sloan-Kettering Cancer Center

Alpha/beta T cell receptor (TCR) recognition of foreign peptides bound to major histocompatibility complex (pMHC) molecules on the surface of antigen presenting cells is a key event in the initiation of adaptive cellular immunity. In vitro, high-affinity binding and/or long-lived interactions between TCRs and pMHC correlate with high-potency T cell activation. However, less is known about the influence of TCR/pMHC interaction parameters on T cell responses in vivo. We studied the influence of TCR/pMHC binding characteristics on in vivo T cell immunity by tracking CD4+ T cell activation, effector, and memory responses to immunization with peptides exhibiting a range of TCR/pMHC half-lives and in vitro T cell activation potencies. Contrary to predictions from in vitro studies, we found that optimal in vivo T cell responses occur to ligands with intermediate TCR/pMHC half-lives. The diminished in vivo responses we observed to the ligand exhibiting the longest TCR/pMHC half-life were associated with attenuation of intracellular signaling, expansion, and function over a broad range of timepoints. Our results reveal a level of control over T cell activation in vivo not recapitulated in in vitro assays, and highlight the importance of considering in vivo efficacy of TCR ligands as part of vaccine design.
Presenter: Dahiya, Yogesh

Role of prostaglandin E2 in peptidoglycan mediated iNOS expression in mouse peritoneal macrophages in vitro.

Yogesh Dahiya, Rajeev K Pandey, Kunal H Bhatt, Ajit Sodhi
School of Biotechnology Banaras Hindu University, Varanasi INDIA

One of the hallmarks of classical activation of macrophages in mice and humans is production of nitric oxide through activation of high output pathway of NO production, iNOS pathway. Many extracellular stimuli, e.g. microbial products, cytokines etc., result in the expression of inducible nitric oxide synthase (iNOS) in macrophages. However, it is not known whether expression of the iNOS gene in response to microbial products is a primary response of macrophages or is the result of paracrine/autocrine signalling induced by endogenous biomolecules that are synthesised as a result of host cell-microbe interaction. We have observed that iNOS expression in mouse peritoneal macrophages in response to bacterial peptidoglycan (PGN) is a secondary effect requiring autocrine/paracrine signalling of endogenously produced prostaglandin E2 (PGE2), and that PGN stimulation is mandatory, but not sufficient in itself, for induction of iNOS expression. In PGN induced iNOS expression PGE2 plays important role along with other autocrine/paracrine signalling molecules like IFNγ, TNFa, and IFNβ. However, PGE2 alone is not sufficient to induce iNOS expression and NO production. Absolute requirement of a microbial product for the induction of iNOS seems to be a evolutionary mechanism to prevent unnecessary damage to the tissues due to NO in absence of microbial infection since PGE2 is produced in many conditions that may not be associated with any microbial infection. Given various anti-inflammatory and immunosuppressive effects of PGE2 it is quite possible that some of them may be due to the ability of PGE2 to induce NO production.
Regulatory T cells (Tregs) are necessary to preserve T cell homeostasis and prevent autoimmunity. Recent studies revealed that Tregs migrate to the afflicted tissues and actively suppress the autoimmune insult at tissue interfaces. Moreover, it is now believed that Tregs tailor their immuno-suppressive activity to suppress a specific type of T effector cell response. The specific sensors that dictate such tailored responses are to be determined, but there is speculation that Tregs somehow survey their environment and are influenced accordingly. In addition to environmental triggers, clear evidence exists that post-translational modifications of Foxp3, a Treg lineage-specification factor, alter the efficiency and efficacy of the response. Moreover, modifications of Foxp3 have been shown to increase the fidelity of Foxp3 transcription and its binding to histone-modifying proteins. Herein, we report that mice whose Tregs bear an N-terminus-modified Foxp3 (Foxp3-fusion GFP, Foxp3-FGFP), whereby a GFP transcript is embedded into the first coding exon of Foxp3, are protected from developing autoimmune rheumatoid arthritis (RA), while succumb to an atypically aggressive type -1 diabetes (T1D). Protection from RA is evidenced by a block in autoantibody formation in the K/BxN model, suggesting attenuation of the immunologic phase of RA. Conversely, despite a normal distribution of Treg cells, NOD Foxp3-FGFP males develop early aggressive insulitis and diabetes. Foxp3-FGFP Tregs have an increased expression of Foxp3 protein, associated with over-expression of a number of signature genes related to Treg function. In particular, they over-express genes controlled by the Irf4 transcription factor, whose expression by Tregs is required for them to suppress Th2-mediated immune responses. Furthermore, we found that Foxp3-FGFP, compared with wild-type Foxp3, is differentially modified at the post-translational level. All in all, our data suggest that a simple modification of the N-terminus of Foxp3 affects the Treg mediated responses contributing to the dichotomous phenotypes observed in two distinct autoimmune diseases. We currently continue to dissect the underlying mechanisms at the molecular level.
Presenter: de Souza, Anjali

An Immunoregulatory role for CD4+ cells expressing Foxp3 and Rorgt.

Anjali de Souza, Steven F. Ziegler

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Abstract not to be posted.
Presenter: Deriu, Elisa

Taking iron from Salmonella: the probiotic Escherichia coli Nissle 1917 competes for iron in the inflamed gut

Elisa Deriu, Milad Pezeshki, Roxanna Ochoa, Heidi Contreras, Robert A. Edwards, Stephen Libby, Ferric Fang and Manuela Raffatellu

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Abstract not to be posted.
Salmonella Typhimurium is a common food-borne pathogen that causes localized enteritis in healthy individuals. A general host strategy to protect against bacterial infection is to reduce the availability of nutrients and essential metal ions. S. Typhimurium replicates to high numbers in the inflamed gut because it can acquire nutrients and essential metals in this hostile environment. Manganese is necessary for S. typhimurium pathogenicity but the role of manganese acquisition in the inflamed gut is not known. The two manganese transport systems that have been characterized in S. Typhimurium, SitABCD and MntH, have different structures and can transport manganese under different environmental conditions. We thus tested whether SitABCD and MntH are essential for acquiring manganese in the inflamed gut. We found that a double deletion mutation of both sitA and mntH in S. Typhimurium attenuated bacterial replication at localized sites of inflammation. Taken together, our data demonstrate that S. Typhimurium requires manganese in the inflamed gut for survival.
We have developed and characterized panels of monoclonal antibodies (mAbs) that recognize human NKG2A, NKG2C, or NKG2D. Balb/c mice were immunized with Ba/F3 cell transfectants that express the inhibitory NKG2A + CD94 receptor, the activating NKG2C + CD94 receptor, or the activating NKG2D + DAP10 receptor. Immunization with the NKG2A + CD94 transfectant generated one mAb (clone 131411) that specifically recognizes NKG2A by flow cytometry. The same fusion yielded several mAbs that cross-react with NKG2C, as well as mAbs specific for CD94. Immunization with Ba/F3 cells expressing NKG2C + CD94, or NKG2D + DAP10, generated mAbs specific for NKG2C or NKG2D, without NKG2A cross-reactivity. Specificity was examined by flow cytometry by screening the mAbs for the ability to stain the immunizing cell, and not irrelevant transfectants expressing other NKG2 family members or CD94. We show here that anti-human NKG2A (clone 131411) specifically recognized NKG2A transfectant cells, and not NKG2C, NKG2E/H, or CD94. In contrast, clone Z199 stained both NKG2A and NKG2E/H-expressing Ba/F3 transfectants, but was not cross-reactive with NKG2C or CD94. Negatively-selected human peripheral blood NK cells co-stained with clones 131411 and Z199. Taken together, these data suggest that clone 131411 is specific for human NKG2A.
Presenter: Dunmire, Samantha K.

Gene Expression Kinetics of Primary Epstein-Barr Virus Infection

Samantha K. Dunmire, Oludare A. Odumade, Jean L. Porter, Emily Bacherel-Gillespie, Henry H. Balfour, Jr., and Kristin A. Hogquist

University of Minnesota

Abstract not to be posted.
All cells have sensors that detect nucleic acids and trigger a cell-intrinsic antiviral response through the production of type I interferons. The RNA helicases RIG-I and MDA5 detect viral RNA, whereas some of the sensor(s) for viral DNA remain unknown. DNA exonucleases, particularly 3 Repair Exonuclease 1 (Trex1), regulate the cell-intrinsic antiviral response to DNA ligands. In contrast, nothing is known about exonuclease regulation of RNA-activated antiviral responses. We therefore hypothesize that a system analogous to Trex1 exists for the metabolism of viral RNA ligands. We examined SKIV2L, a component of the 3->5 RNA exosome and a recently defined lupus susceptibility allele, as a candidate negative regulator of antiviral RNA sensors. Preliminary evidence suggests that loss of SKIV2L dramatically increases the activation of RIG-I by viral RNA ligands. Remarkably, we find that endogenous RNA cleavage products of the IRE-1 endonuclease generated during the unfolded protein response (UPR) trigger an interferon response in SKIV2L-depleted cells but not in control cells. This finding suggests that SKIV2L prevents the activation of antiviral sensors by endogenous RNA products generated during sterile stress responses. This reveals a molecular mechanism by which cellular stress responses could be misinterpreted as infection, with potential implications for autoimmune disease.
Infections with Borrelia burgdorferi, the causative agent of Lyme Disease, result in the systemic enlargement of the lymph nodes with a loss of the demarcation between the T cell B cell areas. Despite a massive increase in cortical B cells and accumulation of Borrelia-specific plasmablasts in the medullary area, germinal centers are largely absent. The mechanisms underlying this lack of T-dependent B cell responses are unknown. Here we provide data indicating that a defect in the quality of T helper cell responses to B. burgdorferi might cause the development of a mainly T-independent Borrelia-specific B cell response. Multicolor FACS-analysis demonstrated the presence of CD4+ CD44hi ICOS+ CXCR5+ T follicular helper cells (TFH) in lymph nodes of Borrelia-infected mice that in frequency resembles those seen in lymph nodes undergoing strong germinal center responses. Thus, suggesting that T cells are activated appropriately following Borrelia-infection. However, purified lymph node T cells from infected, but not immunized, mice were unable to provide help for B cell proliferation when co-cultured with B cells isolated from lymph nodes of mice immunized with Borrelia-antigen in vitro. Thus, indicating the induction of functionally impaired TFH following Borrelia-infection. To begin to identify the mechanisms underlying the potential functional TFH impairment, we asked whether a T-dependent B cell response to an irrelevant co-administered antigen was induced in lymph nodes of Borrelia-infected mice. For that we transferred 106 influenza HA-specific CD4 transgenic T cells i.v. and immunized mice with influenza antigen in adjuvant s.c. in the presence or absence of infection with B. burgdorferi. The results suggest that influenza HA-specific TFH are induced appropriately in lymph nodes unable to support Borrelia-specific TFH development. The results point to a lack of effective priming of Borrelia-specific TFH, potentially explaining the ineffective immune response induced to B. burgdorferi.
Presenter: Enos, Megan

A Role for the Transcription Factor IRF4 in the Development and Function of Innate-like Lymphocytes

Megan Enos, Amanda Prince and Leslie Berg
UMass Medical School

In the process of determining the transcription factors responsible for the development of the innate-like T cell phenotype that arises in the absence of Itk, we found there to be a decrease in IRF4 expression. IRF4 is a protein in the Interferon Responsive Family (IRF) of transcription factors and most are induced downstream of interferon signaling. However, IRF4 is best known to be expressed after B Cell Receptor (BCR) ligation. IRF4 levels in B cells determine the outcome of the Plasma Cell versus Memory B Cell lineage fate choice. Thus, we hypothesized that IRF4 levels were potentially responsible for the Conventional versus Innate-like lineage fate choice in T cells. Itk−/− T cells did not upregulate IRF4 in response to T Cell Receptor (TCR) ligation, but IRF4 could be detected after stimulation with the activating biochemistry, PMA and Ionomycin. Utilizing a T cell-specific conditional knockout, Irf4fl/fl CD4Cre+, we illustrated that the CD8+ T cells had a similar phenotype to the CD8+ T cells in the periphery of the Itk−/− mice. The splenic CD8+ T cells expressed high levels of CD44, CD122 and CXCR3. Additionally, the majority of the cells expressed very high levels of Eomesodermin, a transcription factor partly responsible for IFNγ expression. Not surprisingly, the IRF4 conditional knockout cells produced high amounts of IFNγ when stimulated with PMA and Ionomycin, and cytokine levels were similar to those produced by the Itk−/− cells. However, unlike the Itk-deficient cells, the Irf4fl/fl CD4Cre+ cells made IFNγ in response to TCR-ligation signals. Finally, the innate-like phenotype seen in the Itk−/− CD8+ T cells, as well as in the Id3−/−, Klf2−/− and Cbp−/− T cells, has been attributed to the expression of IL-4 by CD4+ T cells. The cytokine is over-expressed and binds to IL4Ra on the surface of CD8+ T cells, causing the upregulation of Eomesodermin. In contrast, T cells lacking IRF4 have been shown not to respond to IL-4 and, of all the cell types assayed, IL-4 production was not detected in our conditional knock out mice. Thus we have discovered a T cell bearing an innate-like phenotype that cannot be attributed to, or mediated by an over-abundance of IL-4 signaling.
A Role for RBP-J in Regulating the Transition from Innate to Adaptive Immunity

Julia Foldi, Xiaoyu Hu, Lionel B. Ivashkiv

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The master regulator transcription factor of the canonical Notch pathway, RBP-J, is increasingly recognized to have important roles not only in the development of the immune system but also in regulating immune function. Our lab has previously found that RBP-J had an important role in the production of the IL-6, IL-12 family of cytokines in response to Toll like receptor (TLR) stimulation in macrophages and also found a role for RBP-J in TLR-induced expression of the Notch ligand, Jagged1. To identify additional genes regulated by RBP-J, which might have a role in the transition from innate to adaptive immunity, we conducted a microarray analysis of bone marrow derived macrophages (BMDMs) isolated from mice with an inducible deletion of RBP-J and WT littermate controls. To our surprise, we found a group of MHC Class II-related genes including H2-Aa, H2-Ab1 and H2-Dma to be overexpressed at baseline in RBP-J deficient macrophages. Using quantitative real time PCR (qPCR), we have confirmed increased mRNA expression of H2-Ab1 and H2-Dma in RBP-J deficient bone marrow- and spleen-derived macrophages and dendritic cells (DCs). Furthermore, we have found increased surface expression of the MHC Class II molecule as well as the co-stimulatory molecule, CD40, on freshly isolated splenic DCs and macrophages from RBP-J deficient mice. Additionally, in an in vitro mixed lymphocyte reaction, RBP-J deficient DCs and macrophages induced increased OVA-specific proliferation and IL-2 production of CD4+ OT-II transgenic T cells. Taken together, we hypothesize that the Notch master regulator, RBP-J, might negatively regulate certain genes involved in antigen presentation to and co-stimulation of CD4+ T cells, with a possible role in controlling T cell activation. Experiments addressing the possible role of RBP-J in in vivo T cell activation and differentiation are currently under way.
CD4+ T-cells can differentiate into one out of few possible lineages, each invoking a specific immune response. This decision is influenced by the spectrum of cytokine signals the cells sense. IL-12 and IL-4 direct differentiation into the Th1 and Th2 lineages, respectively. This system is usually studied under polarizing conditions applying only one signal, typically together with antibodies against other cytokines. This allows for characterization of relevant molecular pathways and construction of the regulatory network involved. However, this approach is limited in its ability to describe the actual dynamic decision process, in which opposing pathways interact in a complex way. Moreover, in vivo, cells are expected to be simultaneously exposed to many signals with potentially opposing effects. Thus, understanding the logic of the decision process may be facilitated by mapping the system's response to mixtures of input signals. Here, we mapped the response of naïve CD4+ T cells to mixtures of IL-12 and IL-4. We measured, at the single cell level, the expression levels of the two lineage specific master transcription regulators, Tbet and GATA3, as well as the two lineage characteristic cytokines, IFN-Γ and IL-4. Our single cell analysis reveals that under mixed conditions, naïve T cells can be driven into a mixed Th1-Th2 state, in which individual cells co-express both lineage specific transcription factors, Tbet and GATA3. Under these conditions, expression of the lineage specific cytokines is highly heterogeneous, with subgroups of cells producing only IFN-Γ, only IL4, both cytokines, or neither. Interestingly, each cell produces the lineage specific cytokines, IFN-Γ and IL4, following two independent stochastic processes. Our results suggest a novel model for T cell differentiation, where under uncertain conditions, rather than following a hard, binary decision, cells reflect uncertainty through a biased stochastic decision process. This strategy can allow for flexibility of the response at the single-cell level, while maintaining an average, possibly optimized response at the cell population level.
Presenter: Gall, Alevtina

Origins and Progression of a Type 1 Interferon Dependent Autoimmune Disease

Alevtina Gall, Piper Treuting, Daniel Stetson
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Type-1 interferons (IFNs) are essential for antiviral defense, but can also contribute to autoimmune disease. During viral infection, foreign nucleic acids are detected in the cytosol and trigger the interferon stimulatory DNA (ISD) pathway, leading to the induction of type-1 IFNs. Similarly, autoimmune disease can be triggered through the ISD pathway, but in this case the nucleic acids are endogenous instead of foreign. Previous work by our group identified 3 repair exonuclease 1 (Trex1) as an essential negative regulator of the ISD pathway. Mutations in the human trex1 gene cause a spectrum of severe autoimmune diseases, including Aicardi-Goutieres syndrome and chilblain lupus, but the cellular basis of these diseases remains undefined. Using Trex1-deficient mice as a model, we have developed new tools to track the initiation and progression of this IFN-dependent autoimmune disease in vivo. Using mice that report IFN activity, we show the localization of the type-1 IFN response during initiation of disease. We also find that non-hematopoietic cells in the heart first activate type I interferons shortly after birth. Moreover, we establish that both αβ T cells and B cells are essential for disease. Finally, we identify key heart autoantigens that are targeted by the autoantibody response in vivo. Together, these findings provide an integrated picture of autoimmunity from its initiation through pathology, with important implications for the human diseases associated with Trex1 mutations.
Presenter: Garcia-Chagollan, Mariel

Association between serum levels of IL-15, TNF-alpha, MICA and MICB with the frequency of CD4+NKG2D+ T cells in patients with uterine cervix abnormalities

Mariel Garcia-Chagollan, Luis Felipe Jave-Suarez, Pedro Sanchez-Hernandez, Benibelks Albarran-Somoza, Adriana Aguilar-Lemarroy, Jesse Haramati, Angel Cid-Arregui, Susana del Toro-Arreola

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INTRODUCTION. The NKG2D receptor confers important activation signals to NK cells via ligands expressed during cellular stress and viral infection. This receptor has generated great interest because not only is it expressed by NK cells, but it is also seen in virtually all CD8+ cytotoxic T cells and absent in CD4+ T cells. However, recent studies have identified a rare population of CD4+ T cells that do express NKG2D and which could represent a particular cytotoxic effector population involved in viral infections and chronic diseases. Cytokines such as IL-15 and TNF-alpha, as well as soluble NKG2D ligands, have been associated with the expression of NKG2D on CD4+ T cells. OBJECTIVE. To associate the serum levels of IL-15, TNF-alpha, MICA and MICB with the frequency of CD4+NKG2D+ T cells in patients with uterine cervix lesions including intraepithelial neoplasia grade I (CIN-I) with or without infection with human papillomavirus (HPV), as well as invasive cancer. METHODS. Three-color flow cytometry, using blood from CIN-I positive women, cervical cancer patients and a control group was used to assess CD3+CD4+NKG2D+ T cells. Genotyping of the 37 types of HPV found in cervical epithelium was performed using PCR and subsequent hybridization with colorimetric detection. ELISA used to quantify serum levels of cytokines and NKG2D ligands. RESULTS. As expected, more than 50% of the patients diagnosed as CIN-I positive women, practically all women with invasive cancer were positive for HPV infection. The percentage of CD3+CD4+ T cells co-expressing the NKG2D receptor increased in both groups of patients versus controls. Interestingly, this increase was higher in CIN-I patients with HPV infection versus patients negative for HPV. However, the overall levels of CD3+CD4+ cells did not increase. Importantly, serum levels of TNF-alpha were not detected. At the moment, the highest serum levels of IL-15, MICA and MICB have been found in both groups of patients versus controls; however, we still need to increase the number of individuals to associate these levels with the frequency of CD4+NKG2D+ T cell population.
Salmonella enterica serovar Typhimurium (S. Typhimurium) causes acute intestinal inflammation accompanied by an early, IL-23-dependent induction of IL-17A expression. Previous studies have shown that depletion of T cells markedly reduces, but does not abolish, expression of IL-17A in the intestinal mucosa. We noted that IL-23 receptor (IL-23R) was not only expressed by a subset of ΓΔ T cells, NKT cells, Th17 cells and CD8 T cells, but also by a CD3-negative cell population in the intestinal mucosa. To further investigate the contribution of this CD3-negative cell population to IL-17A expression during S. Typhimurium colitis, we utilized tcrbd-/- mice, which are deficient for AB and ΓΔ T cells. Infection of tcrbd-/- mice with S. Typhimurium resulted in pathology and IL-17A cytokine expression. Utilizing flow cytometry we identified a population of cells expressing IL-23R. This population was further characterized and found to be CD3-, CD4-, B220-, Nkp46-, GR-1-, CD11b-, c-Kit- SCA-1+ and CD90.2+ but lacked markers associated with mature lymphocytes. This cell type thus represented innate lymphoid cells, which have recently been identified as producers of IL-17A and mediators of innate inflammatory responses. Subsequent experiments provided data that indicate that these cells can mediate inflammatory responses to S. Typhimurium. We conclude that innate lymphoid cells may be important contributors to the early, innate inflammatory response to bacterial infection in the intestinal mucosa.
TCR ligation is required for the extrathymic differentiation of Foxp3+ regulatory T cells. Several lines of evidence indicate that weak TCR stimulation favors induction of Foxp3 in the periphery; however, it remains to be determined how TCR ligand potency influences this process. We characterized the density and affinity of TCR ligand favorable for Foxp3 induction and found that a low dose of a strong agonist resulted in maximal induction of Foxp3 in vivo. Initial Foxp3 induction by weak agonist peptide could be enhanced by disruption of TCR/peptide-MHC interactions or alteration of peptide dose. However, timecourse experiments revealed that Foxp3 positive cells induced by weak agonist stimulation are deleted, along with their Foxp3 negative counterparts, while Foxp3 positive cells induced by low doses of the strong agonist persist. Our results suggest that together, peptide-MHC ligand potency, density, and duration of TCR interactions define a cumulative quantity of TCR stimulation that determines initial peripheral Foxp3 induction. However, in the persistence of induced Foxp3+ T cells, TCR ligand potency and density are non-interchangeable factors that influence the route to peripheral tolerance.
**Presenter: Greenberg, Milton**

**TLR4-activated B cells out-compete TLR9-activated B cells to establish tolerance via CTLA-4-dependent motile B-T cell conjugates**

*Milton Greenberg, Yan Su and Melanie P. Matheu, Caroline Blanc, Ai-Hong Zhang, Xin Li, Elizabeth Kadavil, Ian Parker, Michael Cahalan and David W. Scott*

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Antigen presentation by B cells can generate long-lived antigen-specific T cell tolerance. Using two-photon microscopy we directly compared T-B cell interactions in the lymph node during tolerance induction or priming of OTII T cells by B cells expressing membrane bound ovalbumin (mOVA B cells). Naïve mOVA B cells and LPS-activated mOVA B cells induced long-lived peripheral tolerance, suppressing OTII T cell proliferation and T-cell-dependent antibody production in response to antigen challenge in vivo. In contrast, CpG-activated mOVA B cells did not induce tolerance to OVA. Naïve, LPS- or CpG-activated mOVA B cells individually were capable of establishing long-lived interactions with OTII-T cells. T-B cognate pairs migrated for up to 2 hours with B cells in the lead. However, CpG-activated mOVA B cell did not form long-lived cognate pairs when tolerogenic LPS-treated mOVA B cells were also present. Moreover, LPS-activated B cells preferentially migrated to the lymph node and disrupted established CpG-activated mOVA T-B pairs. Disruption of CTLA-4 / B7 interactions prevented establishment of long-lived T-B cognates and inhibited tolerance induced by LPS-treated B cells in vivo. Our findings indicate that differential TLR activation of B cells can induce tolerance hierarchically, enabling LPS-activated B cells to out-compete CpG-activated B cells. Furthermore, we demonstrate three potential mechanisms of peripheral tolerance induced by LPS-activated B cells: the establishment of long-lived CTLA-4-dependent cognate pairs between OTII T and mOVA B cells; enhanced CD62L-mediated homing to peripheral immune organs; and a physical mechanism of direct cellular competition.
Presenter: Haberthur, Kristen

Virus-specific CD4+ T cells are necessary for protection against simian varicella virus

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Varicella zoster virus (VZV) is the causative agent of varicella, or chickenpox. The reactivation of latent VZV results in herpes zoster (HZ), or shingles, which causes significant morbidity and occasionally mortality in the elderly and immune compromised. The currently FDA-approved vaccines against chickenpox and HZ have limited efficacy. One of the difficulties in developing a more efficacious vaccine is that the immune correlates of protection against VZV are not fully understood. Clinical observations suggest that cellular immunity is more important than humoral immunity in successfully controlling VZV. However, dissecting the role of T versus B cell immunity in response to VZV infection has been hampered by the lack of an adequate animal model. Our laboratory recently developed a non-human primate model wherein rhesus macaques (RM) are inoculated with simian varicella virus (SVV), a homolog of VZV. This novel model provides a unique opportunity to study host-pathogen interactions during VZV infection. The goal of this study was to identify the immune correlates of protection against primary SVV infection. To accomplish this we compared disease severity and immune response in three groups of RM infected with SVV: (1) CD20+ B cell-depleted; (2) CD8+ T cell-depleted; and (3) CD4+ T cell-depleted. We show that the loss of CD20+ B cells does not affect the ability to effectively respond to SVV infection, nor does it result in increased viral loads when compared to non-depleted animals. We also show that while the depletion of CD8+ T cells results in increased SVV viral titers, these animals were able to successfully respond to and clear acute SVV infection. However, we show for the first time that the depletion of CD4+ T cells results in higher SVV viral titers; disseminated viremia; reduced and delayed T cell, B cell, and antibody responses; and decreased CD8 CM T cell effector functions. Thus, similar to clinical findings regarding VZV control in children, the ability of young rhesus macaques to control acute SVV infection is most likely due to cellular immunity rather than humoral immunity. These data provide the framework for future studies in immune senescence and its role in VZV reactivation.
Presenter: Hammer, Gianna

Dendritic cells require A20 to preserve immune tolerance and intestinal homeostasis
Gianna Hammer, Gianna Elena Hammer, Emre E. Turer, Celia J. Fang, Shigeru Oshima, Eric J. Huang, Barbara A. Malynn, Boris Reizis, Mary C. Nakamura, and Averil Ma
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Abstract not to be posted.
CD4 T cells affect magnitude of B cell responses but not protection following Borrelia burgdorferi infection

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Abstract not to be posted.
A novel immunoglobulin-like receptor, Allergin-1, inhibits IgE-mediated allergic responses.

Kaori Hitomi, Satoko Tahara-Hanaoka, Satoru Someya, Akira Fujiki, Hideaki Tada, Tetsuya Sugiyama, Shiro Shibayama, Kazuko Shibuya, Akira Shibuya

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A novel immunoglobulin-like receptor, Allergin-1, inhibits IgE-mediated allergic responses. Kaori Hitomi(1), Satoko Tahara-Hanaoka(1), Satoru Someya(1), Akira Fujiki(2), Hideaki Tada(2), Tetsuya Sugiyama(2), Shiro Shibayama(2), Kazuko Shibuya(1), and Akira Shibuya(1). (1)Department of Immunology, Institute of Basic Medical Sciences, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan. (2)Exploratory Research Laboratories, Tsukuba Research Institute, Ono Pharmaceutical, Tsukuba, Japan.

Anaphylaxis is a life-threatening immediate hypersensitivity reaction triggered by antigen capture by immunoglobulin E (IgE) bound to the high-affinity IgE receptor (Fc&Episilon;RI) on mast cells. However, the regulatory mechanism of mast cell activation is not completely understood. Here we identify an immunoglobulin-like receptor, Allergin-1, that contains an immunoreceptor tyrosine-based inhibitory motif (ITIM)-like domain, and show it was preferentially expressed on mast cells. Mouse Allergin-1 recruited the tyrosine phosphatases SHP-1 and SHP-2 and the inositol phosphatase SHIP. Coligation of Allergin-1 and Fc&Episilon;RI suppressed IgE-mediated degranulation of bone marrowderived cultured mast cells. Moreover, mice deficient in Allergin-1 developed enhanced passive systemic and cutaneous anaphylaxis. Thus, Allergin-1 suppresses IgE-mediated, mast celldependent anaphylaxis in mice.
Intracellular fate of phagosomes following different receptor engagement in bone marrow-derived dendritic cells

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An efficient initiation of immune responses to invading pathogens is highly dependent on the discrimination of self from non-self antigens. Professional phagocytes, such as dendritic cells (DCs), macrophages and neutrophils, are key players by recognizing, internalizing and destroying both classes of antigens by phagocytosis using specific receptors at their surface to distinguish between different ligands. During maturation, the formed phagosome develops into a functional organelle, in which pH, the activity of hydrolytic enzymes and the production of radical compounds are highly regulated. Especially in DCs, phagosome maturation is required to mount a successful adaptive immune response by an effective processing and presentation of peptides on MHC molecules. However, it is still matter of debate whether each phagosome undergoes a distinct maturation program directly dictated by the involved receptor type, also known as phagosomal autonomy, or whether it is controlled on the cellular level determining the fate of all phagosomes as well as the outcome of the immune response. Here, we present a system using latex beads conjugated with specific ligands, such as IgG, LPS and BSA, engaging different receptors together with OVA as model antigen allowing us to study the control of phagosome maturation simultaneously within the same cell. Bone marrow-derived DCs and macrophages were allowed to internalize these beads, were sorted according to their phagocytic content and phagosomes were isolated from these cells. Subsequently, we used highly quantitative measurements based on flow cytometry approaches to determine the regulation of the phagosomal milieu as well as antigen processing and degradation. Additionally, we analyzed the cross-presentation activity in context of the engaged receptors during internalization and maturation. We found that IgG is able to accelerate the degradation of OVA within phagosomes in comparison to BSA used as a bead ligand. These differences were even more obvious in mature cells stimulated by cytokines where phagosomes containing BSA beads exhibited a delay in phagosome maturation, whereas phagosomes containing IgG beads did not. When both types of phagosomes were present in the same cell, their intracellular fate was dependent on their respective content. The obtained results suggest phagosomal autonomy during maturation restricted to the engaged receptor pathway in both, DCs and macrophages, but also uncover remarkable differences comparing immature and mature cells.
Presenter: Horai, Reiko

Breakdown of immune privilege and spontaneous autoimmunity in retina-specific T cell receptor transgenic mice

Reiko Horai, Phyllis B. Silver, Jun Chen, Ru Zhou, Rajeev K. Agarwal, Mary J. Mattapallil, Peng Wang, Chi-Chao Chan, Rachel R. Caspi
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Abstract not to be posted.
Presenter: Hu, Wei

Priming microenvironments dictate cytokine requirements for Th17 lineage commitment

Wei Hu, Wei Hu, Ty Dale Troutman, Ramakrishna Edukulla and Chandrashekhar Pasare
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Abstract not to be posted.
Presenter: Irena, Ivanovska

Jak signaling pathways in RA: Interrogation of biology with siRNA

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Increased proinflammatory cytokine signaling has been implicated as the molecular mechanism of many autoimmune diseases, including rheumatoid arthritis (RA). Cytokines signal through cell surface receptors by eliciting receptor dimerization, activation of the receptor-associated Janus kinases (Jak) and subsequent phosphorylation of the Signal Transducer and Activators of Transcription (STATs). The cytokine/Jak/STAT signal transduction network is characterized by crosstalk, subunit interdependency and receptor sharing. Determining the significance of individual components to the disease mechanism and identifying inhibitory compounds to specific proteins has been a challenge. We undertook a systematic investigation of the requirement for individual Jak isozymes in a panel of cytokine-activated signaling pathways using siRNA-mediated gene silencing in primary peripheral blood mononucleocytes (PBMCs) and in the erythroleukemia TF-1 cell line. In addition, we employed multiplex assays to monitor up to four read-outs simultaneously. We demonstrated efficient siRNA-mediated gene silencing in both cell systems and confirmed the involvement of several previously-known signaling cascades. Interestingly, in TF-1 cells we found that Jak1 but not Jak3 mediated the activation of STAT6 by IL-4. This result was confirmed by lack of inhibition of IL-4 signaling by a Jak3-specific inhibitor. In contrast, we found that both Jak1 and Jak3 contribute to cytokine signaling in PBMCs, albeit to different degrees. Our systematic analysis in a disease-relevant, primary cell system has expanded our understanding of the subtleties of the complex network interactions in the Jak/STAT signaling pathway.
**Presenter: Jang, Eun Jung**

Lysine 313 of T-bet is essential for DNA association and dimerization with NFAT in T helper cells.

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T-bet, a T-box-containing protein expressed in T cells, is known as a key transcription factor involved in the regulation of T helper (Th) cell differentiation. T-bet-deficient CD4+ Th cells have impaired IFN-γ production and typically differentiate into Th2 or Th17 cells in vitro. However, ectopic expression of T-bet reversed this differentiation through interaction with other transcription factors. In this study, we examined the critical roles of lysine 313 of T-box domain on T-bet function. We identified that lysine 313 is important for DNA interaction of T-bet and subsequent activation of IFN-γ gene transcription. In addition to DNA binding activity, lysine 313 was also involved in the interaction with NFAT, thus affected production of a variety of cytokines in CD4+ Th cells. T-bet mutated in lysine 313 was not able to inhibit NFAT-induced gene transcription of IL-2, Th2 and Th17 cytokines. These results indicate that lysine 313 of T-bet is critical for DNA binding, heterodimerization with NFAT, and subsequent modulation of Th cell development. Key words: T-bet; lysine; T helper cell; DNA binding; NFAT.
Follicular helper T cells (TFH) are the CD4+ T cell effector subset that is specialized to provide B cell help. TFH differentiation is controlled by the transcription factor Bcl6, which is necessary and sufficient for TFH differentiation and function in vivo. It has been proposed that Bcl6 expression is induced by STAT3-mediated IL-6 and IL-21 signals, akin to the cytokine and STAT-driven differentiation pathways of other effector subsets. Here, we have examined roles of STAT signaling in TFH differentiation. Surprisingly, STAT3-deficient CD4+ T cells differentiated normally into TFH cells, expressed IL-21, and drove germinal center formation in response to acute viral infection or protein immunization. Furthermore, constitutive STAT3 signaling was unable to drive TFH differentiation. We next investigated the importance of STAT5, which has also been reported to regulate Bcl6 expression in lymphocytes. We found that constitutive STAT5 signaling in activated CD4+ T cells was a potent inhibitor of TFH differentiation and function. Conversely, STAT5-deficient CD4+ T cells (mature STAT5-floflo CD4+ T cells transduced with a Cre-expressing retrovirus) very preferentially differentiated into TFH cells. To identify the mechanism of STAT5-mediated inhibition of TFH differentiation, we investigated the role of Blimp-1, a direct repressor of Bcl6 expression and TFH differentiation that is known to be induced by STAT5 and IL-2. We found that constitutive STAT5 signaling was unable to inhibit TFH differentiation in Blimp-1 deficient cells, indicating that STAT5 collaborates with Blimp-1 to negatively regulate TFH differentiation.
Presenter: Kaattari, Ilsa M.

Plasmablast, Plasma Cell, and Memory Cell Disposition in the Absence of Bone Marrow and Lymph Nodes

Ilza M. Kaattari, Jianmin Ye, Stephen L. Kaattari

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Structural and functional associations of humoral immunity that transcend evolutionary time and body plans emphasize their critical nature. We have found such associations occur between lymphopoiesis and the maintenance of long-lived plasma cells in teleosts (bony fish) and mammals. This essential juxtaposition, however, occurs in the anterior kidney (AK) of teleosts but in the bone marrow of mammals. Further, at a time point when the teleost lymphopoietic tissue becomes heavily populated with plasma cells (2-3 months post-immunization), the average secretion rate of these cells and the antibody affinity increases ten-fold. After a year, antigen-sensitive precursors (memory cells) increase approximately one thousand-fold in the teleost and are found primarily in the peripheral circulation; however, these memory cells yield only small clone sizes upon antigen stimulation. The class of antigen (T-dependent vs. T-independent) determines unique distributions of antibody secreting cells (ASCs)---both plasmablasts and plasma cells---within immune tissues. Specifically, TNP-KLH, a T-dependent antigen, produces a distribution of ASCs skewed to the AK, while TNP-LPS, a T-independent antigen, induces a more uniform distribution of ASCs throughout the blood, AK, and spleen.
Following acute infection in some mouse models, Th1 memory cells steadily decline over time. Conversely, in humans CD4+ memory T cells can be maintained for many years at rates similar to CD8+ T cells. Because we previously observed that the longevity of Th1 memory cell survival corresponded to their functional avidity, we hypothesized that secondary challenge, which enriches for high functional avidity Th1 responders, would result in more stable Th1 memory populations. We indeed found that secondary Th1 memory cells were maintained at stable levels as compared to primary Th1 memory cells, showing little to no decline after day 75 post-infection. The improved stability of secondary Th1 memory T cells corresponded to enhanced homeostatic turnover, increased expression of the anti-apoptotic molecule Bcl-2 and rapid conversion to high functional avidity following secondary challenge. Our findings suggest that the longevity of Th1 memory T cells is dependent, at least in part, on the combined effects of primary and secondary antigen-driven differentiation.
USP8 controls T-cell development and immune homeostasis

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Abstract not to be posted.
The Equine Immune Response to Corynebacterium pseudotuberculosis

*Marcellina Claudia Kolonas, Marcellina Claudia Kolonas, Dina Abdel-Massih, Luca Valle, and Roberta Pollock*

Occidental College, Department of Biology

Corynebacterium pseudotuberculosis is a facultative intracellular pathogenic bacterium. While two forms or biovars of the bacteria exist, we are studying the equi biovar, which infects horses and cattle. The major secreted exotoxin is phospholipase D (PLD), which catalyzes the hydrolysis of ester bonds in phospholipids, hence eventually contributing to the spread of the bacteria. Since bacteria stay protected inside abscesses within thick enclosing capsules, antibiotics and other forms of treatment are ineffective. This leads to the main goal of the study: the development of a vaccine against C. pseudotuberculosis. One important stepping-stone is the comparison of antibody responses in different manifestations of the disease. We developed an Enzyme Linked ImmunoSorbent Assay (ELISA) to detect antibodies against PLD, and utilized this assay to study the linkage between the levels of IgG and IgA and the different manifestations of the disease. Further understanding of which antibody response triggers the respective manifestations is crucial for vaccine development. The final results were further analyzed for variables such as age group and type of manifestation (i.e. external multiple abscesses vs. external single abscesses).
Determining the cellular targets of TSLP during an allergic response
Ryan Larson, Ryan P Larson and Steven F Ziegler
Department of Immunology, University of Washington

Thymic stromal lymphopoietin (TSLP) is an IL-7 related cytokine produced by epithelial cells in response to tissue damage, inflammation, or TLR ligation. The presence of TSLP in the lesional skin of atopic dermatitis patients and asthmatic lungs suggests a role in the etiology of these allergic Th2 diseases. Overexpression of TSLP in mouse skin or lung leads to the development of atopic dermatitis and asthma, respectively; suggesting that TSLP is sufficient for the development of these diseases, however, these models do not elucidate the function(s) of TSLP. Furthermore, it has been hypothesized that TSLP serves to dampen aberrant Th1 responses at barrier surfaces, specifically in the intestine. We have utilized a contact hypersensitivity (CHS) model to determine what role TSLP plays in Th2 and Th1 responses, as well as to identify potential cellular targets of TSLP. Initially, we have observed a requirement for TSLP-TSLPR interactions for a Th2-type CHS response to the allergen fluorescein isothiocyanate (FITC). Specifically, TSLP receptor deficient (TSLPR-/-) mice lack the hallmark features of a Th2 response to FITC, with reduced IL-4 expression and eosinophilia in allergen challenged skin, as well as reduced serum IgE compared to FITC sensitized and challenged WT mice. The defective response in TSLPR-/- mice appears to be due to a significant reduction in frequency and number of FITC+CD11c+ DCs in the skin draining lymph nodes of FITC-sensitized TSLPR-/- mice. Furthermore, TSLPR-/-FITC+DCs express reduced CD86 and are impaired in their ability to drive proliferation of naïve CD4 T cells. In addition to defective DC function in TSLPR-/-, we have also observed reduced proliferation of CD4 T cells in TSLPR-/- mice, however, this is not a T cell intrinsic defect, as both WT and TSLPR-/- CD4 T cells proliferate less in TSLPR-/- hosts compared to WT hosts. Furthermore, in a mixed bone marrow chimera setting, TSLPR-/- CD4 T cells are equally capable of proliferating and entering inflamed tissue as their WT counterparts after FITC sensitization and challenge. Moreover, CD4 T cells do not require direct TSLP signals to mediate the FITC CHS response, as TCRß-/- mice that have received either WT or TSLPR-/- donor CD4 T cells experience equivalent FITC CHS responses. These data suggest a cell type other than CD4 T cells, such as DCs, are required to respond to TSLP to mediate the CHS response to FITC.
Presenter: Lee, Min Jeoung

Simvastatin acts as an inhibitor of interferon gamma-induced cycloxygenase-2 expression in human THP-1 cells, but not in murine RAW264.7 cells

Min Jeoung Lee, Min Jeoung Lee, Chang Seok Lee, Yong Jae Shin, Cheolhee Won, Yun-Song Lee, Chung-Gyu Park, Sang-Kyu Ye, Myung-Hee Chung
Seoul National University

Cyclooxygenase-2 (COX-2) is a key inflammatory response molecule, and associated with many immune functions of monocytes/macrophages. Particularly, interferon gamma (IFNγ)-induced COX-2 expression appears in inflammatory conditions such as viral infection and autoimmune diseases. Recently, statins have been reported to show variable effects on COX-2 expression, and on their cell and species type dependences. Based on the above description, we compared the effect of simvastatin on IFNγ-induced COX-2 expression in human monocytes versus murine macrophages. In a result, we found that simvastatin suppresses IFNγ-induced COX-2 expression in human THP-1 monocytes, but rather, potentiates IFNγ-induced COX-2 expression in murine RAW264.7 macrophages. However, signal transducer and activator of transcription 1/3 (STAT1/3), known as a transcription factor on COX-2 expression, is inactivated by simvastatin in both cells. Our findings showed that simvastatin is likely to suppress IFNγ-induced COX-2 expression by inhibiting STAT1/3 activation in human THP-1 cells, but not in murine RAW264.7 cells. Thus, we concluded that IFNγ-induced COX-2 expression is differently regulated by simvastatin depending on species specific mechanism.
An attempt was made to test the anti-oxidant activity of 7,8-dihydro-8-oxo-2-deoxyguanosine (8-oxo-dG). 8-Oxo-dG abolished 5,5-dimethylpyrroline-N-oxide-OH signal completely in electron spin resonance spectroscopy and inhibited HO? -induced oxidation of 2,7-dichlorodihydrofluorescein (DCHF). 8-oxo-dG also inhibited DCHF oxidations by peroxynitrite and low density lipoprotein oxidation by 1O2. In all oxidation systems used, 8-oxo-dG showed even stronger inhibition than the anti-oxidants tested, including synthetic: Trolox, N-acetylcysteine, present in the diets: ascorbate, β-carotene, (+)-catechin hydrate, quercetin dihydrate, and synthesized in the body: a-lipoic acid, β-estradiol, a-ketoglutaric acid, guanosine, L-carnosine, bilirubin, melatonin and uric acid. 8-Oxo-dG also showed effective prevention against the UV-induced skin reactions in mice, including increase of protein carbonyl contents, activations of ERK and p38, increase of MMP-9 and -13 expressions, and epidermal hyperplasia. Here, 8-oxo-dG was also more effective than the anti-oxidants tested. The potent anti-oxidant activity of 8-oxo-dG might be a beneficial property that might be used for the modulation of cell functions or treatment of various ROS associated disorders.
Presenter: Liu, Janet

Resistance to calprotectin-mediated zinc withholding promotes Salmonella Typhimurium growth in the inflamed gut.

Janet Liu, Janet Z. Liu(1,2), Adam J. Poe(1), Michele Pesciaroli(1,2,8), Thomas Kehl-Fie(5), Martin Hosking(2,4), Robert A. Edwards(3), Paolo Pasquali(9), Andrea Battistoni(10), Thomas E. Lane(2,4), Walter J. Chazin(6,7), Thomas Vogl(8), Eric P. Skaar(5), and Manuela Raffatellu(1,2)

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Abstract not to be posted.
Presenter: Lu, Rongze

**CEACAM1 down regulates Syk coupled neutrophil NLRP3 inflammasome activation through ITIM and the recruitment of SHP-1**

*Rongze Lu, Hao Pan, John E. Shively*

City of Hope Beckman Research Institute

IL-1ß is a critical pro-inflammatory cytokine produced by innate immune cells, and its maturation is regulated by inflammasome. Studies in macrophages identified several inflammasome activation signals, but whether they are applicable to neutrophils have not been comprehensively studied. Here, we show LPS induced neutrophils NLRP3 inflammasome activation depended on LPS triggered neutrophil ROS production and lysosome damage, both of which are regulated by upstream kinase Syk. Notably, LPS induced neutrophil Syk phosphorylation and inflammasome activation is subject to the negative regulation by CEACAM1. This inhibition required the presence of ITIMs in CEACAM1 long forms and the subsequent recruitment of phosphatase SHP-1. Pharmaceutical inhibition of Syk activation or genetic knock down of Syk attenuated Syk phosphorylation and caspase-1 activation in CEACAM1-/- neutrophils. Therefore, we identify CEACAM1 as a check point of neutrophil NLRP3 inflammasome activation and IL-1ß production.
Presenter: Lu, Rongze

CEACAM1 regulates myeloid-cell-dependent tumor angiogenesis by inhibition of G-CSFR-Bv8 pathway

Rongze Lu, John E. Shively
City of Hope Beckman Research Institute

Gr1+CD11b+ myeloid cells promote tumor angiogenesis and are refractory to VEGF antibodies treatment. Bv8, also known as Prokineticin 2, expressed in Gr1+CD11b+ cells has recently been shown to modulate Gr1+CD11b+ cells mobilization and tumor angiogenesis. However, the signaling pathways in Gr1+CD11b+ to promote tumor angiogenesis are not fully understood. Carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM1) is highly expressed on Gr1+ myeloid cells, however the role of CEACAM1 in myeloid cells mediated tumor angiogenesis has not been investigated. We have demonstrated that tumor growth and angiogenesis was significantly enhanced in Ceacam1-/- mice, which was independent on T and B cells by backcrossing Ceacam1-/- mice with Rag1-/- mice. Meanwhile Gr1+CD11b+ cells infiltration in tumor, spleen and blood was increased. Tumors coinjected with Gr1+CD11b+ cells from Ceacam1-/- mice exhibited increased angiogenesis compared to coinjection with Gr1+CD11b+ cells from control mice. Depletion of Gr1+ cells inhibited tumor growth and angiogenesis in control and Ceacam1-/- mice. Gr1+CD11b+ cells from Ceacam1-/- mice expressed much higher Bv8 in vitro and in vivo. Further we have shown that CEACAM1 recruited Src-homology-phosphatase-1 (SHP-1) to attenuate granulocyte colony-stimulating factor receptor (G-CSFR)-Bv8 pathway in myeloid cells. Reconstitution of CEACAM1 into Ceacam1-/- bone marrow cells restored tumor growth and angiogenesis to normal level, and mutations of immunoreceptor tyrosine-based inhibitory motifs ITIMs abolished this restoration. Therefore, we identified CEACAM1, as an inhibitory regulator of G-CSFR-Bv8 pathway, negatively regulates myeloid cells dependent tumor angiogenesis.
Presenter: Mariana, Xavier

Activation of the Caspase-1 inflammasome by the Type IV secretion system of Brucella spp.

Xavier Mariana, Hortensia G. Rolan, Vidya L. Atluri, Andreas den Hartigh, Renato Santos, Luigi Franchi, Thomas Henry, Denise Monack, Gabriel Nunez, Renee Tsolis

Universidade Federal de Minas Gerais, University of California at Davis

Abstract not to be posted.
Presenter: Matheu, Melanie

Three distinct phases of CD8+ T cell response to influenza A revealed by 2-photon imaging

Melanie Matheu, Melanie P. Matheu, Kevin B. Walsh, John R. Teijaro, David Marsolais, Hugh Rosen, Michael B.A. Oldstone, Ian Parker, and Michael D. Cahalan

University of California, Irvine

Using 2-photon microscopy, we imaged live naïve antigen-specific eGFP+ CD8+ T cells in lung tissue and mediastinal (draining) lymph node preparations from influenza-infected CD11c+-YFP mice over 14 days. Three distinct phases of T cell and CD11c+ dendritic cell (DC) behavior were revealed: 1) Priming (day 1-4); 2) Clearance (day 5-8); and 3) Maintenance (after day 9). During Priming, the lung tissue is cleared of dendritic cells, and the lymph node is populated by both motile and sessile DCs (average velocity of 7.8 \( \text{?m/min} \)) that are briefly contacted by motile T cells (14.2 \( \text{?m/min} \)). By day 3 of viral infection T cells have prolonged interactions with DCs and are proliferating. During Clearance, a second wave of highly motile DCs (7.1 \( \text{?m/min} \)) is seen in the lymph node, while sessile DCs with actively motile processes repopulate the lung. On day 6, slowly motile T cells (5.5 \( \text{?m/min} \)) appear in the lung, coinciding with significant decreases in viral titers. During phase 3 (Maintenance), T cells migrate more rapidly (11.6 \( \text{?m/min on day 10} \)). Progression from phase 1 to 2, defined by localization and behavior of T cell and DCs, is delayed by AAL-R, a sphingosine 1-phosphate receptor (S1P1,3,4,5) agonist prodrug.
Presenter: Miazgowicz, Mike

Inhibition of TSLPR via N-linked Glycosylation

Mike Miazgowicz, Mike Miazgowicz, Hai-Chon Lee, Mark Headley, Steve Ziegler
University of Washington, Benaroya Research Institute

Epithelial cells of the airway and lung have been shown to participate actively in the response to both pathogenic and allergenic environmental stimuli. In particular, the airway epithelium is a potent source of thymic stromal lymphopoietin (TSLP), a cytokine that is strongly associated with asthma and other atopic pathogenesis. In the lung, TSLP acting in concert with an antigenic stimulus is capable of inducing hallmarks of the asthmatic response: eosinophilia and inflammatory cell infiltrates, sub-epithelial fibrosis, goblet cell hyperplasia and mucus production, airway hyperresponsiveness, and Th2 cytokine and chemokine production. The receptor for TSLP, a heterodimer composed of TSLPR and CD127, is known to be expressed on cells of the hematopoietic system, most notably cells of the myeloid lineage such as dendritic cells. We present data here that the TSLPR receptor complex is also expressed on several non-hematopoietic cells, including lung epithelial cells. Interestingly, these non-myeloid and epithelial cells appear to express a heavily glycosylated form of TSLPR. By mutating glycosylation anchor residues in TSLPR, we can allow epithelial cells to respond to TSLP by upregulating TARC, a key mediator of Th2-type inflammatory responses. We speculate that the glycosylation of TSLPR on epithelial cells, and other non-myeloid cells can function as a means to suppress responses to TSLP until the proper environmental conditions are necessary.
**Presenter: Mooney, Jason**

**IL-10 elicited during severe malaria blunts intestinal immune responses and contributes to systemic spread of non-typhoidal Salmonella**

*Jason Mooney, Brian P. Butler, Mariana N. Xavier, Jennifer Y. Chau, Michael D. George, Satya Dandekar, Shirley Luckhart and Renée M. Tsolis*

University of California, Davis

Non-typhoidal Salmonella serotypes (NTS) in pediatric patients with severe Plasmodium falciparum malaria can develop a life threatening bacteremia, a major source of child mortality in Sub-Saharan Africa. Here, we use a mouse model that mimics severe anemia seen in human malaria to address mechanisms by which an underlying malaria parasite infection contributes to the increased risk of developing NTS bacteremia. Plasmodium yoelli infected red blood cells (4x10^7) were administered i.p. in CBA, B6 or B6 il10^-/- mice. At peak parasitemia, Salmonella Typhimurium (1x10^8) was administered by g.g. in a streptomycin-induced colitis model. Characterization of inflammation to NTS in the cecum was assessed by histopathology, microarray and qRT-PCR. Bacterial loads in systemic tissues were followed to 4 days post inoculation. Our results indicate that the malaria parasite infection causes a global suppression of proinflammatory responses in the intestine, blunts the intestinal neutrophil influx normally elicited during NTS gastroenteritis and increases bacterial colonization at systemic sites. Blunting of intestinal inflammatory responses was independent of hemolytic anemia, but required induction of the immunoregulatory cytokine IL-10 by the parasites. Elimination of IL-10 activity restored intestinal inflammation in co-infected mice, and administration of recombinant IL-10 was sufficient to increase systemic colonization by S. Typhimurium in the absence of parasite infection.
Type-1 diabetes (T1D) is primarily a T cell-mediated disease, with both CD4+ and CD8+ T effector (Teff) cells playing important roles, and T regulatory (Treg) cells controlling disease progression. However, precisely how Treg cells impact on the diabetogenic cascade has yet to be fully elucidated. We hypothesize that Treg cells affect the migration and local activity of autoreactive Teff cells in the target organ, thus preventing their destructive potential, and that this impact will be reflected in general and local mobility parameters. Therefore, we have combined performant mouse models of T1D (NOD, BDC2.5/NOD, NOD.Kaede) with flow cytometry and powerful imaging modalities, such as confocal microscopy, to directly but noninvasively visualize Treg and Teff cells in their physiological niche in T1D. Specifically, punctual in vivo cell labeling (through photoconversion of Kaede) and subsequent tracking of labeled cells is utilized. Our findings should have important implications for how Treg cells might be harnessed for cell-based therapy in pre-diabetic or recent-onset patients, or for the design of replacement therapies that mimic Treg cell action.
Presenter: Newell, Evan

Profiling antigen-specific T cell phenotypes with heavy metal labeled pMHC tetramers and single-cell mass-spectroscopy (CyTOF)

Evan Newell, Evan W. Newell, Natalia Sigal, Sean C. Bendall, Garry P. Nolan and Mark M. Davis

Stanford University

The direct detection of antigen-specific T cells using fluorescently tagged tetramers of soluble peptide-major histocompatibility complex (pMHC) molecules is widely used in both basic and clinical immunology. One major benefit of this approach is that it allows for unperturbed assessment of T cell phenotype by concurrent staining with surface and/or intracellular markers. However, the number of T cell specificities and phenotypic markers that can be assessed in a single sample using fluorescent tags is limited by spectral overlap between fluorescent probes. Although accurate compensation is routinely achieved in fluorescence based flow cytometry, this process increases noise and complicates analysis especially when greater than 10-12 colors are used. We recently devised a way to label pMHC tetramers with heavy metals that allows for detection of antigen specific T cells using mass cytometry (a.k.a. Cytometry via Time of Flight - CyTOF). Using 30 or more different heavy metal isotopes as labels, this approach greatly extends the number of T cell specificities and phenotypic markers that can be assessed on single cells without the need for compensations. A recent dataset using 22 phenotypic markers together with a few pMHC tetramer specificities (cells specific for Flu, EBV, CMV, Mart1) illustrates the large number of possible CD8+ T cell phenotypes that exist in humans and vary between individuals. Cells specific for a given antigen displayed a small subset of these phenotypes, which differed depending on the antigen specificity. Nonetheless, each population of T cells specific for the same antigen displayed remarkably heterogenous phenotypes. Looking at a larger cohort of individuals and T cell antigens, we are studying the relationship between the phenotypes of antigen-specific T cells and individual age. We are also working on extending the number of T cell specificities that we can assessed at once by applying heavy metal combinatorial tetramer staining, a technique we previously described using fluorescent labels.
Interaction of Actin with Cell Adhesion Receptor CEACAM1 in Liposomes is Ca2+ and Phospholipid Dependent

Michiel J.M. Niesen, Rongze Lu, Weidong Hu, Vaidehi Nagarajan, John E. Shively
Beckman Research Institute of City of Hope

The regulation of binding of G-actin to cytoplasmic domains of cell surface receptors can be studied by models that mimic the phospholipid bilayer and the effect of Ca2+ on binding. G-actin binds to the short cytoplasmic domain of the cell-cell adhesion molecule CEACAM1 in POPS liposomes only in the presence of Ca2+ and fails to bind in POPC or when a key residue in the peptide, Phe-454, is replaced with Ala. Molecular Dynamics simulations on the peptide in a phospholipid bilayer reveals two conformations for Phe-454 explaining the reversible availability of this residue for G-actin binding. NMR TROSY of 13C-labeled Phe-454 peptide in liposomes with actin further confirmed the existence of two peptide conformers and the Ca2+ dependency of actin binding. These models demonstrate novel approaches to the study of peptide-actin interactions and their dependency on the phospholipid environment and Ca2+ signaling.
Presenter: Oliver, Paula

TGF-[b] silences IL-4 production via an Ndfip1- and Itch-dependent mechanism to provide a window of opportunity for iTreg differentiation.

Paula Oliver, Natalia Ramos, Chris Riling and Allison Beal
University of Pennsylvania

During inducible T regulatory (iTreg) cell differentiation, TGF-B receptor stimulation must induce FoxP3 expression as well as prevent IL-4 production. Here we show that a membrane-tethered E3 ubiquitin ligase adaptor, known as Ndfip1, is expressed in a TGF-B-dependent manner and is required for iTreg differentiation. We showed previously that in the absence of Ndfip1, T cells become activated and promote inflammation at sites of environmental antigen exposure. This correlates with decreased numbers of iTregs in these tissues. T cells lacking Ndfip1 are defective at FoxP3 expression and iTreg differentiation both in vitro and in vivo. Interestingly, during the first 48 hours of iTreg differentiation in vitro, Ndfip1-deficient T cells express normal levels of FoxP3, however these levels consequently decrease and are absent by day 5. iTreg differentiation is aborted because Ndfip1-/- cells begin to produce IL-4 during this early phase of iTreg commitment. Supporting this, when Ndfip1-deficient T cells lack the ability to make IL-4, iTreg differentiation is restored. In wild-type T cells, Ndfip1 is transiently expressed early during iTreg differentiation in a TGF-B dependent manner. Furthermore we showed previously that Ndfip1 promotes the function of the HECT-type E3 ubiquitin ligase known as Itch. Ndfip1 and Itch promote the degradation of Jun-family transcription factors. These factors are known to induce IL-4 expression during T cell differentiation. Based these data, we propose that TGF-B silences IL-4 production via an Ndfip1- and Itch-dependent mechanism to provide a window of opportunity for iTreg differentiation.
The role of miRNAs in the human neonatal immune response

Amy Palin, Amy C. Palin, Shivkumar Venkatasubrahmanyam, Atul Butte, David B. Lewis
Stanford University School of Medicine

Neonates are highly susceptible to severe infection by a variety of intracellular pathogens, including M. tuberculosis and T. gondii. A large body of evidence suggests that a limitation in T helper 1 (Th1) responses accounts for much of the neonatal susceptibility to these pathogens. Our lab has previously defined a number of limitations in naive CD4+ T cells of the neonate obtained from umbilical cord blood that restrict Th1 generation in vitro even in the presence of optimal activating signals. These include decreased expression by T cells of the costimulatory molecule CD40 ligand and IFN-γ. Unexpectedly, we have also observed increased calcium flux in neonatal cells in response to CD3 cross-linking. Because of multiple differences in neonatal naive CD4+ T cell function, we hypothesized that miRNAs account for the impaired generation of Th1 immunity. Consistent with differences in calcium flux, we found that miR-181a, which regulates calcium signaling in T cells, is expressed at higher levels in neonatal naive CD4+ T cells. We are examining the role of miR-181a in regulation of calcium flux in primary human naive CD4+ T cells. We also found that the miR-29 family (miR-29a, b, and c) of tumor suppressor miRNAs is expressed at significantly higher levels in adult naive CD4+ T cells than in neonatal cells. Their predicted mRNA targets are expressed at higher levels in neonatal naive CD4+ T cells, as would be expected if miR-29 were repressing these targets. We previously found high expression of the miR-29 predicted target SOX4 in neonatal naive CD4+ T cells. SOX4 activates transcription of a number of T cell-specific genes during T cell development. We are presently investigating regulation of SOX4 expression by the miR-29 family in primary T cells and cell lines, in addition to pursing other miR-29 targets that may contribute to a reduced Th1 response. [NIH/NIAID R56 AI083757-01, NIH/NIAID R01 AI 83757-01, Jeffrey Modell Foundation to DBL NIH T32 Training Grant HD07249, Stanford Graduate Fellowships/Lucille P. Markey Charitable Trust to ACP]
Presenter: Parker, Clare

B cells that respond to an allograft following aCD20 depletion have an activated phenotype and produce antibody

Clare Parker, Clare Parker, Lindsay Hilken, Dylan Farnsworth, Jagdeep Obhrai

OHSU

Antibody-mediated rejection is a phenotype seen in transplant patients and experimental settings. Depletion of B cells could be hypothesized to improve clinical outcomes. That hypothesis may be wrong. Clatworthy et al. found that B cell depletion as induction therapy led to more rejection of kidney transplants in a clinical setting (New Engl J Med, 2009). However, sixty percent of transplant patients still receive some form of B cell depletion. We used a mouse model to assess the role of B cells that recover after depletion in transplant rejection. Using an aCD20 antibody, we depleted B cells from B6 mice and then performed a heterotopic cardiac transplant from BALB/c mice. We harvested at two weeks after transplantation and collected spleens for flow cytometry and immunofluorescence and blood plasma to measure alloantibodies. We found that while B cells remained depleted in control mice, allografts induced a partial recovery of B cells. Furthermore, these recovered B cells had predominantly activated phenotype and produced anti-BALB/c antibodies.
The Collaborative Cross (CC) is a large panel of recombinant inbred (RI) lines in the mouse, derived from eight diverse founder strains, including 3 wild derived strains. Here we describe the multicolor flow cytometric analysis of the resting immune phenotype of splenocytes in 66 partially inbred CC lines (the pre-CC mice) and the eight founder strains. Markers included in our 9 color panel cover basic subsets of B-cells, T-cells, and antigen presenting cells. Analysis of quantitative immunophenotypic traits in the founder strains, such as the ratio of B-cells to T-cells, reveals remarkable intra-strain consistency with wide inter-strain variation among the founders. Meanwhile, analysis of the pre-CC strains reveals variation greatly exceeding that of the founder strains from which they were originally derived. Whole genome scanning for quantitative trait loci (QTLs) associated with selected quantitative traits yields novel QTLs for B-cell to T-cell ratios, and CD23 surface antigen density (MFI). The dominant QTL for CD23 surface antigen density encompasses the gene for CD23 itself, an expected result which lends credibility to our methods. Here we discuss selected traits with the greatest consistency and which produced the most significant QTLs. Our data demonstrate the potential value of the Collaborative Cross and of our analytical methods as tools for the study of the genetics of immune homeostasis.
Presenter: Poholek, Amanda

In Vivo Regulation of Bcl6 and T Follicular Helper Cell Development

Amanda Poholek, Kyle Hansen, Sairy G. Hernandez, Danelle Eto, Anmol Chandele, Jason S. Weinstein, Xuemei Dong, Jared M. Odegard, Susan M. Kaech, Alexander L. Dent, Shane Crotty, Joe Craft

NIAID/NIH & Yale University

Follicular helper T (TFH) cells, defined by expression of the surface markers CXCR5 and programmed death receptor-1 (PD-1), require upregulation of the transcriptional repressor Bcl6 for their development and function. Although B cells, and the cytokines IL-21 and IL-6 have been indicated as important inducers of TFH cell development, the regulation if Bcl6 in response to these signals is unclear. We have explored the role of B cells, IL-6 and IL-21 in the in vivo regulation of Bcl6 expression and TFH cell development. We found that TFH cells are characterized by a Bcl6-dependent downregulation of P-selectin glycoprotein ligand 1 (PSGL1, a CCL19- and CCL21-binding protein), indicating that modulation of PSGL1 expression is part of the TFH cell program of differentiation. B cells were not required for initial upregulation of Bcl6 or PSGL1 downregulation, suggesting these events preceded T cell interactions. However, B cells were required for full development of the TFH cell phenotype, including CXCR5 and PD-1 upregulation, and IL-21 synthesis, suggesting B cells play a more important role in maintenance of TFH cells. Importantly, Bcl6 upregulation and TFH cell differentiation were independent of IL-6 and IL-21, revealing that either cytokine is not absolutely required for development of Bcl6+ TFH cells in vivo. These data increase our understanding of Bcl6 regulation in TFH cells and their differentiation in vivo and identifies a new surface marker that may be functionally relevant in this subset.
Presenter: Priatel, John

The long lasting-type calcium channel CaV1.4 is a critical regulator of T cell receptor signaling and naïve T cell homeostasis

John Priatel, Kyla Omilusik, Xiaoxi Chen, Kyung Bok Choi, Rayshad Gopaul, Adam McIntyre-Smith, Hung-Sia Teh, Rusung Tan, N. Torben Bech-Hansen, Simon V. Hunt, Wilfred A. Jefferies
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Abstract not to be posted.
Presenter: Price, April

Tracking in vivo Interleukin-17A Production during Infection and Autoimmunity

April Price, Hong-Erh Liang, R. Lee Reinhardt, Richard Locksley

University of California San Francisco

Abstract not to be posted.
Presenter: Probst, Hans Christian

Release of dendritic cells from cognate CD4+ T-cell recognition results in impaired peripheral tolerance and leads to fatal CTL autoimmunity

Hans Christian Probst, Sabine Muth, Hansjörg Schild
Institute for Immunology, University Medical Center Mainz, Germany

Dendritic Cells (DC) are professional antigen presenting cells (APC) that play a dual role in the control of adaptive immune responses. DC that have been activated through recognition of pathogen- or danger associated molecules can prime naïve T cells. In contrast, non-activated DC in the steady state induce peripheral T cell tolerance. We have recently shown that CD4+Foxp3+ regulatory T cells (Treg) are important for tolerance induction by steady state DC. Depletion of Treg results in an activated phenotype of steady state DC and these DC induce priming instead of tolerance. However, whether DC activation and the concomitant loss of tolerizing capacity is a result of general autoimmunity that develops in the absence of regulatory T cells, or whether direct interactions between DC and Treg are necessary to allow steady state DC to induce tolerance has remained unclear. To address this question in vivo, we have generated mixed bone marrow chimeric mice in which a part of the APC are negative for MHC class-II and thus cannot make cognate interaction with CD4+ T cells. We show that Dendritic Cells that cannot interact with regulatory T cells are completely unable to induce peripheral CD8+ T cell tolerance. As a consequence, mice in which part of the APC cannot make cognate interactions with CD4+ T cells develop fatal CTL mediated autoimmunity.
Presenter: Rahman, Prof.Dr.Md.Zakiur

Diagnosis of Rheumatoid Arthritis in Bangladesh

Prof.Dr.Md.Zakiur Rahman,
Sapporo Dental College, Bangladesh.

Objectives: This study was conducted to assess the utility of anti-Cyclic Citrullinated Peptide (Anti-CCP) antibodies in the diagnosis of early RA (disease duration less than 2 yrs.) and sero-negative RA in our population. Place and period of study: The study was conducted in Department of Physical medicine and Rehabilitation of Bangabandhu Sheikh Mujib Medical University (BSMMU) and Department of Physical medicine and Rehabilitation of Bangladesh Institute of Diabetes, Endocrinology and Metabolic Disorders (BIRDEM), Bangladesh from July 2005-June 2009. Materials and methods: Serum Anti-CCP antibodies and RF were evaluated in 80 RA patients diagnosed by ACR criteria, 40 non RA disease control and 40 healthy control. Anti-CCP antibodies and RF were detected by second generation anti-CCP-2 enzyme linked immunosorbent assay and immunonephelometry respectively. Detection of ACCP titre after giving treatment by DMARD. Results: Anti-CCP antibodies were detected in 58 out of 80 (72.5%) cases in early rheumatoid arthritis and in disease control group in 2 out of 40 (5%) and none out of 40 healthy control. Conversely RF was detected 43 out of 80 (54%) in early RA, and 10 out of 40 (25%) disease control and 2 out of 40 (5%) healthy control. In case of sero-negative RA cases, 17 (22%) were positive. The sensitivity and specificity of Anti-CCP in early RA was 72.5% and 97.5% respectively. The sensitivity and specificity of RF in early RA was 53.75% and 85% respectively. Decrease ACCP titre after giving treatment significantly. Conclusion: Detection of anti-CCP antibody were found to be more sensitive and highly specific for the diagnosis of early rheumatoid arthritis.
Mammalian Target of Rapamycin (mTOR) regulates FoxO1 for T-bet dependent effector differentiation of CD8+ T cells

Rajesh R Rao, Qingsheng Li, Protul A Shrikant
Roswell Park Cancer Institute

The mammalian target of rapamycin (mTOR) plays a central role in instructing T-bet dependent type I effector maturation of CD8+ T cells; however, the mechanisms underpinning its ability to regulate gene programs are not well understood. Herein, we demonstrate that type I effector OT-I cells generated by Ag/B7.1 and IL-12 stimulation show Akt dependent increases in phosphorylation and nuclear exclusion of transcription factor FoxO1; leading to inhibition of downstream target genes. The decrease in activity of FoxO1 was mTOR dependent, as addition of rapamycin inhibited IL-12 enhanced Akt-FoxO1 phosphorylation, nuclear exclusion, and resulted in a loss of T-bet dependent CD8+ T cell effector maturation. Furthermore, Akt deficiency or over expression of FoxO1 in IL-12 stimulated OT-I cells inhibited T-bet expression and IFN-γ production, whereas Akt over expression or FoxO1 knockdown restored T-bet expression and type I effector functions in rapamycin treated IL-12 conditioned OT-I cells. These findings implicate FoxO1 as a repressor of T-bet expression and type I effector differentiation of CD8+ T cells, and uncovers mechanisms by which mTOR governs functional differentiation of CD8+ T cells. Support by NIH 5R01CA104645-05, Alliance Foundation and OCRF to PAS
Presenter: Raubitschek, Antony

Genome-wide identification of cis-Regulatory elements in human CD4 T cell subsets

Antony Raubitschek, Antony Raubitschek, Sean Thomas, Theresa K Canfield, Mercedes Perez-Melgosa, Pete Sabo, Chris Wilson, John Stamatoyannopoulos, Steve Ziegler

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Recent evidence suggests a degree of plasticity whereby T cells of a given subset are able to express cytokines characteristic of other T cell subsets. We hypothesize that an important underlying mechanism for maintenance of specific effector subsets, as well as any flexibility in these subsets and their phenotype, is epigenetic regulation of transcription factor interactions with cis-regulatory elements. By isolating helper T cell subsets from the blood and expanding them in vitro in conditions which support the maintenance of the various subsets, we obtained phenotypically pure T cell subset populations. Cis-regulatory elements were identified in genome-wide manner by isolating nuclei, treating with DNase I, purifying fragments via a density gradient, and performing next-generation sequencing on the resulting DNA fragments. Global analysis of purified T cell subsets has identified approximately 80,000 regions that show differences in DNase I sensitivity. We have identified numerous regions in proximity to cytokine, chemokine receptor, and lineage-associated transcription factor genes. These regions represent specific genomic regions, which interact with transcription factors in order to regulate gene expression, of which only a small fraction have undergone any previous characterization. We are focusing now on characterizing identified regulatory regions in the IFNG-IL26-IL22 and IL17A-IL17F loci.
Presenter: Reinhardt, R. Lee

Divergent expression patterns of IL-4 and IL-13 define unique functions in type 2 immunity

R. Lee Reinhardt, Hong-Erh Liang, Richard M. Locksley
University of California San Francisco

Abstract not to be posted.
Differential infection of regulatory T cells by R5 vs. X4 HIV-1

Rachel Resop, Marc Douasi, Christel Uittenbogaart
University of California, Los Angeles

Differential infection of regulatory T cells by R5 vs. X4 HIV-1 Resop, Rachel S., Douasi, M. and Christel Uittenbogaart University of California, Los Angeles T Regulatory cells (Treg), characterized by FoxP3 expression, develop in the thymus and modulate the immune response during infections such as Human Immunodeficiency Virus (HIV). We and others have shown Treg to be targets for HIV infection. In addition we discovered through in vivo experiments using the SCID-hu mouse model that thymic Treg are more productively infected than non-Treg, developing T cells (thymocytes) by CCR5-tropic (R5) HIV-1, thereby contributing to Treg depletion and inappropriate immune activation. In contrast, CXCR4-tropic (X4) HIV-1 infects thymocytes more productively than Treg. Because Treg play a pivotal role in immunity, it is essential to characterize the viral factors that contribute to the differential impact of R5 and X4 HIV-1 on Treg. Several infections of whole thymocytes (prepared from human thymus tissue) with NL4-3 (X4-tropic lab strain), JR-CSF (R5-tropic lab strain) and a mock were performed; however, results were inconclusive. Infection with JR-CSF, as evaluated by p24 ELISA, did not enter exponential growth until approx. 21 days of culture, at which point cell viability was too poor to perform flow cytometric analysis to examine infection of FoxP3+ (Treg) cells. The proposed experiments will test the hypothesis that viral proteins as well as localization of Treg in the thymic medulla, the location of primary R5 HIV-1 targets, contribute to Treg infection and ultimate depletion. In vitro (Treg) cultures and in vivo experimental models (SCID-hu mouse), various molecularly cloned HIV-1 isolates, Alu-Gag quantitative PCR and multi (10-11) color flow cytometry will be used to further address our hypothesis.
TLR2 regulates Th17 responses and autoimmunity directly through signaling in CD4+ T lymphocytes

Joseph Reynolds, Bhanu P. Pappu, Gustavo J. Martinez, Yongliang Zhang, and Chen Dong
MD Anderson Cancer Center

Toll-like receptor (TLR) activation provides a potent mechanism for the rapid recognition of microbial pathogens leading to the initiation of immune responses. Although thought to be limited to cells of the innate immune response, here we found that TLR2 is expressed by T lymphocytes and plays a role in promoting T helper 17 (Th17) cell responses. Activation through the TLR2 pathway not only promoted Th17 cytokine production in vitro but enhanced the proliferative capacity of these cells as well. To assess the role of TLR2 signaling directly on CD4+ T lymphocytes in vivo, we analyzed the function of wild type and TLR2-deficient CD4+ T cells in the experimental autoimmune encephalomyelitis (EAE) model. TLR2 expression promoted CNS inflammation whereas the loss of TLR2 in CD4+ T cells dramatically reduced EAE severity. Interestingly, we found that endogenous TLR2 activation signals were required for the full development of EAE in a CD4+ T cell dependent manner. Taken together, our results reveal a novel pathway involving a TLR in the direct regulation of adaptive immune responses.
Presenter: Schmidt, Timothy

S1P2 promotes germinal center homeostasis by linking B cell localization and survival
Timothy Schmidt, Jesse A. Green, Kazuhiro Suzuki, Bryan Cho, David Willison, Daniel Palmer, Christopher D.C. Allen, Timothy H. Schmidt, Ying Xu, Richard Proia, Shaun R. Coughlin and Jason G. Cyster
UCSF

Abstract not to be posted.
Partial protection against enterovirus 71 (EV71) infection in newborn mice immunized with recombinant Newcastle disease virus capsids displaying EV71 VP1 fragment

Norazizah Shafee, Wei-Chooing Chng, Eric J. Stanbridge, Khatijah Yusoff

Universiti Putra Malaysia

Abstract not to be posted.
Regulating the regulators? Killer cell lectin-like receptor G1 (KLRG1) expression on Foxp3+ regulatory T cells.
Kate Smigiel, Dan Campbell
University of Washington

Mechanisms governing Foxp3+ regulatory T cell mediated immune suppression have been extensively studied but mechanisms regulating these regulatory cells are not well characterized. Recent work on the killer cell lectin-like receptor G1 (KLRG1) has identified it as an ITIM-bearing inhibitory receptor on NK and CD8+ T cells that can modulate signals delivered through activating ITAM-containing receptors. The ligand for KLRG1 is E-cadherin, which is expressed by epithelial cells, keratinocytes and Langerhans cells. Although a significant fraction of mouse Treg express KLRG1, little is published on this subject. We have hypothesized that KLRG1 expression by Treg functions to negatively regulate the responses of activated, effector-like Treg in peripheral tissues where levels of E-cadherin are greatest. We have found KLRG1+ Treg to be greatly enriched in the skin and gut, and unlike NK or CD8 T cells, KLRG1 expression by Treg does not require T-bet, IFN-?, or IL-12 signaling. KLRG1 expression by Treg is consistently associated with high expression of the effector molecules CD44 and CTLA-4, as well as peripheral tissue homing molecules such as CD103 and P-selectin ligand. KLRG1 expression by Treg correlates with greater proliferative history in vivo, but impaired activation and proliferation in vitro. We are currently working to understand the functional consequences of Treg KLRG1 expression and signaling both in vitro and in vivo.
Presenter: Spahn, Jessica

Immune-mediated chronic hepatitis in a mouse model

Jessica Spahn, I. Nicholas Crispe

Department of Pathology, University of Rochester; Seattle Biomedical Research Institute

Hepatitis B and C viruses (HBV and HCV, respectively) chronically infect millions of people worldwide. Immune responses to these viruses are frequently unable to eliminate the pathogens. Sustained infection renders patients vulnerable to fibrosis, end-stage liver disease, and hepatocellular carcinoma emphasizing the importance of understanding the molecular mechanisms that lead to clearance or persistence to more effectively treat disease. In an attempt to understand these processes, we have developed a mouse model in which an AAV vector expressing an antigen is directly injected into the liver. After the administration of antigen-specific CD8+ T cells, sustained hepatitis occurs and vector RNA expression is significantly decreased in a majority of mice while vector DNA remains unaffected. Interferon IFNγ is an important mediator in the decrease in vector RNA as this effect is not seen using IFNγ KO OT-1 cells or IFNγ Receptor deficient host mice. Several features of this model are similar to human hepatitis including sustained hepatitis, stellate cell activation, fibrogenesis, and the inability of partially exhausted CD8+ T cells to eliminate the vector. We believe this model will be important in determining the mechanisms that lead to clearance of virus rather than sustained viral presence.
Elucidating the Effects of Salmonella enterica serovar Typhi Virulence Antigen on Host Response

Alanna M Spees, Ivan Godinez, Andreas Baumler
University of California Davis

Elucidating the Effects of Salmonella enterica serovar Typhi Virulence Antigen on Host Response Alanna M. Spees, Ivan Godinez, and Andreas Bäumler Department of Medical Microbiology and Immunology, University of California at Davis, One Shields Ave., Davis, CA. Each year Salmonella enterica serotype Typhi causes around 600,000 deaths and 16,000,000 cases of the systemic infection known as typhoid fever (1). Disease caused by S. Typhi contrasts with the self-limited inflammatory diarrhea that is associated with the other major non-typhoidal serotypes (2). The pathogenesis and systemic spread of S. Typhi is currently not well understood. Although the mechanisms of pathogenesis for S. Typhi have remained mysterious, recent studies suggest the virulence capsule (Vi) of Salmonella Typhi, a surface-bound polysaccharide capsule that has been shown to interfere with the host innate immunity, is a major contributing factor to pathogenesis (3, 4). Work with human and murine models have demonstrated that the Vi capsule is a major contributing factor to a reduced host immune response including decreased TLR4 signaling and impaired complement fragment 3 deposition on the bacterial surface (5, 6). Our group has been interested in studying the influence of the Vi capsule on host adaptive immunity. Recent in vitro studies with primary macrophages demonstrate decreased activation of antigen presenting cells upon infection with Vi+ S. Typhi. To investigate host-pathogen interaction we utilized an in vivo mouse model. Compared to mice infected with a Vi-negative S. Typhi strain, mice infected with wild type S. Typhi showed decreased IFNγ production 21 days post infection. Mice that received multiple vaccinations of either strain showed distinctive differences in levels of IFNg production suggesting impaired Th1 development over multiple exposures to S. Typhi. This data suggests that S. Typhis Vi capsule impairs robust Th1 development. In addition to Salmonella Typhi, these studies may prove important for understanding the pathogenesis of other encapsulated pathogenic bacteria and their effects on host immunity.
T-bet Target Genes are Differentially Expressed in Regulatory and Effector T Cells

Shivani Srivastava, Shivani Srivastava, Meghan A. Koch, Todd J. Suscovich, Daniel J. Campbell
University of Washington

T helper cells undergo functional specialization into Th1, Th2, and Th17 subsets to combat infection with intracellular pathogens, mucosal parasites, and extracellular bacteria/fungi, respectively. More recently, Foxp3+ regulatory T cells (Tregs) have also been shown to functionally specialize in order to inhibit specific T helper immune responses. The expression of the Th1-associated transcription factor T-bet in a subset of Tregs has been shown to be important for their ability to regulate Th1 responses. Interestingly, T-bet target genes are differentially expressed in Th1 and T-bet+ Tregs. While both subsets express similar levels of the chemokine receptor CXCR3, T-bet+ Tregs do not express IL12Rb2 or the prototypical Th1 cytokine IFN?.

As Tbet+ Tregs express T-bet at nearly 10-fold lower levels than Th1 cells, we hypothesized that the level of T-bet expression may affect which T-bet target genes are expressed in Tregs versus Th1 cells. To test this, we sorted CD4+Foxp3+ Tregs and CD4+Foxp3- T effectors (Teffs) from Foxp3-GFP Tbet-/- mice and transduced cells with a retrovirus carrying a Tbet-estrogen receptor (ER) fusion construct. We then treated cells with varying concentrations of 4-hydroxytamoxifen (4-HT) to control the level of T-bet active in the nucleus. The same dose of 4-HT drove similar levels of surface CXCR3 expression in both Tregs and Teffs. However, similar doses of 4-HT drove IL12Rb2 and IFN? expression only in Teffs but not in Tregs, indicating that differences in T-bet target gene expression in Tregs and Th1 cells cannot be explained by differing levels of T-bet.
**Foxo1 controls T regulatory cell development and function**


Molecular Biology Section, Division of Biological Sciences, and Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, USA, 92093

Foxo transcription factors integrate extrinsic signals to regulate cell division, differentiation and survival, and in addition, specific functions of lymphoid and myeloid cells. T cell specific deletion of Foxo1 results in autoimmunity. We show that this autoimmunity is due to a loss of dominant tolerance, as wild-type bone marrow is able to rescue Foxo1 deficient T cell activation in mixed bone marrow chimeras. Furthermore, Foxo1f/f Cd4Cre bone marrow is unable to rescue the development of autoimmunity due to Foxp3 deficiency. Thus, Foxo1-deficient regulatory T cells are not functional. This loss of function included diminished CTLA-4 expression as the Ctl4 gene was shown to be a direct target of Foxo1. Additionally, Foxo1 controls Treg cell development. Loss of Foxo1 in T cells results in a misdirected TGFβ response, lacking in T-bet suppression and characterized by IFNγ secretion. These studies reveal that Foxo transcription factors guide the contingencies of T cell differentiation and specific functions of effector populations.
**Presenter: Teh, Charis**

**Central tolerance deficiency due to Aire co-operates with the peripheral tolerance defect in Cblb but not Fasl, Rc3h1 or Card11 to precipitate lethal autoimmunity**

*Charis Teh, Charis E. Teh, Stephen R. Daley, Anselm Enders and, Christopher C. Goodnow*

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Although autoimmune diseases have a strong genetic basis, they vary widely in their timing of onset, clinical presentation and target organs. The heterogeneous nature of these diseases suggests that compounding genetic defects in multiple tolerance mechanisms co-operate to precipitate autoimmunity. To explore this hypothesis, we investigated the interaction between well-defined mild defects in central and peripheral tolerance mechanisms. Aire-deficient mice with crippled thymic deletion of organ specific T cells - were crossed with mice carrying genetic defects in one of four peripheral tolerance mechanisms: decreased T regulatory cells (Card11um/unm), apoptosis (Faslgld/gld), anergy (Cbl-b-/-), or deregulated T follicular helper cells (Rc3h1san/san). Cblb-deficiency was unique among these four in precipitating rapid clinical autoimmunity when combined with Aire-deficiency, resulting in lethal destruction of the exocrine pancreas and salivary gland within weeks after birth. Changing the MHC haplotype and frequency of the autoreactive cells using a T cell transgenic model did not influence the spectrum of organs targeted. This study provides the evidence for a multistep pathogenesis model of autoimmune disease, but poses mechanistic questions about why only certain combinations of defects are synergistic. Understanding how individual mechanisms fit together for robust tolerance is critical for interpreting patterns of genetic and phenotypic variability in human autoimmune disease.
Presenter: Tellez, Martha C.

Endosulfan shows antiapoptotic effect via ERK1/2 in vitro, increases in vivo IL-2L and IgM of tilapia challenged with Aeromonas.

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BACKGROUND. Recent studies have shown an increase in the incidence of autoimmune diseases and cancer related with the indiscriminate use of pesticides. Endosulfan is an insecticide in general use in Mexico but it has been restricted in several countries for its adverse effects on the environment and health. OBJECTIVE. This study evaluated the in vivo effects of acute exposure to sublethal concentrations of endosulfan in juvenile tilapia (Oreochromis niloticus). This study measured serum levels of the cytokine interleukin-2 like (IL-2L), and immunoglobulin M specific for Aeromonas hydrophila. Additionally, this study measured in vitro expression of pERK1/2, apoptosis and senescence induction in splenic lymphocytes. METHODS. ELISA was used for measuring the serum IL-2L and IgM, as well as lymphoproliferative activity. Flow cytometric analysis was used to quantify the expression of pERK1/2, apoptosis and senescence in splenic cells. RESULTS. In vivo endosulfan exposure induced exacerbated synthesis of IL-2L and significant increases in secretion of IgM specific for Aeromonas hydrophila. In vitro exposure of splenocytes induced base line proliferation yet decreased the proliferative response after mitogenic stimulation, and demonstrated antiapoptotic effects and the promotion of senescence. CONCLUSIONS. Endosulfan is a xenoestrogen that alters key cell signaling pathways (via sustained phosphorylation of pERK1/2, antiapoptotic effects and the induction of senescence) the same way that the humoral (IL 2L and IgM) in tilapia increases the immune response in juvenile tilapia. We conclude that exposure to endosulfan is a high risk factor for health.
Role of microRNAs in the pathogenesis of AIDS-related non-Hodgkins lymphomas

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Background: Individuals infected by HIV are at an increased risk for developing non-Hodgkins lymphomas (AIDS-NHL). Even though effective anti-retroviral treatment exists, the overall incidence of AIDS-NHL has not decreased as much that of other AIDS-related clinical conditions, and AIDS-NHL remain a significant clinical problem and cause of death in people living with HIV. Various etiologic factors contribute to the genesis of AIDS-NHL genetic lesions, chronic B cell stimulation, cytokine deregulation and association with Epstein-Barr virus (EBV) and Kaposis sarcoma associated virus (KSHV). Recently, a lot of attention has been directed towards the role of microRNAs (miRNA) in cancer, including NHLs. miRNAs are small non-coding RNAs that can bind to mRNAs and inhibit translation. Thus, miRNAs contribute to the development and growth of cancers by inhibiting crucial proteins involved in cell cycle, growth, differentiation, and apoptosis. We hypothesized that miRNAs are deregulated in AIDS-NHLs and that these deregulated miRNAs serve not only as useful biomarkers but also provide additional insight into the molecular mechanism of NHL pathogenesis.

Methods: Global miRNA profiling of viral and cellular miRNAs was done using Exiqons miRCURYTM LNA microRNA v.11.0 array. miRNAs were profiled from a total of 26 primary AIDS-NHL tumors derived from 4 lymphoma subtypesBurkitts lymphoma (BL, n=6); primary central nervous system lymphoma (PCNS, n=6); diffuse large B cell lymphoma (DLBCL, n=9); and primary effusion lymphoma (PEL, n=5). For comparison, miRNAs were also profiled from non-neoplastic B cell subsetsnaïve, germinal center (GC), and memoryisolated from tonsillar B cells. Microarray data for select miRNAs was further confirmed by quantitative real-time PCR. Predicted miRNA target was verified by 3UTR luciferase assay and Western blot/qPCR assay post-transfection of synthetic precursor miRNAs into B cell lines. Results: We show that naïve, GC, and memory B cell subsets from tonsils have a distinct differentiation stage specific miRNA pattern with groups of miRNAs either upregulated or downregulated in a stage specific fashion. This shows that miRNAs play a role in the maturation and differentiation of activated B cells as it progresses through the GC reaction. Of particular interest was miRNAs from the miR-17-92 paralog clusters which was specifically upregulated only during the GC stage. miRNA profiling of AIDS-NHLs was able to discriminate between the GC derived BL/DLBCL and post-GC derived PCNS/PEL lymphomas. Importantly, miR-17-92 paralog cluster miRNAs were also found to be upregulated in AIDS-BL/DLBCL types suggesting that this miRNA cluster play a role in its pathogenesis. Target analysis for this miRNA cluster showed that it plays a critical role in tumor proliferation by targeting the cell cycle inhibitor, p21. Conclusions: miRNAs play a crucial role in the normal differentiation process of B cells and it is also deregulated in AIDS-NHLs. We find that select miRNAs from the miR-17-92 family are overexpressed in AIDS-NHLs and that these miRNAs contribute to tumorigenesis by targeting the tumor suppressor p21.
Suppression iNKT cell cytokine secretion by Kupffer cells

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Invariant NKT (iNKT) cells are a unique subset of T lymphocytes that rapidly produce and secrete large amounts of cytokines following activation. These cytokines have a profound impact on a variety of different immune reactions. However, little is known how iNKT cell activation is regulated at steady state or curtailed to avoid the escalation of an out-of-control inflammatory response. Our preliminary data indicates that purified Kupffer cells, liver resident macrophages, produce soluble factors that can block the cytokine production of primary iNKT cells. Surprisingly, this inhibition acts directly on iNKT cells stimulated either by cytokine-mediated activation or antigen-specific TCR activation. The cytokine suppression is unique to Kupffer cells as macrophages purified from the spleen are able to stimulate iNKT cells and have no inhibitory action. These studies have revealed a unique mechanism of iNKT cell regulation in the liver.
Presenter: Van Dyken(1,2), Steven J.

**Fungal chitin from asthma-associated home environments induces murine eosinophilic lung infiltration**

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Development of asthma and allergic inflammation involves innate immunity but the environmental contributions remain incompletely defined. Fungi and fungal-derived bioactive agents, however, are prominent as ubiquitous constituents of airborne particulate matter and inhaled house dust, and have been linked to severe asthma and allergic conditions. Chitin, a polysaccharide that interconnects with β-glucans, galactomannans, and mannoproteins to form the structural foundation of fungal cell walls, is a recognition element capable of initiating innate immune responses associated with allergy and asthma, but whether chitin in natural environments retains biologic activity is unknown. Analysis of dust collected from the homes of asthmatic individuals revealed that chitin is environmentally widespread, and associated with β-glucans, possibly from ubiquitous fungi. Cell wall preparations of Aspergillus isolated from housedust induced robust recruitment of eosinophils into mouse lung, an effect that was attenuated by enzymatic degradation of cell wall chitin and β-glucans. Mice expressing constitutively active acidic mammalian chitinase (AMCase) in the lungs demonstrated a significant reduction in eosinophil infiltration after fungal challenge. Conversely, chitinase inhibition prolonged the duration of tissue eosinophilia. Thus, fungal chitin derived from home environments associated with asthma induces eosinophilic allergic inflammation in the lung, and mammalian chitinases, including AMCase, limit this process.
All organisms have viral pathogens, and the ability to mount an effective antiviral response relies on detection of viral nucleic acids that accumulate during infection. Recognition of cytosolic DNA by the interferon stimulatory DNA (ISD) pathway triggers a robust type I interferon (IFN) response independent of other known innate immune pattern receptors including the Toll-like receptors (TLRs). Whereas this type I IFN response is essential for protection against viral infection, dysregulated IFN production can lead to autoimmunity. Recently it has been shown that mutations in RNaseH2 cause the autoimmune disease Aircardi-Goutieres Syndrome (AGS), which is characterized by accumulation of self nucleic acids in the cytosol and chronic activation of the ISD pathway. While RNaseH2 is thought to metabolize nucleic acids, nothing is known about RNaseH2 function in vivo. We have created a system to determine localization of RNaseH2, its endogenous nucleic acid substrates, and how mutations in RNaseH2 that cause AGS alter its function. This will further our understanding of the underlying mechanisms of cell-intrinsic nucleic acid detection and autoimmune disease.
Regulation of Autoreactive B cells During Innate Immune Responses in Mouse and Human

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Systemic lupus erythematosus (SLE) is a severe systemic autoimmune disease characterized by the activation of autoreactive B cells and resulting formation of pathogenic immune complexes. Efforts to effectively treat SLE have been impeded because the mechanisms that normally regulate autoreactive B cells are still largely unknown. Using murine models of autoimmunity, we showed that during an innate immune response to LPS, dendritic cells and macrophages regulate autoreactive B cells. Activation of Toll-like receptor 4 (TLR4) on these cells induces the secretion of cytokines (IL-6, CD40L and TNF?) that synergistically repress autoreactive B cells, yet allow naïve B cells to secrete non-self antibodies. On the lupus-prone MRL/lpr background, dendritic cells and macrophages are defective in secreting repressive factors and therefore cannot properly control the autoreactive B cells that cause disease. Likewise, autoreactive B cells from the MRL/lpr background fail to be repressed by recombinant factors. Our current goal is to translate these findings to human SLE by asking if similar mechanisms exist in humans, using the anergic 9G4 B cell population as a sentinel for autoreactive B cells. Human B cells express very little TLR4, but respond robustly to stimulation through TLR7 and TLR9, both of which are highly implicated in SLE. We found that the TLR7 response by human autoreactive B cells is repressed by one or more cell types within the peripheral blood mononuclear cell population at a high ratios of B cells : PBMCs, whereas total Ig secretion is unaffected. Similarly, transgenic autoreactive murine B cells are repressed by splenocytes at such ratios, and wild-type B cells are not. These data indicate that a more complex network of cell interactions is regulating the TLR7 response by autoreactive B cells. Our goals are to identify the players involved in this network, define the mechanisms, and determine whether these mechanisms are defective in SLE patients.
Presenter: Won, hee yeon

Prominent bone loss mediated by RANKL and IL-17 produced by CD4+ T cells in TallyHo/JngJ mice.

hee yeon Won, Zong Sik Park, Jin Sook Song, Seong-Hwan Kim, Hee Yun Kim, Sung-Eun Yoo, Youmi Rhee, Myung Ae Bae*, and Eun Sook Hwang*

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Increasing evidence that decreased bone density and increased bone fracture are associated with abnormal metabolic states like hyperglycemia and insulin resistance indicates diabetes to be a risk factor for osteoporosis. In this study, we observed that a polygenic model of type II diabetes, TallyHo/JngJ (TH) mice spontaneously developed bone deformities with osteoporotic features. Female and male TH mice significantly gained more body weight than control C57BL6 (B6) mice upon aging. Interestingly, bone density was considerably decreased in male TH mice which display hyperglycemia. The osteoblasts-specific bone forming markers osteocalcin and osteoprotegerin were decreased in TH mice, whereas osteoclasts-driven bone resorption markers such as IL-6 and RANKL were significantly elevated in bone marrow and blood of TH mice. In addition, RANKL expression was prominently increased in CD4+ T cells of TH mice upon T cell receptor stimulation, which was in accordance with enhanced IL-17 production. IL-17 production in CD4+ T cells was directly promoted by treatment with leptin while IFN-γ production was not. Moreover, blocking IFN-γ further increased RANKL expression and IL-17 production in TH-CD4+ T cells. In addition, the osteoporotic phenotype of TH mice was improved by treatment with parathyroid hormone or alendronate. These results strongly indicate that increased leptin in TH mice may act in conjunction with IL-6 to preferentially stimulate IL-17 production in CD4+ T cells and induce RANKL-mediated osteoclastogenesis. We also propose that TH mice could provide a beneficial model for osteoporosis.
Notch has been proposed to play a critical role in Th1/Th2 differentiation either by directly regulating expression of master transcription factors and lineage-specific cytokines or by facilitating cellular proliferation without direct regulation of Th1/Th2-related genes. To address this controversy on the role of Notch in Th lineage commitment, we conducted a loss-of-function study using mice with a conditional deletion of Rbpj gene in mature T cells by LoxP-Cre system (Rbpj-cKO), because the Rbpj gene product CSL is indispensable for canonical Notch pathway and its null mutation results in embryonic lethality. Rbpj-cKO naïve CD4 T cells showed markedly diminished IL-2 production, which indirectly impaired Th2 differentiation due to a failure to up-regulate IL-4-dependent IL-4 and GATA-3 expression during late polarization phase. However, we found an aberrant expression of Calcipressin-3, an endogenous inhibitor of Calcineurin, in freshly isolated Rbpj-cKO naïve CD4 T cells, leading to impaired NFATc2 translocation in response to an acute weak TCR signal without Notch activation. To re-examine the Notch effect on Th2 differentiation of normal CD4 T cells, wild-type naïve CD4 T cells were stimulated with low concentrations of peptide on myeloid DC (mDC). Although mDC expressed two different Notch ligands Jag1 and Dll4, the blockade of Notch/Dll4, but not Notch/Jag1, interaction decreased Th2 differentiation. To better understand the role of Notch/Dll4 interaction in Th2 polarization, we generated an artificial fibroblast APC expressing MHC class II, CD54, CD80 and Dll4 to mimic mDC. Real-time PCR analysis revealed that Notch/Dll4 interaction did not influence IL-4-independent GATA-3 and IL-4 expression during early induction phase but increased IL-2 production, essential for IL-4-dependent up-regulation of GATA-3 and IL-4 during late polarization phase. We found that Notch/Dll4 interaction rendered activated CD4 T cells more sensitive to limiting amounts of IL-4 as judged by STAT6 tyrosine phosphorylation. Furthermore, Notch/Dll4 interaction prevented activated CD4 T cells from up-regulating the expression of PTP1B, a tyrosine phosphatase that down-modulates the IL-4R/STAT6 pathway. We conclude that Notch does not directly regulate either GATA-3 or IL-4 expression but increases IL-2 production and sensitivity to IL-4, leading to enhanced Th2 differentiation.
Inhibition of Double-Stranded RNA-Induced Inducible Nitric Oxide Synthase Expression by Fraxinellone and Sauchinone in Murine Microglia

Eun Hee YI, Eun Hee YI, Chang Seok LEE, Cheolhee WON, Hyouna YOO, Yuri CHO, Jung Woo MAENG, Sang Hyun SUNG, Sang-Kyu YE*, Myung-Hee CHUNG

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Fraxinellone and sauchinone, isolated from natural substance, are known to have an anti-inflammatory effect in inflammatory conditions. However, the anti-inflammatory actions of these compounds have been insufficiently demonstrated in viral-induced neuroinflammation. A viral component (double-stranded (ds)RNA) triggers a toll-like receptor 3-dependent inflammatory response that stimulates pro-inflammatory mediators in the brain. In present study, we initially examined the biological effects of fraxinellone and sauchinone on anti-inflammatory actions in dsRNA-stimulated microglia. Both compounds inhibited dsRNA-induced inducible nitric oxide synthase (iNOS) expression, a major pro-inflammatory enzyme. To demonstrate the mechanism of inhibitory effect on iNOS expression, we further examined the signaling pathway induced by dsRNA in microglia. Our data show that dsRNA promotes the expression of signal transducers and activators of transcription (STAT)1/3 identified as major inflammatory transcription factors as well as activates c-Jun N-terminal kinase (JNK) in an early time. Moreover, both compounds suppressed activation of JNK-STAT1/3 signaling pathway. These results suggest that an anti-inflammatory effect by fraxinellone and sauchinone is mediated via blockade of the JNKSTAT1/3-iNOS signaling pathway in viral-infected microglia.
Cytotoxic effect of methanolic extract from sweet potato leaves (Ipomoea batatas Lam.) in human cancer cell lines and PBMCs.

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Cancer is a major public health problem and worldwide is a leading cause of death; in 2007 cancer accounted for 7.9 million deaths. Despite advances in early diagnosis and treatment, in 2009 malignant tumors were the third leading cause of death in Mexico. A diet in plant-based food has been shown to be protective against various types of cancers. Previous studies have established that a plant endemic to central Mexico, the Mexican Sweet Potato (Ipomoea batatas), contains compounds that are active against certain diseases and that the extract of the root has been shown to have cytotoxic activity against several cancer cell lines including breast and neuroepithelial cancer and leukemia. Due to these findings, we investigated the cytotoxic effect of a methanol extract from Ipomoea batatas leaves in the colon cancer cell line SW-480, B lymphoma cell line BJAB and cervical cancer cell lines SiHa, HeLa and C33a. Cell proliferation was evaluated using the WST1 assay and apoptosis was evaluated by annexin V and PI staining using flow cytometry. Etoposide was used as a positive control for induction of cell death. The crude extract displayed strong antiproliferative effects on the growth of SW-480 cells, with estimated IC50 values of 450 µg/mL and demonstrated 90 % inhibition with 1000 µg/mL. An enhancement of the antiproliferative effect was seen in BJAB cells shown with 90 % of inhibition after treatment with 200 µg/mL. In the cervical cancer cell lines an antiproliferative effect of between 60 and 70 % with was seen after treatment of 800 µg/mL. However, this dose did not alter proliferation in human PBMCs. The extract showed variable apoptotic effects in the differing cancer cell lines (17-49%). The above findings suggest that components of the methanolic extract of the Mexican sweet potato have potential anti-cancer therapeutic effects.
Environmental stimuli may trigger spontaneous uveitis by retina-specific T cells

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The protease UBP43 is a regulator of IFN-I signalling in the brain and controls acute microglial immune response underlying lethal tissue injury

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To date therapeutic interventions using type I interferons (IFN-I) are a major treatment option for multiple sclerosis potentially by exerting silencing effects on myeloid cells during brain autoimmunity. Here we identified UBP43, an IFN-inducible cysteine protease, which is highly expressed in microglial cells, as a novel negative regulator of IFN-I signalling essentially modulating brain homeostasis in vivo. UBP43 controls the activation of STAT1, thereby regulating the expression of IFN-I target genes in microglia. IFN-I hyperactivated microglia lacking UBP43 induce severe brain degeneration by myelin destruction leading to premature death of the animals. Importantly, early lethality, neuropathology and IFN-I hypersensitivity can be completely rescued by genetic depletion of IFN-I receptor. Therefore, controlled regulation of IFN-I signalling is essential for tissue homeostasis in the central nervous system.
Bone generation and maintenance involve osteoblasts, osteoclasts, and osteocytes which originate from unique precursors and rely on key growth factors for differentiation. However, an incomplete understanding of bone forming cells during wound healing has led to an unfilled clinical need such as nonunion of bone fractures. Since circulating monocytes are often recruited to sites of injury and may differentiate into various cell types including osteoclasts, we investigated the possibility that circulating monocytes in the context of tissue injury may also contribute to bone repair. In particular, we hypothesized that LL-37 (produced from hCAP-18, cathelicidin), which recruits circulating monocytes during injury, may play a role in bone repair. Treatment of monocytes from blood with LL-37 for 6 days resulted in their differentiation to large adherent cells. Growth of LL-37-differentiated monocytes on osteologic discs reveals bone-like nodule formation by scanning electron microscopy (SEM). In vivo transplantation studies in NOD/SCID mice show that LL-37-differentiated monocytes form bone-like structures similar to endochondral bone formation. Importantly, LL-37-differentiated monocytes are distinct from conventional monocyte-derived osteoclasts, macrophages, and dendritic cells and do not express markers of the mesenchymal stem cells (MSC) lineage, distinguishing them from the conventional precursors of osteoblasts. Furthermore, LL-37 differentiated monocytes express intracellular proteins of both the osteoblast and osteoclast lineage including osteocalcin (OC), osteonectin (ON), bone sialoprotein II (BSP II), osteopontin (OP), RANK, RANKL, MMP-9, tartrate resistant acid phosphatase (TRAP), and cathepsin K (CK). Blood derived monocytes treated with LL-37 can be differentiated into a novel bone forming cell that functions both in vitro and in vivo. We propose the name monoosteophil to indicate their monocyte derived lineage and their bone forming phenotype. These cells may have wide ranging implications in the clinic including repair of broken bones and treatment of osteoporosis.