Are there new and undiscovered enzymes that contribute to lymphocyte-mediated anti-viral and anti-tumor immunity? Here we examine the role of cytotoxic T cell lipases in the killing of pathogenic cells. These lipases include a digestive pancreatic lipase, pancreatic lipase related protein 2 (PLRP2) that is induced by IL-4 (Grusby M, Glimcher L, Cell vol: page 1990). We compared tumor cell death by CTLs generated from Balb/c wild type (WT) and PLRP2-/-(KO) littermate mice. Concanavalin-A activated splenic T cells were cultured with IL-2 or with IL-4 (each 500 u/ml) for 6 days. We assayed cytotoxicity by anti-CD3 redirected lysis of P815 cells, monitoring 51Cr release. We also determined the frequency of Grz B+ CD8+ CTLs and their Grz B levels by flow cytometry. PLRP2 was detected by RT-PCR and by immunoblots developed with cross-reacting antibodies to human PLRP2. Lipase activity was monitored by labeling the P815 lipids with 3H-oleic acid or by enzymatically monitoring released lipase from the CTL upon stimulation with phorbol myristic acid and ionophore. We found that the WT CTLs were consistently 2-4 times more cytotoxic than the PLRP2-/-. CTLs, with greatest differences from cultures with IL-4. The frequency and mean fluorescent intensity of Grz B+ CTLs was similar for WT and KO cells cultured with identical cytokines. Thus the differences responsible for the higher activity of the WT cells were likely to be attributed to other factors. There was extensive 3H released into the cell-free supernatant from the oleic-acid labeled P815s. The released label (lysolipids or fatty acid) could reach 30% of the total label of the P815s. The release correlated with the effector to target cell ratios and was absent without anti-CD3. It was blocked by the triglyceride lipase irreversible inhibitor, tetrahydrolipstatin. The 3H release appeared regardless of the WT or KO genotype of the CTL, suggesting that the lipase(s) mediating the target cell lipid degradation are independent of PLRP2. However, PLRP2 is secreted and could mediate selective damage. Upon stimulation, the WT CTLs induced with IL-4 released activity that degraded the triolein substrate that is selectively hydrolysed by lipases like PLRP2 while the IL-4 cultured PLRP2-/-. CTLs lacked this activity. From these data, we conclude that at least two lipases participate in T cell-mediated death and extensively hydrolyze target cell lipids in the process.

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AIDS as immune system activation: Seeing through the paradigm
Michael S. Ascher, Haynes W. Sheppard

California Department of Health Services

A satisfactory scientific explanation for something as complex as AIDS pathogenesis must incorporate a very large amount of ever-changing information. Initial attempts at models of AIDS pathogenesis used then current, but incomplete, views of the immune system, the natural history of AIDS, and lentivirus biology. Examples include: an apparent long clinical and virologic latency followed by collapse; an emphasis on viral cytopathicity under artificial in vitro conditions; views that the immune system was overall suppressed, that the T cell population was regulated at the global level with a finite source, that the CD4 receptor was nonfunctional, that the proviral reservoir was maintained through reinfection, and the idea that high-level apoptosis and T cell turnover were special HIV-related phenomena. Based on these assumptions, early therapy focused on reverse transcriptase inhibition and attempts to activate the immune system. Such models predicted a rapid and complete rebound of the immune system and elimination of the viral reservoir under effective therapy aimed at killing the virus.

Our first studies in HIV-infected patients in the 1980’s revealed that many of these assumptions were incorrect. There was abundant clinical and laboratory evidence of immune system activation such as fever, adenopathy, elevated neopterin and beta-2 microglobulin. Natural history data revealed a very gradual but inexorable T cell decline over many years with relatively constant virus concentrations. In vitro experiments under physiologic conditions showed little or no cytopathicity and, ironically, using HIV, the CD4 receptor was shown to be functional and the key to CD4 T cell activation. We found evidence of two types of immune system activation, virus-specific and nonspecific with the former “good for you” and the latter “bad for you”. Most importantly, the immune system rebound seen with therapy was modest at best and the provirus reservoir has been shown to persist unaltered after years of apparent total virus suppression.

These conflicts led us to consider alternative hypotheses for AIDS pathogenesis and in 1988 resulted in the concept of AIDS as immune system activation in which HIV presents a signal to the CD4 cell population that disrupts homeostasis of these cells. Further explanation of this theory and the supporting evidence are presented elsewhere at this meeting by Chip Sheppard. Corollaries of this hypothesis include the idea that the provirus reservoir is antigen-specific, could be enhanced by vaccine with adverse consequences but could be a target for specific elimination through the T cell idiotyp(s) found on HIV antigen-specific cells.
Presenter: Bensinger, Steven

LXRβ signaling intrinsically regulates lymphocyte proliferation and function
Steven J. Bensinger, Michelle S. Bradley, Sean B. Joseph and Peter Tontonoz

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Per Steven Bensinger, abstract not available on the website.
BCR-induced Rac1 activation and Rac1-dependent spreading are impaired in transitional immature B cells due to levels of membrane cholesterol
Randall J. Brezski, Fredrick Karnell and John G. Monroe

The BCR-triggered response of mature and transitional immature B cells is different. We show here that in mature B cells, BCR signaling triggers Vav phosphorylation and Rac1 activation. Furthermore, downstream actin-dependent BCR capping was Rac1-independent whereas spreading and ruffling were Rac1-dependent processes. In contrast, Vav phosphorylation and Rac1 activation is impaired in transitional immature B cells, resulting in defects in BCR-induced actin polymerization-dependent spreading and membrane ruffling whereas capping was unimpaired. Since transitional immature B cells maintain lower steady-state levels of plasma membrane cholesterol, we augmented their levels to that of mature B cells and found that BCR-induced Rac1 activation and Rac1-dependent processes were restored. These studies provide a direct link between B cell cholesterol levels and downstream cellular signaling processes.
Presenter: Carlyle, James

Cytomegalovirus Evasion of Innate Immunity by Subversion of the NKR-PIB:OCIL/CLR-B Missing-Self Axis
Aruz Meschi1, Sebastian Voigt2, Jason Fine1, Peter Chen1, Wayne Chou1, Jakob Ettinger2, & James R. Carlyle1

1 Sunnybrook Research Institute, University of Toronto, ON, Canada
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Per James Carlyle, abstract not available on the website.
Glutamine Metabolism and Activation of T Lymphocytes
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Activation of a naïve T cell is a high energy consuming event which leads to an increase in metabolism. Upon stimulation, T cells must increase in size, rapidly proliferate and differentiate, all of which lead to a high demand for energetic and biosynthetic precursors. Even though amino acids are the basic building blocks of protein biosynthesis, the role of amino acid metabolism in this process has not been well characterized. We began investigating this by stimulating T cells in media lacking individual amino acids. Glutamine, in particular, was found to be important for proliferation as well as for cytokine production. Glutamate, a precursor of glutamine could not compensate for glutamine deprivation when equal concentrations were supplemented. We have begun studying signaling pathways that are affected by glutamine depletion. We have examined mTOR signaling as well as GCN2 signaling, both known to be involved in amino acid sensing, in T cells grown in the absence of glutamine. Our data suggest that glutamine does not regulate either of these pathways. However, we found that MAPK signaling is regulated by glutamine. We also found that T cells up-regulate expression of key amino acid transporters upon activation. Furthermore, we found that glutamine is rapidly taken up by lymphoma cells. Taken together, these data suggest that glutamine is essential for T cell activation. A better understanding of glutamine utilization and transport into T cells is important because of its future therapeutic implications in diseases caused by malfunctioning of T cell activation.
Presenter: Chao, Cheng-Chi

Therapeutic IL-23 Antagonism of Animal Arthritis
Cheng-Chi Chao, Smiley Chen, Gil Asio and Eddie Bowman,
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Interleukin (IL)-23 is a heterodimeric cytokine composed of a unique p19 subunit, and a common p40 subunit shared with IL-12. IL-23 drives the development of IL-17-producing T cells and promotes chronic inflammation dominated by IL-17, IL-6, IL-8 and tumor necrosis factor. IL-23 plays a key role in inflammatory diseases such as experimental allergic encephalomyelitis and inflammatory bowel disease.

We assessed the in vivo efficacy of therapeutic IL-23 neutralization in collagen-induced arthritis. IL-23 antagonism inhibited disease progression assessed by visual disease scores and diminished the associated joint histopathologic changes such as cartilage destruction and bone erosion. Modulation of bone erosion was independently confirmed using micro-CT analysis. These results indicate IL-23 plays a major role in driving joint destruction and that IL-23 neutralization may provide an additional therapeutic strategy for the treatment of rheumatoid arthritis.
**Presenter: Chen, Shi-Juan**

**IL-23 Contributes to Intestinal Inflammation in Acute TNBS-Induced Colitis**

Shi-Juan Chen¹, Frederique Poulet², Terri McClanahan¹, Wendy Blumenschein¹, Cheng-Chi Chao¹, Gil Asio¹, Lisa Oldham¹, Edward Bowman¹

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IL-23, a heterodimeric cytokine consisting of a novel p19 molecule and the p40 subunit of IL-12, plays a major role in the pathogenesis of inflammatory bowel diseases in animal models. We investigated the pathogenic role of IL-23 in the development of 2,4,6-trinitrobenzene sulfonic acid (TNBS) induced colitis using mice genetically deficient in IL-23p19, IL-12p40, IL 12p35, or SJL mice treated with anti IL 23p19 or anti-IL-12p40 mAbs. Our studies show that IL-23p19/-mice are more resistant to TNBS-induced colitis compared to wildtype, IL-12p40/- and IL-12p35/- mice. Exogenous mouse IL 23 reconstitution accelerates disease progression and colonic histological change in wildtype and IL-23p19/- mice. The intestinal inflammation is associated with colonic Th17 gene up-regulation (IL-17A, IL-17F, IL-6, TNF and GM-CSF). IL 23p19 neutralization not only inhibits disease development, but also attenuates established colonic histological scores and serum Th17 cytokine production. These results suggest that IL-23 contributes to the development of TNBS-induced colitis and blocking IL-23/Th17 pathway may be a new therapeutic approach to the treatment of inflammatory bowel diseases in humans.
Presenter: Cho, John

GITR-L Mediated Inhibition of CD25+CD4+ Regulatory T cells in the Tumor Microenvironment
John Cho¹, Jeffrey Hsu¹, Sherie Morrison¹.²

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Naturally occurring CD4+CD25+ regulatory T cells (Treg) are not only involved in the maintenance of peripheral tolerance, but also inhibit anti-tumor immunity. To better understand the role of Treg cells in cancer development, we have generated tumor cells expressing the ligand for glucocorticoid-induced tumor necrosis factor receptor family related (GITR), GITR-L, on the cell surface. Signaling through GITR has been shown to abrogate the suppressive effects of Treg cells. Due to the nature of GITR signaling in regulating Treg activity, it is expected that the effects of localized GITR-L expression is sufficient to shift the balance of tumor specific suppression of effector T cells towards activation. Using the CT26 colon carcinoma model, we have generated stable tumor clones that constitutively express different levels of GFP fused to GITR-L. GITR-L expression on the tumor cell surface was determined using GFP as a surrogate marker and confirmed using recombinant GITR-Fc. While subcutaneous injection of 105 control tumor cells into BALB/c mice results in rapid tumor growth, injection of GITRL expressing tumor cells leads to delayed tumor growth and in most cases complete protection. Protected mice remained tumor free after subsequent rechallenge with unmodified parental CT26 tumor cells, indicating long term immunity against tumor specific antigens. GITR-L expression does not lead to significant changes in the absolute number or proportion of CD4, CD8, or Treg cells in the tumor draining lymph nodes. Current studies are aimed at determining if GITR-L expression alters intratumoral infiltration of lymphocytes and/or affects the functional state of the infiltrating cells.
Presenter: Cho, Yan

CpG-containing Immunostimulatory DNA Sequences (ISS) induce cytokine responses from diverse cell populations in murine lungs
Yan Cho, Darren Campbell, Robert Coffman, & Edith Hessel

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Per Yan Cho, abstract not available on the website.
Presenter: Chtanova, Tatyana

Visualizing the immune response against T. gondii
Tatyana Chtanova, Marie Schaeffer, Giel Van Dooren, Marc-Jean Gubbels, Boris Striepen and Ellen Robey.

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Immune responses against pathogens involve a complex interplay between the pathogen and the host’s immune system. Although 2-photon imaging has provided many important insights into the interactions between different immune cells during responses to model antigens, the immune response to real pathogens has not yet been well characterized. We have selected Toxoplasma gondii, an intracellular parasite which causes Toxoplasmosis, as a model pathogen due to its genetic tractability and the availability of a good mouse model of disease. Using mice with various cells of the innate immune system labeled with fluorescent proteins, together with parasites which have been engineered to express a different fluorescent label, we have set up a system which allowed us to visualize the interactions between T. gondii and the host immune system.

To visualize the adaptive immune response to T. gondii, we took advantage of fluorescently-labeled parasites which also expressed a model peptide, ovalbumin. To study antigen-specific T cell responses in vivo, we used 2-photon microscopy to track the behavior of ovalbumin-specific CD8 T cells in the lymph nodes of infected mice. Taken together these results provide an exciting glimpse into the mechanisms of the innate and adaptive immune responses to natural pathogens.

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T cell stimulation triggers a signal cascade that causes the activation of various transcription factors. From these transcription factors NF-κB is especially important because it is necessary for expression and secretion of T cell growth factor IL-2. In this study we are interested in comparing the regulatory pattern of NF-κB proteins p65 and c-Rel. NF-κB is composed of the protein subunits p50, p52, p65, c-Rel, and RelB which form dimers that bind via the κB domain. In resting T cells, the NF-κB complex remains in the cytosol bound to the inhibitory molecule IκB. Once the T cell is activated, IκB is degraded and the NF-κB complex is translocated into the nucleus where it binds to the IL-2 promoter to initiate transcription. We compared the localization of NF-κB protein subunits p65 and c-Rel during T cells stimulation. First, we studied degradation of IκB and NF-κB proteins at different time points by Western blot. Here we found degradation of IκBα but not IκBβ during T cell activation. We also observed no degradation of either total p65 or c-Rel after 60 minutes of stimulation. We also analyzed nuclear translocation by immunofluorescence in stimulated T cells using antibodies against p65 and c-Rel. We found that p65 moves from the cytosol to the nucleus within 15 minutes and 45 minutes of stimulation. Data from immunofluorescence was also confirmed by nuclear fractionation assay. c-Rel shows a different pattern since it appears nuclear at all time points. Future studies will compare these patterns with anergic cells to determine how NF-κB is regulated in T cell anergy.
Macrophages have been shown to be closely associated with tumor cells, comprising up to 80% of the malignant mass in some human tumors. To investigate the role of macrophages we used a mouse model that allows for the selective deletion of macrophages. MaFIA (Macrophage Fas Induced Apoptosis) mice have been transgenically engineered to contain a receptor for the drug AP20187 (Ariad Pharmaceuticals) on the surface of cells in the macrophage lineage. Macrophages expressing this receptor die through Fas mediated apoptosis when exposed to the drug AP20187. This receptor and the GFP reporter gene are under the control of the cfms promoter, therefore all macrophages expressing the receptor will also express GFP. In order to deplete macrophages, intra peritoneal injections of the drug AP20187 were given for five consecutive days, and then three times a week to maintain macrophage depletion. To confirm macrophage depletion peritoneal lavages were preformed and analyzed by flow cytometry. Using this model, tumors were introduced at various stages of depletion. 3 different groups of 9 mice were given subdermal injections of mouse melanoma cells. One group received the subdermal injection, but no depletion, to serve as a tumor positive control group. Receptor negative mice were also used in each group as controls. Another group received the subdermal injection and then started depletion three days later, and the last group completed five days of depletion and then received the subdermal injection on the fifth day. Two weeks following the subdermal injection, the mice were sacrificed: tumor mass was assessed, depletion of macrophages was confirmed, and organs were removed for potential metastases. Our data indicate differences in tumor mass in the presence or absence of macrophages. We have found the average mass of tumor control groups to be 1.1 g while the macrophage-depleted mice have an average mass of 0.67g. In the control mice an average of 12% of the tumor mass was composed of macrophages while in macrophage depleted mice an average of 3% was observed. Organs (kidney, lung and brain) were removed from each mouse and examined for the presence of metastatic melanoma cells using a cocktail of antibodies specific for the melanoma cell surface. In control mice melanoma cells were found in high numbers in the lungs and kidneys and in low numbers in the brain. In macrophage depleted mice differences were found that indicate the absence of macrophages not only affects tumor growth but also metastatic progression.
Presenter: Corse, Emily

TCR signal quality and in vivo T cell activation
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Per Emily Corse, abstract not available on the website.
Presenter: Crampton, Steve

Wnt Signaling Regulates T cell Migration
Steve P. Crampton, Beibei Wu, and Christopher C.W. Hughes

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LEF/TCF-dependent wnt signaling is important for the development of immature T cells in the thymus. We have found that wnt signaling also regulates migration of mature T cells into inflamed tissues and that, contrary to earlier reports, β-catenin is expressed in these cells. Here we examined in more detail the β-catenin-dependent wnt pathway in peripheral blood T cells and show that several components are induced by stimulation of resting cells. Furthermore, soluble wnt protein stabilizes β-catenin and activates a wnt-dependent reporter in effector T cells. Overexpression of the adaptor protein dishevelled-2 or the co-receptor LRP6, also activates the reporter. Additionally, Wnt signaling enhances transmigration of effector T cells through a collagen IV layer in vitro. This enhancement correlates with higher mRNA levels of the gelatinase MMP2. Lastly, microarray analysis has revealed several candidate genes regulated by wnt signaling in effector T cells that may be important for transmigration and proliferation. Thus, our data suggest that inflammatory diseases may be amenable to treatment with drugs that target wnt signaling.
TLR7 Gene Dosage is Critical for Autoantibody Production and Pathology in Mouse Models of Lupus
Jonathan A. Deane¹, Prapaporn Pisitkun¹, Rebecca Barrett¹, Michael J. Difilippantonio², and Silvia Bolland¹

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Toll Like Receptors (TLRs) are components of the innate immune system that have been shown to modulate the adaptive immune response in many contexts. The current study addresses the role of TLRs in autoimmunity using a murine model of lupus. In this system, mice lacking FcγRIIB develop antibodies to nuclear proteins and DNA, as well as lethal glomerulonephritis at 9 months of age. When FcγRIIB deficient mice are bred to the Y-linked autoimmune accelerator (Yaa) strain of mice, pathology is enhanced and mortality ensues within 5 months. Furthermore, the autoantibody profile in the FcγRIIB-/-Yaa mouse is altered to possess an anti-nucleolar pattern, as opposed to a homogenous nuclear appearance. We recently showed that the difference in the Yaa mouse is due to a genomic duplication, such that the Y chromosome of the Yaa strain possesses a 4.5 Mb sequence that is normally found only on the X chromosome. Analysis of the region reveals 13 known and 4 unknown duplicated genes, including the RNA-binding innate immune receptor TLR7. In this study, we investigated the importance of TLR7 gene dosage has on pathology, mortality, and autoimmunity in vivo, using pharmacological and genetic techniques. We find that pharmacological inhibition of TLRs dramatically alters the autoantibody production, but that lifespan is only increased when oligonucleotides specific to TLRs 7&9 are used. We also find that breeding the Yaa mouse to the TLR7-ko is sufficient to restore the marginal zone defect seen in the Yaa mouse. Furthermore, we generated transgenic mice that overexpress TLR7 alone. In this system, we see a recapitulation of the Yaa phenotype and even more strikingly, we see autoimmunity in mice that overexpress TLR7 at levels higher than 2-fold Thus, while the Yaa mouse can accelerate mouse models of autoimmunity due to a duplication of TLR7, we find that even greater levels of TLR7 gene dosage are alone sufficient to break tolerance. Overall, our model is that TLR7 signaling can synergize with BCR signaling to promote autoimmune response to RNA, and our findings imply that differences in gene dosage can have a dramatic impact on the pathology of autoimmunity.

This work is funded by the Intramural Research Program of the National Institutes of Health.
Presenter: deSouza, Anjali

TIM-1 Mediated Co-stimulation for T Cell Activation
Anjali J. de Souza and Lawrence P. Kane

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T cell Ig and mucin-1 (TIM-1) is a member of the recently discovered TIM family of proteins. Recent studies have implicated TIM-1 in the regulation of immune responses associated with asthma and atopic diseases. We have examined the function and signaling capabilities of TIM-1. Our results show that TIM-1 is present on in-vivo activated CD4 T cells following intranasal immunization. Further, ectopic expression of TIM-1 on murine CD4+ T cells after primary activation enhances the frequency of IL-4 producing cells and not IFN-gamma.

Until now, there has been no information regarding the signaling capabilities of the TIM molecules. Through a series of reporter experiments in Jurkat and D10 T cells and using mutant constructs of TIM-1, we show that TIM-1 co-stimulates NFAT/AP-1 dependent transcription. This effect is significantly decreased with the mutant construct lacking the cytoplasmic tail. A similar decrease in co-stimulation is observed when tyrosine 276 contained in a conserved tyrosine kinase phosphorylation motif of the cytoplasmic tail is mutated to phenylalanine. Finally, we show that this tyrosine can be inducibly phosphorylated. These results provide the first pieces of evidence that TIM-1 provides a co-stimulatory signal for NFAT/AP-1 dependent transcription in a manner that requires that cytoplasmic tail and is dependent on tyrosine 276 phosphorylation. We are currently attempting to delineate how tyrosine 276 mediates TIM-1 costimulatory signaling and its connection to the role of this molecule in T cell activation and differentiation.
CD4+CD25+ regulatory T cells (TReg) are a distinct subset of CD4+ T cells which play an important role in the generation and maintenance of peripheral tolerance. Natural TReg as well as adaptive TReg are present in peripheral blood and lymphoid organs. Natural TReg (CD25+/hi, FoxP3+) develop intrathymically at the CD4+CD8+ stage during transition of CD27- to CD27+ stage and acquire their regulatory function while still in the thymus. Natural TReg leave the thymus and enter secondary lymphoid tissues, lymph nodes and spleen, where they acquire a memory/activated phenotype. In contrast, adaptive TReg develop in periphery, either from conventional T cells or from natural TReg, in response to activation of mature T cells by pathogens or foreign antigens. A role for TReg has been implicated in viral infections, including HIV-1 infection. However, the role of TReg in HIV-1 infection is still controversial and may depend on the stage of infection. Peripheral TReg may be either protective or harmful in HIV-1 infected individuals. There are no data on TReg in the thymus during HIV 1 infection. In order to evaluate the impact of HIV-1 infection on TReg in the thymus, we used the SCID-hu mouse model. Human fetal thymus and liver were implanted in SCID-hu mice and infected with well characterized primary HIV-1 isolates. Our preliminary data strongly suggest an expansion of TReg early post-infection. Such a dysregulation of TReg production in the thymus could lead to a premature down-modulation of the immune response against HIV-1 and an impaired T cell (re)generation.
Presenter: Eriksson, Anna

Langerhans Cell Impairments in a Mouse model of Autoimmune Dermatitis are Improved by the NKT cell ligand αGalCer
Eriksson, Anna U. Singh, Ram R

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Mechanisms underlying autoimmune skin inflammation remain unclear. During steady state, the constant migration of Langerhans cells (LC) to cutaneous LNs (CLNs) has been proposed as a mechanism to maintain peripheral T cell tolerance to skin. We have previously reported that CD1d-deficient MRL-lpr mice that lack NKT cells experience more severe dermatitis than their wild-type littermates, whereas NKT cell activation with the glycolipid αGalCer reduces dermatitis. These observations led us to hypothesize that interactions between NKT cells and skin DCs/LCs play a crucial role in NKT cell-mediated prevention of lupus dermatitis. To test these hypotheses we used lupus-prone MRL-lpr mice that develops dermatitis, and control strains including congenic MRL-Fas and healthy MHC-matched C3H and B10.BR mice. Analyses of CLNs show that MRL-lpr mice have markedly reduced proportion of skin-derived DCs compared to controls. We reasoned that such reduction might be due to impaired migration of LC/DC from skin to lymph node, or due to reduced numbers in the skin. In situ staining of epidermal sheets revealed that although expressing lower levels of MHCII, the numbers of LCs in MRL-lpr mice appeared to be equivalent to control strains. Both in vitro and in vivo experiments, however, indicated that LC/DC in MRL-lpr mice have reduced migratory capacity in response to activation. Interestingly, one week after αGalCer injection, the skin DC populations begin to reappear in MRL-lpr CLNs and the activation induced migration of LC/DC was restored both in vitro and in vivo.
Presenter: Feuerer, Markus

MHC restriction of Foxp3+ regulatory T cells
Markus Feuerer, Christophe Benoist and Diane Mathis

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Regulatory T cells are important players in the control of peripheral self-tolerance. Most if not all Treg cells in normal, unmanipulated mice are generated in the thymus. More than 95% of peripheral Foxp3+ Treg cells are CD4–single-positive whereas; only 0.3% are CD8-singlepositive. The TCR-MHC-class-II interaction seems to be the central interplay for successful Treg cell differentiation. We analyzed mice devoid of MHC-class-II molecules and found that Foxp3+ Treg cells were easily detectable. Peripheral Treg cells were CD4-single-positive, CD4,CD8- double-positive or CD8-single-positive. The Foxp3+ T cells were neither NKT cells nor CD1d-restricted. The TCR repertoire was polyclonal based on expression of TCR variable regions. Introducing the β-2m deficiency into MHC-class-II KO mice revealed that CD8-single-positive as well as CD4, CD8-double-positive Treg cells were β-2m-dependent, whereas CD4-single-positive Tregs were still detectable. A distinct “signature” in their gene expression profile characterizes Treg cells. To our surprise, that signature was independent of the different TCR ligands. Thus, distinct MHC restriction did not alter the overall Treg-cell-specific profile. However, our data did not favor a pure affinity/avidity-based process of Treg cell differentiation since the ability of CD8+ T cells to enter the Treg lineage seems to differ from that of CD4+ T cells, and NKT or non α/β T cells were not able to differentiate into Foxp3+ Treg cells.
**Presenter: Fousteri, Georgia**

**Peptide-based immunotherapy for type 1 diabetes; the antigen, the dose, the immunization route and the state of the disease can make a difference**

Georgia Fousteri, Damien Bresson, Amy Dave, Michael Croft and Matthias von Herrath

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Autoimmune diabetes is one of the most common autoimmune diseases. Although blood sugar levels can be controlled by exogenous insulin administration, diabetes strongly impacts life quality and longevity. Therefore therapeutic approaches which either prevent or delay the disease are currently under investigation. Among them, immunization with beta-cell derived antigens such as insulin and other targets of the autoimmune process bear promise. This strategy is of particular interest since the administration of immunosuppressive drugs can be reduced and maybe circumvented, limiting in this way negative side-effects. In our current study, we compared the efficacy of diabetes prevention following administration of several islet-antigen derived peptides (insulin and IGRP) alone or in combination by two different routes (intranasally [i.n.] or subcutaneously [s.c.]) in ten week-old prediabetic NOD mice.

Surprisingly, we found that neither intranasal nor subcutaneous administration of human pro-insulin II peptide B24-C36 (hpII) affected the disease progress, whereas insB-chain 9-23 (B9-23) and its altered-peptide ligand (Ala 16,19) (APL) accelerated diabetes development significantly following intranasal administration. This acceleration was not observed when the s.c. route was chosen, and more interestingly, the APL conferred significant delay. Combination of the three CD4 epitopes did not affect disease progress when administered by either route. When insB-chain 15-23 (B15-23) and 24-36 (B24-C36) CD8 epitopes were tested (s.c. route), opposing effects were observed; B15-23 delayed and B24-36 accelerated the disease. Lastly, when two IGRP-derived altered peptide ligands NRPI4 and NRPV7 were compared for their protective effect following s.c. immunization, NRPV7 provided the greatest delay.

From our study it is evident that neither nasal nor subcutaneous administration of insulin- and IGRP-derived peptides conferred strong protection from the diabetes development. In constrast, mucosal immunizations with whole proteins (such as oral or intranasal insulin) or insB9-23 peptide combined with suitable adjuvants (i.e. IFA) were able to prevent diabetes efficiently in previous studies, especially when given at a much earlier stage (4-6 weeks old NODs). It will be important to consider these observations for the design of future clinical trials.
What is the Key Molecule that Induce NKT Cells in the Liver?
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Juzentaihoto (JTT), one of the Japanese botanical medicines was reported that it could induce the NKT cells and had the anti-tumor effects. These key molecules might be IL-12 and/or IL-18 that induce and activate the NKT cells. In this study, we first estimated the serum concentration of some cytokines from the JTT treated patients in our hospital with their consent. It resulted that interleukin (IL) -18 had been up regulated. In the other hand, the lymphocyte culture method revealed that the JTT stimulation resulted to high production of IL -12 within 48 hours. These liver tissues from the JTT administrated mice have infiltrated massive mononuclear cells with cluster formation. Immuno-histochemical and in-situ-hybridization staining showed that there are many NKT cells in that cluster and abundant cytokine expression of IL -12 and IL -18 in the JTT treated mice liver.

These studies show that the JTT stimulate the APC cells and produce of IL -12 in early phase, and IL -18 in chronic phase. These cytokines induce the NKT proliferation and activation and result in the immunological restoration of the liver.

JTT, Japanese botanical medicine might have the anti-tumor effect by NKT induction in the liver.
Presenter: Goldrath, Ananda

A novel role for Id2 in the regulation of CD8+ T cell immunity
Ananda W. Goldrath¹, Michael A. Cannarile¹, Nicholas A. Lind¹, Richard Rivera², Alison D. Sheridan¹, Kristin A. Camfield¹, Bei Bei Wu¹, Kitty P. Cheung¹, and Zhaoqing Ding¹

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Transcriptional programs that initiate and sustain the proliferation, differentiation and survival of CD8+ T cells during the immune response are not completely understood. We found that Inhibitor-of-DNA-binding-2 (Id2), an antagonist of E-protein transcription factors, was upregulated by CD8+ T cells during infection and maintained in memory cells. Whereas Id2-deficient CD8+ T cells recognized antigen and proliferated normally after infection, they failed to accumulate and demonstrated high levels of apoptosis, resulting in the premature contraction of the effector population. Id2-deficiency also diminished effector memory T cell formation and altered expression of genes that influence survival. These data highlight a novel function for Id2 in regulating the magnitude of CD8+ T cell responses and the formation of memory cells and suggest a mechanism where Id and E proteins regulate mature T cell survival and differentiation.

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Highly motile natural killer cells form stable conjugates to eliminate MHCmismatched target cells in peripheral lymph nodes
Kym R. Garrod, Ian Parker, and Michael D. Cahalan

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Natural killer (NK) cells are known to reject MHC-mismatched targets within blood organs, yet their role in peripheral lymph nodes (pLN) remains unresolved. Recently, NK cells isolated by positive selection were examined by two-photon microscopy. In comparison with T or B lymphocytes that are highly motile (10-12 and 6 µm/min, respectively), NK cells were reported to move with slow velocity (2.75 ± 0.17 µm/min). However, a recent report found that enhanced recruitment of NK cells to pLN facilitated the induction of a productive immune response, implying a more active role for NK cells.

We have assessed the capacity of NK cells to home to lymph nodes and used two-photon microscopy to image cell motility and interaction dynamics within the pLN compartment. Adoptively transferred, unmanipulated NK cells localized adjacent to the B cell follicle and were highly motile (6–8 µm/min), whereas DX5-positively selected NK cells exhibited diminished motility (2–3 µm/min). NK cells made transient contacts with both syngeneic and allogeneic B cells; long-lasting contacts (5 to > 50 min) formed exclusively with allogeneic cells, resulting in diminished motility and subsequent elimination of the target cell following dissociation. These data demonstrate that NK cells are remarkably dynamic and are a functionally important component in the lymph node environment.
Presenter: Gray, Daniel

Turnover of aire expressing thymic epithelial cells
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Aire expression by thymic medullary epithelial cells (MECs) is required for central tolerance of self. To better understand the mechanism by which these rare cells impose tolerance on the thymocyte repertoire, we examined the proliferation and half-life of aire+ and aire- MEC subsets using BrdU incorporation and flow cytometric analysis. These experiments indicated the aire+ MEC subset was the only population that was largely post-mitotic. This population appeared to be derived from and have a similar turnover to its aire-negative counterparts. In addition, despite the correlation between proliferative arrest and aire expression, we found that the aire protein itself did not have a direct impact on cell proliferation in vitro. Overall, these data indicate that, although most aire+ MECs do not divide, they have a surprisingly high turnover. This property places strict temporal limits on the function of any single aire+ MEC in central tolerance.
Antigen-Specific Modulations of CTL-Response by Nonprofessional APC Determine Pool Sizes and Memory Cell Frequencies
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Upon recognition of cognate antigen, naïve CD8 T cells expand and gain effector functions to eliminate pathogens, virus infected cells or tumors. The few T cell clones recognizing antigen do expand massively by cell division, however, most of them will, upon clearance of the pathogen, die by apoptosis. Only a small percentage will finally form the long-lived memory CD8 T cell pool, able to respond more optimally upon a second encounter with the same antigen. Understanding the rules governing these processes is important for the development of vaccines and comprehension of dysfunction of CD8 T cells during chronic viral infections. Dendritic cells (DC) as potent antigen presenting cells suffice to provide all required signals to fully activate antigen-specific T cells. After a latency phase of 24 hours T cells proliferate for several days and it has been demonstrated that proliferation is independent of antigen, a phenomenon described as "autopilot" or "early programming". While the "autopilot" hypothesis is widely accepted, it is unclear how and if further encounter of antigen (on non-DC) might influence the post priming behavior of CTL (i.e.: peak expansion levels, memory T cell frequencies). In order to investigate this question, we compared CTL-generation in transgenic mice, where selectively DC present Ag to the wild type situation, where Ag is presented more ubiquitously. While tolerance induction by abortive CD8 T cell proliferation under non-inflammatory conditions was identical in both situations, the magnitude of T cell expansion differed greatly under immunizing conditions. The peak expansion levels as well as frequencies of memory CD8 T cells were several-fold higher, when Agpresentation was restricted to DC only. We demonstrate that increased expansion of CTL by DC was not due to enhanced proliferation of CD8 T cells, but rather due to their reduced apoptosis rates early after priming. These data demonstrate, that DC are sufficient to induce a functional CTL response, but other cell types cells might negatively affect the outcome and net-result of CTL-priming. Our findings have direct implications for development of vaccines aiming at optimal CTL-priming and efficient establishment of CD8 T cell memory.
**Presenter: Guerau-de-Arellano, Mireia**

**The impact of developmentally or spatially controlled expression of Aire on autoimmunity and gene expression**
Mireia Guerau-de-Arellano, Christophe Benoist and Diane Mathis

*Section on Immunology and Immunogenetics, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215, USA*

AIRE deficiency in humans underlies the autoimmune polyendocrine syndrome APECED. Absence of the analogous murine protein, Aire, also results in multi-organ autoimmune disease. Aire induces ectopic expression of peripheral-tissue antigens in thymic medullary epithelial cells, although the exact mechanism and kinetics of this activity remain unknown. We recently generated transgenic mice that conditionally express Aire in the thymic epithelium (via the Tet system) in order to address a number of important outstanding issues concerning its function, e.g. thymic versus peripheral requirements, kinetics of aire dependence, etc. Transgene-mediated expression of Aire specifically in the thymic epithelium throughout development protected aire-knockout mice from their characteristic autoimmune disease. Turn-off of Aire via doxycycline treatment led instead to the full array of autoimmune manifestations shown by aireknockout mice. Secondly, we have addressed the role of cellular context in the induction of Aire-dependent transcripts by engineering mice that express Aire specifically in pancreatic islet beta islets. Microarray analysis comparing wildtype vs. Aire-expressing beta cells is currently underway. These novel tools and approaches should allow important new insights into Aire function.
Presenter: Guiolucci, Cristiana

The Nature of human PDC Response is Regulated by Endosomal Activation of TLR9
Cristiana Guiducci¹, Gary Ott¹, Jean H. Chan¹, Emily Damon¹, Thea Meeker¹, Carlo Calacsan¹, Tracy Matray¹, Kyung-Dall Lee², Robert L. Coffman¹ and Franck J. Barrat¹

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Human PDCs can produce IFN-α and/or mature and participate in the adaptive immune response. We show using three classes of CpGs that can differentially induce IFN-α or maturation that the nature of the PDC response is determined by the higher order structure and endosomal location of the CpG oligonucleotide. Activation of TLR9 by the multimeric CpG-A occurs in TfR-positive endosomes and leads exclusively to IFN-α production while monomeric CpG-B oligonucleotides localize to LAMP-1-positive endosomes and promote maturation of PDC. However, CpG-B when complexed into microparticles localize in TfR-positive endosomes and induce IFN-α from PDC, while monomeric forms of CpG-A localize to LAMP-1-positive endosomes accompanied by the loss of IFN-α production and a gain in PDC maturation activity. CpG-C sequences which induce both IFN-α and maturation of PDC, are distributed in both type of endosomes. Encapsulation of CpG-C in liposomes stable above pH 5.75 completely abrogate the IFN-α response while increasing PDC maturation. This establishes that the primary determinant of TLR-9 signaling is not valency but endosomal location and demonstrates a strict compartmentalization of the biological response to TLR9 activation in human PDC.
Presenter: Hagenbeek, Thijs

Pten negatively regulates T cell development
Thijs J. Hagenbeek1,2, Wendy Dontje1, Maho Nagasawa1, Ramon Gimeno1, Marianne Naspetti1 and Hergen Spits1,2

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During the CD4+CD8- stages of thymocyte development, cells depend on IL-7 to survive, proliferate and differentiate. Following productive rearrangements at the TCR locus, the translated TCR chain is expressed at the cell surface in complex with pre-T cell receptor (pT), CD3 and CD3 to form the pre-TCR. The pre-TCR mediates survival, proliferation and differentiation into CD4+CD8+ thymocytes. Previously, we documented that phosphatidylinositol 3 kinase (PI-3K) is involved in IL-7-mediated cell survival. PI-3K converts phosphatidylinositol-(4,5)-biphosphate (PIP2) to phosphatidylinositol-(3,4,5)-triphosphate (PIP3). PI-3K may also be involved in pre-TCR signaling since activation of T cells through the mature TCR results in activation of PI-3K and Protein Kinase B (PKB), a downstream target of PI-3K. Moreover, we recently showed that defective T cell development in mice that lack CD3 or Rag2 can be restored by loss of PTEN (phosphatase and tensin homolog deleted on chromosome 10). PTEN is a tumor suppressor whose lipid phosphatase activity is associated with tumor suppression. PTEN directly counteracts the PI-3K signal transduction pathway by converting PIP3 to PIP2, thereby negatively regulating signaling mediated by downstream targets of PIP3. Thus, PTEN might be an important regulator of thymocyte development. During murine ontogeny in vivo, an increased number of CD4+CD8+ cells was observed in thymuses of T cell-specific PTEN deficient mice when compared to littermate controls. Furthermore, whole Pten-/- thymocyte populations showed an enhanced survival/resistance to apoptosis in vitro. In addition, PTEN deficient murine fetal liver stem cells were co-cultured with OP9 stromal cells that express murine Delta Like 1 (OP9-mDL1) in vitro, which resulted in an increase in CD4+CD8+ cells. Moreover, using RNA interference, PTEN was knocked down in human postnatal thymic stem cells which resulted in more CD4+CD8+ cells when co-cultured with OP9-hDL1 cells in vitro. These data implicate an important regulatory role for PTEN in the development of thymocytes.
Presenter: Hamblin, Amanda

Critical Parameters for Induction of Apoptosis in Cells of the Lymphoid Lineage
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Apoptotic cell death plays a critical role in organism development, maintenance of homeostasis, and proper immune function. Disregulation of this process, including failure of a cell to undergo apoptosis when it receives initiatory signals, has been implicated in the development of disease, including cancer. As understanding of the significance of the apoptotic process increases, so to does the frequency with which it is studied. To elucidate the parameters for induction of apoptosis and determine heterogeneity of response in cells, apoptosis was induced by heat shock (incubation for 1 hour at 43°C) in multiple cell lines. The comet assay was used to distinguish between apoptotic and necrotic cell death. We varied multiple parameters including lymphoid cell line, growth phase, heat shock temperature, recovery time, prior exposure to heat stimulus, and prior protein inhibition. Different cell lines exhibited pronounced differences in the level of response to heat shock stimulus, with the percentage of apoptotic cells ranging between 1.3% and 96.3%. Although this difference in response was conserved in later experiments, variation in heat shock procedures produced trends consistent across cell types. Plateau phase cells had increased sensitivity to apoptotic induction, although this sensitivity reverted nearly to exponential phase levels when cultures were washed and supplemented with fresh media and serum. Apoptotic response was confined to a narrow temperature window between 41°C and 45°C, with maximum response exhibited at 43°C. Above 45°C cell death was virtually 100% necrotic. Detection of apoptotic response was found to be time dependant, with maximal apoptotic response 12 hours after heat shock. Incubation for 30 minutes at 43°C followed by a 6 hour recovery period exerted a protective effect, reducing levels of cell death. Additionally, moderate inhibition of protein synthesis by cycloheximide and actinomycin D reduced cell death, including apoptotic response. However, greater levels of inhibition potentiated apoptotic response. The neutral comet assay proved highly effective at differentiating between apoptotic and necrotic cell death. These results indicate variation of response in lymphoid cells to heat shock induced apoptosis and provide guidelines to maximize apoptotic cell death experimentally. They also indicate significant differences in apoptotic response which merit further investigation.
Dissecting the human to mouse trans-vivo DTH assay: Cross-communication between human cytokines and mouse chemokines.

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The human to SCID or monkey-to-SCID mouse trans-vivo DTH assay (TV-DTH) is one of the few immunologic assays currently available that reliably detects donor antigen-specific, adaptive T regulatory cells in organ transplant recipients. However, attempts to replicate the TV-DTH "linked suppression" using in vitro assays have been unsuccessful thus far. What distinguishes the TV-DTH and in vitro assays is a) the involvement of chemokines and their receptors in the allo-response, and b) the cross-communication between human T and APC on the one hand with mouse accessory cells on the other. We hypothesized that cross communication occurs because human cytokines induce murine endothelial cells in the footpad to release chemokines, which mobilize murine accessory cells to the injection site (footpad). An alternative hypothesis is that human cytokines induce human chemokines which directly bind mouse chemokine receptors.

In an effort to dissect the human-to-mouse TV-DTH assay we neutralized human cytokines, mouse and human chemokines and mouse and human chemokine receptors during the TV-DTH assay. We also performed in vitro culture of human PBMC or murine endothelial cells to determine the key cytokines/chemokines involved in the human-mouse cross-communication. We found that antibodies neutralizing certain human cytokines (TNFα, IFNγ, and/or IL-1β), or murine chemokine receptors (CCR5 and CXCR3) were equally effective in blocking the DTH swelling response. We also found that both human TNFα and IL-1β are produced by EBV-stimulated human PBMC in vitro, and that both cytokines were potent stimulators of release of IP-10 and RANTES from the murine EC cell line 2F2B. Neither IFNγ nor IL-6, which are also up-regulated in EBV-stimulated human PBMC tissue culture supernatant, could directly affect mouse chemokine release. Interestingly, we also found that different T memory cell responses (i.e. to tetanus, EBV or collagen V) tended to induce different cytokines to be released in vitro and were differentially blocked by cytokine-neutralizing antibodies in the trans-vivo DTH response.

The data so far favor the hypothesis that human T-APC interactions result in cytokine releases that induce mouse endothelial cells to produce chemokines. These mouse chemokines in turn drive CCR5- and CXCR3-dependent recruitment of PMNs (Gr1+ cells by IHC) into the footpad. Whether human TNFα or IL-1β are indeed the cytokines which drive the chemokine production in vivo remains unclear. These experiments will form the basis of design of surrogate assays that replicate TV-DTH and its characteristic feature of "linked-suppression" seen in cases of organ allograft tolerance.
Presenter: Headley, Mark

TSLP-dependent induction of airway inflammatory disease is antigen dependent in an acute model of allergic airway inflammation
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The cytokine, Thymic Stromal Lymphopoietin (TSLP), has been shown to play an important role in the mediation of Th2-biased inflammatory diseases in particular the atopic diseases: asthma and atopic dermatitis. Mice expressing a TSLP-transgene driven by the lung-specific Surfactant Protein C promoter (SPC-TSLP) develop a severe and chronic asthma-like disease characterized by airway hyperresponsiveness, leukocytic infiltration of the lung, eosinophilia, goblet cell hyperplasia, sub-epithelial fibrosis, and elevated serum IgE. Previous studies have indicated that TSLP alone is sufficient to induce all of the hallmark features of asthma in mice. Here we show that an airway inflammatory disease, similar both to that seen in asthmatics as well as SPC-TSLP mice, can be induced in BALB/c mice through direct intranasal administration of TSLP in conjunction with a foreign antigen. This has been observed with multiple antigens, including ovalbumin (OVA), chicken gamma globulin (CGG), and bovine serum albumin (BSA). In contrast, mice treated with either TSLP alone, the antigens alone, or TSLP in conjunction with the "self" protein, mouse serum albumin (MSA); fail to develop disease. This data demonstrates for the first time that in an acute model of TSLP-mediated airway inflammation, both thymic stromal lymphopoietin and antigen are required in order to develop full disease. We plan to utilize this model in the future to further elucidate the exact contributions of both TSLP and foreign antigen in the pathogenesis of allergic airway inflammation.
Intraepithelial lymphocytes (IELs) of the small intestine represent a large population of T cells, whose location provides potential exposure to countless immunological insults. IELs possess several characteristics that distinguish them from other peripheral T cells: an oligoclonal TCR repertoire, an antigen-experienced phenotype and a tendency to express the CD8αα homodimer. Despite being predominately CD8+ T cells, IELs are heterogeneous in terms of function, TCR and co-receptor expression, and developmental pathway. The “conventional” (Type A) subset of intestinal IELs, comprised of T cells expressing the αβ TCR along with the CD8αβ or CD4 co-receptors, follows the same developmental pathway as peripheral T cells, undergoing positive selection in the thymus. They may then be exposed to antigen in the GALT and migrate into the intestine as antigen-experienced cells. The “non-conventional” (Type B) subset expresses the αβ (or γδ) TCR along with the CD8αα homodimer. TCRαβ CD8αα IELs possess a self-reactive repertoire, which may be the result of agonist selection in the thymus. Although the exact stage at which these cells exit the thymus is controversial, there is general agreement that CD8αα IELs represent an early lineage of T cell development. These properties, along with their oligoclonal repertoire and appearance early in ontogeny, suggest that the TCRαβ CD8αα IELs express the products of initial TCRα rearrangements.

We hypothesize that CD8αα IELs rely on early TCRα rearrangements, perhaps utilizing a limited repertoire of Jα-proximal Vα genes. To test this hypothesis, we have generated CD4p-Cre Tg RAG-2fl/fl mice, in which TCRα rearrangements are curtailed in late double negative (DN) thymocytes by Cre-mediated deletion of RAG2. Our preliminary analyses of Thy-1+ CD8αα IELs in CD4p-Cre Tg RAG-2fl/fl mice reveal this compartment is of normal size, while the conventional splenic T cell compartment is severely depleted compared to wildtype mice. These preliminary findings suggest Thy-1+ CD8αα IELs do arise from DN thymocytes and rely on early TCRα rearrangements. Spectratype analyses of the expressed TCR repertoire are currently being undertaken and should reveal the impact on the CD8αα IEL repertoire of limiting TCR rearrangements to the DN stage of thymocyte development.
Influence of HIV-1 infection on thymic output *in vitro*

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The thymus plays an important role in human and simian immunodeficiency virus (HIV and SIV) infections. Though for long controversial (1), its contribution to T cell regeneration has been shown during the low but persistent replication that occurs in attenuated SIV infection (2), and more importantly during the immune reconstitution observed in HIV-infected patients on antiretroviral therapy (3). However, mechanisms underlying modulation of the thymic output during an adaptive immune response are unknown.

**Objective:** To determine the early effects of acute HIV-infection on the egress of T-cells from the thymus.

**Methods:** T cell migration from post-natal human thymus organ cultures to CXCL-12 (stromal derived factor, SDF-1) as chemoattractant was measured in transwell plates. Post-natal thymuses were infected with R5-tropic (NFNSX, JR-CSF) or X4-tropic (NL4-3, NL4-3 Delta-nef) HIV-1. Migrating cells were counted and immunophenotyped after 24 hours of migration.

**Results:** Acute infection of human post-natal thymic tissue with HIV-1 lead to early changes in the quantity that egress the organ. Both R-5 and X-4-tropic HIV induce an increase in the numbers of thymocytes that spontaneously exit the thymus. Surprisingly, in response to an acute X4 HIV-1 infection, the chemotaxis of thymocytes towards CXCL-12 is increased. In contrast, our data show that R5-tropic HIV-1 lead to a reduction of the chemotaxis towards SDF-1.

**Conclusion:** Our in vitro data suggest that R5 and X4 HIV-1 have differential impact on egress of mature T cells from the thymus. As both X4 and R5-tropic viruses induce a raise in early thymic output, X4-tropic viruses may impair further T-cell development by perturbing the complex chemotaxis balance that lead the thymocytes through their maturation process. Obviously, host factors intervene in the complex regulation of the numbers of T-cells that egress the thymus in the context of HIV-1 infection, and mechanisms of this regulation are under investigation.

**References:**
(1) Ho Tsong Fang et al, AIDS 2006, HIV and the hidden face of the thymus
(2) Solomon et al, J Infec Disease, 2003
(3) Ho Tsong Fang et al, AIDS 2005
MHC Class II Derived Recombinant T-cell Receptor Ligands Protect DBA/1LacJ Mice from Collagen-Induced Arthritis

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We previously demonstrated the therapeutic effects of MHC class II derived recombinant T cell receptor ligands (RTL), single chain molecular complexes of the α1 and β1 domains of MHC class II molecules genetically linked with an immunodominant peptide, in experimental autoimmune encephalomyelitis (EAE). In the current study, we produced a monomeric murine IAq/bCII 257-270 peptide construct suitable for use in DBA1/LacJ mice that develop collagen-induced arthritis (CIA), an animal model of human rheumatoid arthritis, after immunization with bovine collagen type II (bCII) protein in CFA. Protection with recombinant IAq/bCII257-270 molecule (RTL2001MII) suppressed the clinical and histological signs of CIA and induced a long-term T cell tolerance against arthritogenic antigens. Our results showed that RTL2001MII treatment could both reduce the message and protein secretion of IFN-γ and significantly increase the anti-inflammatory cytokines, IL-10 and IL-13, suggesting that the RTL treatment may lead to a switch from pro-inflammatory to anti-inflammatory cytokines. Moreover, the RTL treatment led to a significant suppression of IL-6, IL-17 and IL-23 in synovial joint tissue. Also the expression of FOXP3 was significantly up regulated after the RTL treatment. Our results demonstrated that the RTL treatment shifted the bCII-specific antibody response from proinflammatory to anti-inflammatory isotypes. Furthermore, HLA-DR4-derived RTL with hCII261-273 peptide has recently been constructed. Protection study showed that human DR4-derived RTL significantly reduced the incidence of the CIA induced by human CII protein in DR4 transgenic mice and induced a long term tolerance against the arthritogenic antigen. This is the first report describing effective treatment of joint inflammation in CIA with a two-domain recombinant IAq and DR4-derived RTL constructs, thus supporting its possible clinical use for treating subjects with rheumatoid arthritis.
Presenter: Huse, Morgan

Spatial and Temporal Dynamics of T Cell Receptor Signaling Revealed Using a Novel Photocaged Agonist
Morgan Huse, Lawrence O. Klein, Andrew T. Girvin, Joycelyn M. Faraj, Qi-Jing Li, Michael S. Kuhns, and Mark M. Davis

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Per Morgan Huse, abstract not available on the website.
Presenter: Hwang, Eun Sook

T-bet protects OVA-induced asthmatic phenotype
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T-bet is a master regulator for Th1 cell differentiation by directly activating IFN gene transcription and by interfering with GATA-3 activity which induces Th2 cytokine production. T-bet-deficient mice developed the spontaneous asthmatic phenotype in lung tissue, which may be resulted from the increased Th2 cytokines. In order to examine the function of T-bet in the progression of asthma, we produced inducible T-bet transgenic in T-bet-null (double Tg/KO) mouse, which re-expresses T-bet in tetracycline-dependent and T cell-specific manner. Using the inducible T-bet transgenic in T-bet-null mice with or without feeding doxycyclin water, we assessed whether induction of T-bet prevents or treats OVA-induced asthmatic progression. OVA challenge induced methacholine airway hyperresponsiveness, eosinophilia, and IL-13 production in BAL fluid and increased mucus secretion in T-bet-deficient mice compared to wild type mice. However, T cell-specific T-bet induction concurrent with OVA injection blocked development of asthmatic phenotype, while inducible T-bet transgenic in T-bet-null mice without feeding doxycyclin water didn’t reveal any changes with conventional T-bet-null mice, suggesting T cell-specific T-bet induction can be a critical immune modulator for asthma.
Presenter: Ikeda, Minako

Immunohistochemical Studies of Hematopoiesis in Two Different Types of Ossification, Endochondral and Intramembranous

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Introduction: It has been well recognized that the body skeleton is formed by two different types of ossification systems, endochondral and intramembranous, and that the bone marrow is the site of active hematopoiesis. However, it has been still uncertain whether the hematopoiesis in the bone marrow in these different ossifications is regulated by the same systems or not. In this study, we studied the ontogenetical development of hematopoiesis in these two different types of ossification with immunohistochemical methods.

Materials and Methods: Humeral long bones (endochondral) and mandibles (intramembranous) from embryonic stage 16 days (E16) to 2 days postnatal (2dPN) of ICR strain mice were fixed, decalcified, embedded in CRYOform, and then quick-frozen in cold acetone with Dry Ice. Sections were processed for the immunohistochemical staining by usual methods. The antibodies were TER-119 for erythropoiesis and Gr-1 for granulopoiesis.

Results: Bone marrow cavity was formed in the humeral bone at E16, but not hematopoiesis. At E17, clusters of Gr-1 positive cells were appeared, erythropoietic islands were not yet. Both granulopoietic and erythropoietic clusters were detected at E18. In the case of mandibles, erythropoietic islands were observed at 2dPN, but no granulopoiesis was detected.

Discussion: Our studies indicate the ontogenetical development of hematopoiesis in the bone marrow was different between two types of ossification, and suggest the microenvironment systems of bone marrow to support hematopoiesis might be different in two types of occurrences.
Human antigen-specific responses tend to default to a Th1 phenotype. In the presence of Interleukin (IL)-4, or in the absence of Th1 cytokines such as Interferon (IFN)-γ and IL-12, these responses can be skewed towards a Th2 phenotype. We have developed an in vitro culture system in which antigen-specific human CD4+ T cells can be expanded from peripheral blood mononuclear cells (PBMC) using House Dust Mite allergen and IL-4. The resulting Th2 T cells secrete high levels of IL-4, IL-5 and IL-13 upon restimulation with antigen. Thymic Stromal Lymphopoietin (TSLP) is a Th2-associated cytokine that is expressed by epithelial and stromal cells. TSLP activates CD11c+ dendritic cells (DC) stimulating production of CCL17/TARC and CCL22/MDC. TSLP-stimulated DC have been shown to expand T cells that produce TNFα and Th2 cytokines. It was of interest to determine whether TSLP could influence the function of blood-derived allergen-specific Th2 cells, specifically whether the presence of TSLP during the expansion phase had an effect on the resulting Th2 T cells, both in terms of phenotype and the potential to produce cytokines. TSLP did not have a significant effect on the numbers of allergen-specific T cells grown out in these cultures but did enhance the levels of IL-4, IL-5 and IL-13 produced upon subsequent restimulation with antigen. Depletion of DC from the PBMC prior to initiation of cultures resulted in a substantial reduction in Th2 cytokine production and the loss of any detectable effect of TSLP, confirming DC as the likely target for TSLP activity. Interestingly, depletion of B cells prior to initiation of cultures also resulted in a reduction of secreted Th2 cytokines although the enhancing effect of TSLP remained intact. The joint role of DC and B cells as antigen presenting cells for memory T cells was confirmed by the lack of any effect on Th2 cytokine production observed after the removal of monocytes. Increases in Th2 cytokine production correlate well with the increase in the number of cytoplasmic IL-4+ cells generated in this culture system. Interestingly, it was found that T cells containing cytoplasmic IL-4 did not secrete this cytokine spontaneously but required restimulation in order for IL-4, IL-5 and IL-13 to be detected in the culture supernatant. The results of these studies demonstrates that TSLP can promote the expansion of human CD4+ memory T cells and that this activity is dependent on the presence of dendritic cells.
FoxP3+ Regulatory T cells Upregulate CXCR5 and Migrate to Germinal Centers Following GC Induction
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While several studies have demonstrated a role for Foxp3+ regulatory T cells (Tregs) in controlling antibody responses in vivo, the mechanisms by which this regulation occurs remain unclear. One possibility is that Tregs migrate to B cell follicles and germinal centers (GC) to directly inhibit the differentiation and activation of B cells. We have identified a subset of Foxp3+ Tregs that express the follicular-homing receptor CXCR5 at steady state levels in naïve mice. This Foxp3+CXCR5+ subset expanded following GC induction with similar kinetics to their CXCR5+CD4+ T helper counterparts. These Tregs were chemotactic toward the B-cell zone chemokine CXC13, and could be visualized in the T cell areas proximal to GC, as well as in the follicular mantle and within the GC itself. The follicular homing of Tregs following GC induction suggests a potential mechanism of regulation whereby Tregs directly inhibit B cells at the site of their affinity maturation and activation.
A role for “self” in the amplification of T cell receptor signaling

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Recently, we showed that to achieve that ‘self’-peptide-MHC (pMHC) complexes are necessary to achieve the greatest sensitivity to foreign (agonist) antigens. In addition, we demonstrated that T-cells could be stimulated by heterodimers containing one strong and one very weak ligand. Mutagenesis of the CD4 binding site on the MHC class II molecule demonstrated that the contribution of CD4 was limited to interaction with the agonist-pMHC. This strongly supports a ‘modified pseudodimer’ model of T-cell activation in which agonist-endogenous pMHC heterodimers, stabilized by the co-receptor CD4, are crucial intermediates for triggering T lymphocytes (Irvine et al., Nature 2002, Krogsgaard et al., Nature 2005). In the context of this model, it remains unclear as to what the precise role of endogenous ligand might be. One possibility is that endogenous ligand cannot induce signaling by themselves, but rather induce an overall conformation that permits phosphorylation of the TCR/CD3 complex that binds agonist. A second possibility is that endogenous ligand-TCR interaction can trigger TCR/CD3 phosphorylation directly in the presence of agonist interaction in a pseudodimer. In this case the rapid turnover of TCR/CD3 in this very unstable interaction with endogenous ligands could contribute more to T cell stimulation than the agonist-TCR/CD3 binding alone. This is analogous to the “serial triggering” model of Lanzavecchia and Valitutti, but because it depends on an agonist ‘anchor’ or more nearly resembles enzymatic catalysis where the agonist ligand-TCR forms an active site. To test these models, we assessed the synergistic effect of a hierarchy of ligands with different potencies from strong agonist to null-peptide. We measured activation in three different I-Eκ-restricted systems using three-dimensional video microscopy to study both early (synapse formation, calcium flux, PI3K localization, CD3ζ phosphorylation) and late (IL-2 translation and loading on secretory vesicles). Here we show that only self-peptides or weak agonist peptides can efficiently synergize with agonist ligand indicating that there is an optimal half-life range for the synergistic ligand. In situ modeling studies confirmed our experimental observations. This optimal half-life range is consistent with a molecular mechanism underlying serial triggering and signal amplification in which binding of endogenous pMHC to the signaling complex enhance the generation of triggered TCR in response to minute amounts of antigens. This consistent with the “catalytic” mechanism discussed above.
Presenter: Kuhns, Michael

Extracellular regions of the TCR constant domains mediate TCR/CD3 complex stability, immunological synapse formation, and TCR signaling
Michael S. Kuhns, Andrew T. Girvin, Johannes B. Huppa, Lawrence O. Klein, Bjorn F. Lillemoeir and Mark M. Davis

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Per Michael Kuhns, abstract not available on the website.
Presenter: Ladi, Ena

Visualizing Dendritic Cell Interactions of Migrating Thymocytes in Real-time Using Two-Photon Microscopy
Ena Ladi, Tanja Schwickert, Tatyana Chtanova, Michel C. Nussenzweig, Nigel Killeen, and Ellen A. Robey

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Using Two-Photon Laser Scanning microscopy, we are able to simultaneously visualize thymocytes and dendritic cells (DCs) in an intact thymus enabling us to quantitate and characterize thymocyte migration and thymocyte-DC interactions that occur in the cortex. We found that thymocytes expressing a TCR transgene (P14) that are more efficiently positively selected also spend more time in contact with DCs as compared to wild type thymocytes with polyclonal TCRs. Additionally, overexpression of CCR7 in thymocytes expressing polyclonal TCRs results in a significant increase in DC-thymocyte interactions. Intriguingly, the cortical source of CCR7 ligand, CCL21, is not DCs but rather the blood vessels of the cortex which are in close contact with the cortical DCs. Based on these observations, we propose a novel role for CCR7 in which CCR7 is upregulated in response to positive selection and the cortical source of CCL21 drives thymocyte-DC interactions that may represent the initiation of scanning for negative selecting signals in the cortex after positive selection.
Presenter: Lambolez, Florence

Identification of Pre- and Post- Selection TCRαβ Intraepithelial Lymphocyte Precursors in the Thymus
Florence Lambolez, Denise Gangadharan, Antoine Attinger, Yiran Wang-Zhu, Barbara A.Sullivan and Hilde Cheroutre

La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037

Per Florence Lambolez, abstract not available on the website.
Presenter: Lopes, Jared

Analysis of FOXP3 Reveals Multiple Domains Required for Its Function as a Transcriptional Repressor
Jared E. Lopes\textsuperscript{1,2}, Lisa A. Schubert\textsuperscript{3}, Troy R. Torgerson\textsuperscript{4}, Stephanie D. Anover\textsuperscript{4}, Elizabeth L. Ocheltree\textsuperscript{4}, Hans D. Ochs\textsuperscript{4} and Steve F. Ziegler\textsuperscript{1,2}

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Per Jared Lopes, abstract not available on the website.
Presenter: Marko, Aimee Joy

Regulation of Glucose Metabolism in Primary T cells
Aimee Joy Marko and Kenneth Frauwirth

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A critical role in a cell-mediated immune response is played by T lymphocytes. During activation, cell-cell interactions and intracellular signaling pathways alter the metabolism and direct the fate of responding T cells. Naïve T cells may become tolerized or activated depending upon the signals they receive. At the same time, metabolism increases in conjunction with receptor stimulation and activation of signal transduction pathways in a resting T cell in order to support T cell function. It is known that metabolism increases upon initiation of activation in order to meet the increased metabolic needs of a proliferating, metabolically active cell, and another idea – that metabolism can be regulated directly via receptor-mediated signals and can feed back in turn to modify cellular signaling - is being investigated. While major signaling pathways and changes in gene expression have been identified, little is known about the cellular control of metabolic changes that enable the cell to sustain its increased growth and proliferation programs and to choose between anergic and activated fates. In order to define the role of metabolism in T cell activation, I will modulate signaling through the T cell receptor (TCR) and CD28 stimulatory pathway in T cells and monitor metabolic changes induced by these signals.
Presenter: McBride, Sara

Symbiotic Bacteria Network with their Mammalian Hosts to Protect from Intestinal Disease
Sara W. McBride\textsuperscript{1}, Dennis L. Kasper\textsuperscript{2}, Sarkis K. Mazmanian\textsuperscript{1}

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Per Sara McBride, abstract not available on the website.
Rheumatoid arthritis (RA) is a systemic inflammatory disease that targets the joints and affects approximately 1% of the worldwide population. Despite this prevalence, the etiology of RA is still unclear. We have discovered a novel mouse strain that spontaneously develops arthritis, and we are currently assessing its viability as a new model of immune-mediated arthritis. After the initial cross between an arthritic male and SJL females, the phenotype has been stable for over 7 generations of inbreeding. Clinical disease is characterized by inflammation in the larger distal joints progressing often to joint deformation. Digits and tail are also occasionally involved. Disease is typically progressive, although mice vary in age of onset (as early as 25 post-natal days), rate of progression, the peak clinical score, and the number of joints involved. Total penetrance has varied by breeder pair but has reached over 90% in some. The mice began on a predominantly SJL background, backcrossed at least 7 times to SJL from an FVB background. Further backcrossing to SJL has only lowered incidence and therefore the line is being maintained by inbreeding. Crosses have now been made with 4 other strains: FVB, Balb/C, C3H, and DBA. In the F1 generations, incidence has been low (FVB: 1/35; Balb/C: 0/31; C3H: 1/31; DBA: 0/33). The F2 generations are currently being tracked for disease. Histological analysis confirmed both cartilage and bone degradation in the larger distal joints with robust infiltration of polymorphonuclear cells and synoviocyte hyper-proliferation. Immunohistochemistry confirms the presence of CD4+ T cells, neutrophils, and F4/80+ macrophage in the inflamed joints. Flow cytometric analysis of cells isolated from the joints confirms the presence of these cell types but also reveals a significant number of B220+ B cells. In arthritic mice, mean serum levels of total Ig and IgG1, but not IgG2a, are elevated compared to non-arthritic littermates. Mean serum levels of Ig specific for collagen type II and joint homogenate are also elevated. Nevertheless, serum transfers into naïve SJL recipients failed to induce disease. Current experiments are assessing the ability of splenocytes to transfer disease into immune-competent and –deficient recipients. Together, this data preliminarily indicates that the arthritis observed in this novel strain is likely immune-mediated and represents a new model.
Presenter: Meeker, Thea

Inhibition of Both TLR7 and TLR9 in Lupus Prone Mice Lead to Reduction of Autoantibody Production and Amelioration of Disease Symptoms
Thea Meeker, Cristiana Guiducci, Jean H. Chan, Robert L. Coffman and Franck J. Barrat

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Per Thea Meeker, abstract not available on the website.
Presenter: Miazgowicz, Michael

Determining the mechanisms by which T-box proteins regulate cell-type and activation dependent target gene expression
Michael M. Miazgowicz, Megan D. Lewis, Kristin M. Beima, and Amy S. Weinmann

Department of Immunology, University of Washington

T-bet is a T-box family transcription factor that plays a critical role in Th1 cell fate development. It has also been shown to play unique, cell type intrinsic roles in a variety of other cells, including CD8+ T cells, B cells, NK cells and dendritic cells. Using a ChIP-based promoter microarray approach, we previously identified a broad set of gene promoters with which T-bet associates, including CXCR3, CCL3 and IL2Rβ. T-bet binding to these promoters occurred independently of cell type. The T-bet dependent regulation of these targets, however, varied dramatically across different cell types. We have therefore set out to identify mechanisms downstream of the DNA binding event that may account for T-bet’s cell type specific functional activity.

To this end, we have used additional promoter microarray analysis to identify novel T-bet targets and have isolated several targets that are either positively or negatively regulated by Tbet. To determine the mechanisms by which T-bet regulates these promoters, we are studying Tbet dependent transcriptional activity in an overexpression system. Using various mutant constructs containing C- and N-terminal truncations of T-bet, we are examining activation and cell-type dependent modes of regulation. The data indicate that multiple domains, independent of the T-box DNA binding domain, are required for functional activity in model T and B cell lines. Further refinement of these domains will allow us to identify relevant protein interactions that govern T-bet’s activity in unique cell types during development and under physiological conditions of activation.
Presenter: Milojevic, Diana

Immune cell expression in systemic and polyarticular juvenile idiopathic arthritis
Claudia Macaubas, Diana Milojevic, Khoa Nguyen, Christy Sandborg, Elizabeth Mellins

Stanford University and UCSF, CA

Juvenile idiopathic arthritis (JIA) is the most prevalent chronic disease of childhood. Unlike isolated joint inflammation of polyarticular form of the disease (poly JIA), systemic juvenile idiopathic arthritis (SJIA) is characterized by severe systemic inflammation causing arthritis, fever, serositis, rash, lymphadenopathy and hepatosplenomegaly. In order to better understand JIA immunopathogenesis, we compared the frequencies of different immune cells in SJIA and poly JIA and studied patients' samples at flare and quiescence. We hypothesized that by comparing samples at these different time points, immune dysfunctions intrinsically related to the disease could be distinguished from ones triggered by generalized immune cell activation during flare.

We assayed cell distribution and markers of immune activation using flow cytometry based assays in peripheral blood mononuclear cells from SJIA patients at systemic flare and during quiescence. As a disease control, we tested patients with poly JIA at flare and at quiescence. We found evidence of expansion of NK cells and monocytes, two innate system cell populations. The percentage of NK cells was consistently higher in SJIA at both flare and quiescence, compared to poly JIA. Specific to SJIA, we observed a higher percentage of the CD14low/CD16high monocyte subset during SJIA quiescence compared to SJIA flare. Following LPS stimulation, higher intracellular pro-IL-1 beta production by monocytes was associated with SJIA flare, but not quiescence, while higher intracellular TNF was associated with both SJIA flare and quiescence.

Prolonged systemic inflammation in SJIA may also be due to ineffective suppressor functions of the immune system. Therefore, we examined the frequencies of “natural” T regulatory cells (Tregs) defined by cell surface expression of CD4 and CD25, and the absence of CD127 (IL-7 receptor). The percentage of T regulatory cells in SJIA was higher than in polyJIA, but not significantly different from controls. However, compared to controls, the percentage of “naïve” CD45+ Tregs was higher in SJIA and lower in poly JIA. Our results suggest an altered distribution of innate cell populations as well as Treg subsets may be a feature of SJIA.
An anti-apoptotic protein, c-FLIPL directly binds to MKK7 and inhibits JNK and ROS accumulation
Akihito Nakajima, Hideo Yagita, Ko Okumura and Hiroyasu Nakano
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Inhibition of NF-κB activation increases susceptibility to tumor necrosis factor (TNF)α-induced cell death, concurrent with caspases and prolonged c-Jun N-terminal kinase (JNK) activation, and reactive oxygen species (ROS) accumulation. However, the detailed mechanisms are unclear. Here we show that cellular FLICE-inhibitory protein (c-FLIP) is rapidly lost in NF-κB activation-deficient, but not wild-type fibroblasts upon TNFα stimulation, indicating that NF-κB normally maintains the cellular levels of c-FLIP. The ectopic expression of the long form of c-FLIP (c-FLIPL) inhibits TNFα-induced prolonged JNK activation and ROS accumulation in NF-κB activation-deficient fibroblasts. Conversely, TNFα induces prolonged JNK activation and ROS accumulation in c-Flip-/- fibroblasts. Moreover, c-FLIPL directly interacts with a JNK activator, MKK7 in a TNFα-dependent manner and inhibits the interactions of MKK7 with MEKK1, ASK1, and TAK1. This stimulus-dependent interaction of c-FLIPL with MKK7 might selectively suppress the prolonged phase of JNK activation. Taken that ROS promote JNK activation and activation of the JNK pathway may promote ROS accumulation, c-FLIPL might block this positive feedback loop, thereby suppressing ROS accumulation. Therefore, c-FLIPL-dependent inhibition of the JNK pathway may be a novel target to treat inflammation and cancers, in which JNK activation is critically involved.
Presenter: Okumura, Shigeru

Analysis and Prediction of signaling events in single cell
Shigeru Okumura, Byron Ellis, Garry Nolan

Stanford University School of Medicine, Baxter Laboratory in Genetic Pharmacology, Microbiology and Immunology

The stress-activated protein kinase (SAPK) and mitogen-activated protein kinase (MAPK) subfamilies are crucial to environmental stress responses and responses to growth factors that cause transcriptional activation of genes required for cell proliferation, differentiation and programmed cell death. These pathway are well studied as individual pathways and one protein, or two at a time. However no one has been able to observe all three pathways at once (9 proteins) and at the single cell level. In other words, the underlying subtleties of crosstalk would be very difficult to discern by standard approaches. We hypothesized that significant crosstalk would exist some crosstalk pathway between ERK, JNK, P38 MAPK (even though none has previously been seen). To address this question, we analyzed these phosphorylation events using with multi color phospho flow cytometry. To initiate the project we started with the human monocytic cell line U937. Recombinant hTNF-alpha were used for stimulation. Time points are 5min, 15min, and 30min. We also use kinase specific inhibitor for this experiment. We used SB203580 for P38 inhibition, u0126 for MEK inhibition and JNK inhibitor I for JNK/SAPK inhibition. To analyze the relationships between all of these proteins as a whole we rely on a machine learning technique known as Bayesian Network Structure Learning. In this analysis, protein phosphorylation is taken to be a proxy for protein activity except under inhibition which allows the construction of a model for the phosphorylation of downstream proteins given the activity level of upstream proteins. We have found the some possibilities which are crosstalk pathway and unknown pathway.
Presenter: Ozeki, Munetaka

Cell senescence-like growth arrest in neutrophilic-HL60 cells
Munetaka Ozeki and John E. Shively

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Retinoid, natural and synthetic derivatives of vitamin A, regulate various important cellular functions in the body through specific nuclear retinoic acid receptors and retinoid-X receptors, which are encoded by separate genes. Furthermore, all-trans retinoic acid, the natural isomer of 13-cis retinoic acid, has shown to be an effective agent in acute promyelocytic leukemia, with induction of complete remissions in the majority of patients. HL60 human promyelocytic leukemia cells can be differentiated into neutrophil with retinoic acid treatment, and used for many kind of experiments, for example cancer chemotherapy, drug discovery, cell differentiation, apoptosis, etc.

On the other hand, Chang et al reported that retinoic acid-treated MCF7 human breast carcinoma cells showed senescence-like phenotype using senescence associated beta-galactosidase (SA-Beta-Gal) activity at pH 6.0 as a surrogate marker of senescence. In general, senescent cells are characterized as followings: i) Senescent cells arrest growth with a G1 DNA content and don not enter S phase in response to physiologic mitogens. ii) Senescent cells remain metabolically active, and resist apoptotic death, for long periods of time. Etc.

Retinoic acid-treated HL60 cells predominantly differentiated into neutrophil and died from apoptosis within a week. At Day 1-2, retinoic acid caused G1 cell cycle arrest. The G1/S cell cycle check point molecules including cyclin D1 and E1 expression were dramatically decreased. Then pro-apoptotic proteins were induced. At the same time CD66a expression was also induced. At day 4-5, cells started to die from apoptosis. However we observed that CD66a positive neutrophilic-HL60 cells showed anti-apoptotic phenotype and lived longer than CD66a negative neutrophilic-HL60 cells in our preliminary experiments. Interestingly, these cells showed SA-Beta-Gal activity. Transient treatment with retinoic acid caused G1 cell cycle arrest and CD66a induction, but not apoptosis. Moreover, cells can survive longer as G1 state and were positive for cell SA-Beta-Gal staining. These results suggested that CD66a positive neutrophilic-HL60 cells had cell senescence-like phenotype.
Presenter: Pakpour, Nazzy

Central memory CD4+ T cells require IL-12 to become Th1 cells capable of contributing to immunity against Leishmania major
Nazzy Pakpour, Colby Zaph, and Phillip Scott

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Control of the parasite Leishmania major occurs through the production of IFNγ by Th1 effector cells and leads to life-long immunity. We have previously shown that immunity to re-infection is mediated primarily by two populations of cells: effector and central memory CD4+ T (Tcm) cells. It remains unclear whether the Tcm cells generated during Leishmania infection are non-polarized and retain their plasticity or whether these cells are predisposed to a particular set cytokine profile. Since we have previously demonstrated that IL-12 is crucial for the maintenance of immunity to L. major, we hypothesized that the Tcm cells generated during infection are non-polarized and that these cells require IL-12 in order to become the Th1 effector cells capable of contributing to immunity. To test this, we transferred donor Tcm cells from immune C57BL/6 mice into naïve wild-type or IL-12p35/- mice, which we then infected with L. major. Proliferation and cytokine production of donor cells was assessed two weeks after infection. Tcm cells proliferated equally well in the presence or absence of IL-12, and in IL-12 sufficient mice 44% of proliferating cells were capable of producing IFNγ. However, when these same cells were transferred into IL-12p35/- mice only 7% became IFNγ+. Moreover, a significant percentage of Tcm cells in IL-12p35/- mice became IL-4 producers. Our results show that IL-12 is required for the differentiation of Tcm cells into Th1 cells, suggesting that the Tcm cell population generated during L. major infection is primarily composed of non-polarized cells.

This work was supported by NIH grants RO1 35914 and 2-T32-AI007532.
Presenter: Parker, David

Differential Ability of B-1, Marginal Zone, and Follicular B cells to Induce Peripheral CD4 T Cell Tolerance In Vivo
David C. Parker and Susan E. Murray

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Aside from dendritic cells (DC), B cells are the only other antigen presenting cells (APC) that express high levels of MHC class II and B7 costimulatory molecules in the resting state. While DC are very efficient at presenting antigen via non-specific uptake, B cells can concentrate and present antigen that binds to their BCR with much higher efficiency than DC. Teleologically, B cells may be important tolerizing APC to prevent activation of autoreactive T cells specific for low concentration soluble self-antigens or self-antigens for which there is a high B cell precursor frequency (i.e., some nuclear antigens and glycoproteins).

In the absence of adjuvants or other danger signals, antigen presented by B cells can induce CD4 T cell tolerance. However, antigen targeted to B cells can be transferred to other APC in vivo and it is unclear whether naïve T and B cells at physiologically low precursor frequencies interact in vivo. In addition, the relative efficiency of different B cell subsets in inducing T cell tolerance has not been assessed. In order to address the above questions, we have developed an antigen transgenic system in which peptide is covalently linked to MHC class II.

Using this system in conjunction with antigen-specific CD4 T cells, we are investigating the ability of B-1, marginal zone (MZ), and follicular (Fo) B cells to induce CD4 T cell tolerance in vivo. We created mixed BM chimeras in which very small numbers (<1% of splenocytes) of either B-1, MZ, or Fo B cells express the antigen transgene. Upon injection of CFSE-labeled antigen-specific CD4 T cells under tolerizing conditions, we found that naïve T cells do encounter antigen on rare antigen-presenting B cells as assessed by CFSE dilution. Moreover, antigen-expressing B-1 B cells were far superior APC for naïve T cells than were equal numbers of MZ or Fo B cells. We are currently investigating the quality of tolerance induced in these naive T cells that have encountered antigen on B-1 versus MZ and Fo B cells.

The ability of different B cell subsets to induce or maintain peripheral CD4 T cell tolerance may have important implications for autoimmune disease, and we are concurrently investigating T cell tolerance in the absence of certain B cell subsets.
Expression of FOXP3 in Attenuated IPEX
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IPEX syndrome is a genetic autoimmune disease characterized by immune mediated polyendocrinopathy, enteropathy, and X-linked inheritance. As described in previous publications, most IPEX patients lack expression of FOXP3 mRNA and protein. Therefore, CD4+ CD25+ Tregs fail to develop in these individuals. As a consequence these patients develop overwhelming and fatal autoimmune disease. This case represents the only mutation that has been associated with an attenuated phenotype. This mutation was found in a family originally described by Powell et al (1982) and has been localized to the first polyadenylation signal down stream of the stop codon for the FOXP3 gene. The goal of the present study was to determine if FOXP3 mRNA and protein were expressed at birth in this IPEX patient. Prenatal genetic diagnosis was performed at 16 weeks gestation due to extensive family history and indicated the patient was affected. FOXP3 mRNA was measured by RT-PCR and protein expression was determined with fluorescent antibody staining and flow cytometry. The absolute number and percentage of all lymphocyte subpopulations were determined using routine immunophenotyping methods. The study included cord blood from the IPEX patient and normal male controls, as well as peripheral blood from normal adult male controls. Cord blood from this IPEX patient contained normal numbers of T-cells, including CD4+CD25+ regulatory T-cells (Tregs), compared to normal cord blood control ranges. T-cells from the IPEX patient expressed normal levels of both FOXP3 mRNA and protein compared to both normal cord blood and normal adult peripheral blood controls. This study illustrates expression of FOXP3 mRNA and protein in a case of attenuated IPEX. The 13 mutations reported for classical IPEX occur within the coding region. These mutations prevent production of functional FOXP3 protein. In contrast, attenuated IPEX is characterized by a mutation in the polyadenylation site. The patient reported in this study expressed FOXP3 mRNA and protein in cord blood lymphocytes. This patient and others in the extended family develop IPEX later (3 months-5 years), often after an immune stimulus. Therefore, we speculate that the Tregs present at birth in attenuated IPEX have limited function and do not provide adequate suppression after immune stimulation. Alternatively, this case may illustrate differences in the development of “natural” and “acquired” Tregs. Further studies are needed to clarify this issue.
Distinct sources of sphingosine-1-phosphate promote lymphocyte egress into blood and lymph

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Lymphocytes require sphingosine-1-phosphate (S1P)-receptor-1 to exit lymphoid organs, but the source(s) of extracellular S1P and whether it directly promotes egress are unknown. Two kinases, Sphk1 and Sphk2, synthesize S1P. We inactivated Sphk1 using Mx1-Cre-mediated recombination in newborn Sphk2−/− mice. S1P was undetectable in plasma and lymph in these mice, and lymphocyte egress from thymus and secondary lymphoid organs was markedly reduced. Bone marrow chimera analysis revealed that plasma S1P is predominantly hematopoietic in origin whereas lymph S1P arises from a distinct source. Red blood cells (RBC) were identified as a major source of plasma S1P, and transfusion of RBCs and intravenous infusion of S1P restored egress into blood but not lymph. Our results suggest that lymphocyte egress occurs in response to S1P presented at exit sites and that separate sources supply S1P to plasma and lymph to help direct exit.
Presenter: Rosen, David

Lectin-like transcript-1 (LLT1) is a ligand for the human CD161 receptor, NKR-P1A
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TACI positively regulates APRIL-induced IgA production in concert with HSPG
Daisuke Sakurai

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APRIL is a member of the TNF family that has been implicated in several immunological phenomena such as peripheral B-cell survival, CD40L-independent antibody isotype switching and the induction of self-reactive B cells. APRIL engages two TNF receptor family proteins named TACI and BCMA, and interacts with heparan sulfate proteoglycans (HSPGs). However, no functional study on the role of APRIL-binding to HSPG in B-cell responses is reported. Although TACI is thought to serve as a negative regulator for B-cell responses, some studies showed that TACI is a positive regulator for IgA response. To assess the functional aspects of the TACI and HSPG in B-cell responses, we attempted to describe the mutual roles between TACI and HSPG in APRIL-induced B-cell responses.

To evaluate the role of HSPG in APRIL-induced B-cell responses, we initially assessed the dependency of TACI and HSPG in APRIL-binding to human peripheral blood B cells by utilizing siRNA approach to knock down TACI and/or the treatment with heparitinase to cut the HS side chain of HSPG. Flow cytometric analysis indicated that the ratio of APRIL bound to B cells was equally reduced by either the depletion of TACI or denaturation of HSPG, both of which showed complete loss of its binding. Next, to determine the contribution of HSPG and TACI to APRIL-induced B-cell responses, we evaluated B-cell proliferation, secretion of IgG and IgA in heparitinase-treated B cells with or without TACI siRNA. BAFF-induced B-cell proliferation was significantly increased by the depletion of TACI compared to the control. In contrast, APRIL-induced cell growth was inhibited by heparitinase treatment. BAFF-induced IgG and IgA secretion was enhanced by the knockdown of TACI, whereas APRIL-induced IgA secretion required both TACI and HSPG. In addition, immunoblot analysis revealed that collaboration of TACI with HSPG was required for phosphorylation PKA, a physiological kinase for AID while HSPG alone was sufficient for AID upregulation. Importantly, simultaneous co-ligation of TACI and HSPG by specific antibodies resulted in the phosphorylation of PKA and IgA secretion instead of APRIL. These results suggest that TACI provides a synergistic signal for IgA production with APRIL-HSPG binding.
Wiskott Aldrich Syndrome Protein is Required for Regulatory T Cell Homeostasis
Stephanie Humblet-Baron¹,², Blythe Sather³,⁴*, Stephanie Anover¹, Shirly Becker-Herman¹, Debora J. Kasprowicz⁴, Socheath Khim¹, Thuc Nguyen³, Kelly Hudkins-Loya⁵, Charles E. Alpers⁵, Steve Ziegler³,⁴, Hans Ochs¹, Troy Torgerson¹, Daniel J. Campbell³,⁴, and David J. Rawlings¹,³,⁶

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Wiskott Aldrich Syndrome protein (WASp) is essential for optimal T cell activation. Patients with WAS exhibit both immunodeficiency and a marked susceptibility for systemic autoimmunity. We investigated whether alterations in T regulatory cell (TR) function might explain these paradoxical observations. While WASp deficient (WASp⁻/⁻) mice exhibit normal thymic TR generation, the competitive fitness of peripheral TR was severely compromised. WASp⁻/⁻ TR numbers were decreased in all peripheral lymphoid tissues; and rapidly out-competed by wild type TR in vivo. These findings correlated with reduced expression of markers associated with self-antigen driven peripheral TR activation and homing to inflamed tissue. Consistent with these findings, WASp⁻/⁻ TR showed a reduced ability to control aberrant T cell activation and autoimmune pathology in FOXP3⁻/⁻ Scurfy (Sf) mice. Finally, WASp+ TR exhibited a marked selective advantage in vivo in a WAS patient with a spontaneous revertant mutation, indicating that altered TR fitness likely explains the autoimmune features in human WAS.
Presenter: Savage, Peter

Spontaneous CD8+ T lymphocyte responses to a ubiquitous self antigen in a transgenic mouse prostate cancer model
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Per Peter Savage, abstract not available on the website.
Loss of GATA-3 at the thymic precursor and early DN stages impairs early T cell development
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The T regulatory transcription factor GATA-3 is expressed throughout intrathymic T cell development as well as during peripheral T cell development (where it has a role in promoting the shift to the TH2 phenotype). Our previous studies suggest that GATA-3 is important in the earliest stages of T cell development, but an absolute requirement for this factor at each of the earliest stages has not been proven. Using a variety of gene expression knock down techniques (including retroviral expression of shRNA and electroporation of siRNA or morpholinos specific for GATA-3), we seek to determine whether GATA-3 is required in each of the earliest DN stages. We are also identifying target genes regulated by GATA-3, and thus defining the control exerted by this factor over the formation and composition of the gene expression networks controlling T cell development.

Employing an OP-9 co-culture system to generate early thymocytes derived from fetal liver precursors (FLDNs) in numbers sufficient for knock-down experiments, we find that basal gene expression patterns found in FLDNs are similar to the known gene expression patterns seen in DN1-DN4 in fresh fetal thymocytes. In this same system, loss of GATA-3 at the early T precursor stage, as well as at DN1, DN2, and DN3, results in stage specific viability and progression defects. These defects cannot be overcome by providing a bcl-2 transgene, although decreases in viability can be somewhat relieved at the precursor and DN1 stages using this method. The delay in T cell development due to loss of GATA-3 from the early Kit+lin-27+ cell is observable even at the single cell level, as GATA-3 shRNA expressing clones failed in all cases to progress beyond DN2. We have now extended our knock down studies to include freshly isolated fetal thymic DNs, and have further confirmed a delayed developmental phenotype.
We are currently conducting gene expression analysis within each stage of T development, and will use this information to understand the precise GATA-3 mechanism in action at each developmental phase.
Production of Bioactive IL-16 by Infiltrating Lymphocytes Correlates to Damage of Axonal Cytoskeleton in Multiple Sclerosis (MS) Lesions

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Multiple sclerosis (MS) is a central nervous system–specific autoimmune, demyelinating and neurodegenerative disease. Infiltration of lesions by autoaggressive, myelin-specific CD4+Th1 cells correlates with clinical manifestations of disease. The cytokine IL-16 is a CD4+ T cell-specific chemoattractant that is biased towards CD4+Th1 cells. IL-16 precursor is constitutively expressed in lymphocytes and during CD4+ T cell activation; active caspase-3 cleaves and releases C-terminal bioactive IL-16. Previously, we used an animal model of MS to demonstrate an important role for IL-16 in regulation of autoimmune inflammation and subsequent axonal damage. This role of IL-16 in MS is largely unexplored. Here we examine the regulation of IL-16 in relation to CD4+ Th1 infiltration and inflammation-related changes of axonal cytoskeleton in MS lesions. We measured relative levels of IL-16, active caspase-3, T-bet, Stat-1 (Tyr 701), and phosphorylated NF(M+H), in brain and spinal cord lesions from MS autopsies, using western blot analysis. We examined samples from 39 MS cases, which included acute, subacute and chronic lesions, as well as adjacent, normal-appearing white and grey matter. All samples were taken from patients with relapsing remitting clinical disease. We employed two-color immunostaining and confocal microscopy to identify phenotypes of IL-16-containing cells in frozen tissue sections from MS lesions. We found markedly increased levels of pro- and secreted IL-16 (80 kD and 22 kD, respectively) in MS lesions compared to controls. Levels of IL-16 peaked in acute, diminished in subacute, and were elevated again in chronic active lesions. Compared to lesions, lower but still appreciable IL-6 levels were measured in normal-appearing white matter adjacent to active lesions. Levels of IL-16 corresponded to increases in active-caspase-3, T-bet and phosphorylated Stat-1. In MS lesions, we readily observed IL-16 immunoreactivity confined to infiltrating CD3+, T-bet+ and active caspase-3+ mononuclear cells. We present evidence suggesting that IL-16 production occurs in MS lesions. We show correlations between increased levels of secreted IL-16, CD4+ Th1 cell inflammation, and phosphorylation of axonal cytoskeleton in MS lesions. Overall, the data suggest a possible role for IL-16 in regulation of inflammation and of subsequent changes in the axonal cytoskeleton in MS.

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Presenter: Smyth, Jeff

TOLAMBA™ Shows Reduced Allergenicity in IgE Binding Assay
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Background
Immunostimulatory DNA Sequences (ISS) linked to the major human ragweed antigen, Amb a 1, decrease IgE recognition of the Amb a 1 antigen and create an immunogen that generates protective Th1 responses and reduces harmful Th2 responses. TOLAMBA is an Amb a 1-ISS conjugate currently in late stage clinical trials that is aimed at reducing the human ragweed allergic response.

Methods
An allergenicity assay was developed to titrate the concentration of TOLAMBA or unmodified Amb a 1 material required to compete for binding of a constant amount of IgE purified from the sera of ragweed allergic subjects. After allowing the materials to react, signal from unbound IgE is detected by binding to an Amb a 1 coated ELISA plate. High signal indicates that TOLAMBA or Amb a 1 materials from the first reaction bound very little of the IgE leaving much of it to bind to the Amb a 1 coated ELISA plate. Low signal indicates binding of the IgE in the first reaction leaving little antibody to bind to the Amb a 1 coated ELISA plate. A titration of each material in the competition ELISA is performed and then the ratio of TOLAMBA versus Amb a 1 is calculated for the linear portion of the titration curves.

Results
Assay results show that TOLAMBA demonstrates greater than a five-fold reduction in IgE antibody binding compared to the unmodified Amb a 1 antigen alone. Conjugate lots having a similar ratio of ISS molecules conjugated to the Amb a 1 antigen show similar reductions in allergenicity.

Conclusions
The allergenicity assay demonstrates that TOLAMBA has a reduced ability to bind IgE from sera of ragweed allergic donors in a competition ELISA as compared to unmodified Amb a 1. This reduced ability to bind or react with IgE represents a reduction in the reactogenicity of the TOLAMBA material in ragweed allergic subjects, indicating a level of safety for TOLAMBA material in this patient population.
Presenter: Soper, David

IL-2Rβ links IL-2R signaling with Foxp3 expression
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Per David Soper, abstract not available on the website.
Presenter: Tamang, David

**CxRP2, a novel protein that regulates perforin cytolytic activity**

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It has become an unholy grail to determine how cytotoxic T cells avoid killing themselves while attacking and killing their targets using cytotoxic granules. The protein perforin (Pfn) is an essential component of the granule-mediated cytotoxic lymphocyte pathway, permeabilizing target cell plasma membranes to allow entry of apoptotic granule proteins and contributing to osmotic lysis. Here we describe a regulatory protein within the granules that ablates perforin-dependent cell membrane damage in cytotoxic granules, that reduces lysis by recombinant perforin, and that also blocks allogeneic tumor killing by T lymphocytes. The source of CxRP2 was the dense granules of murine Pfn-/- T lymphocytes that were expanded with 1000 u/ml human r-IL-2, that were purified using PercollTM gradients and then extracted. The perforin regulatory activity was limited to the dense Pfn-/- granule fraction. The ability of wild type granules to lyse cells was reduced several fold by Pfn-/- granules at less than 3 ug/ml total granule protein. Recombinant perforin was similarly reduced in lytic capability by Pfn-/- granules. When Pfn-/- granules were included in allogeneic cytotoxic T lymphocyte reactions against P815 target cells, the effector cells demonstrated more than a 2 fold reduction in efficacy. CxRP2 is calcium insensitive, unlike perforin. CxRP2 is heat-inactivated, which suggests that the activity is independent of sulfated glycans which would be unaffected by heating. The regulatory protein may specifically cleave perforin protein as assayed by immunoblot. CxRP2 is insensitive to E64, DCI, or CA072, suggesting it is something other than a serine/cysteine protease. Further, using immobilized RNK-16 rat granules containing perforin, we depleted the bio-activity from Pfn-/- granules. Fractionation of pfn-/- granules has shown that CxRP2 bioactivity resides with proteins between 50-100 kDa. Our working hypothesis is that CxRP2 contributes to intricate mechanisms that regulate T cell cytotoxic processes and that attenuation of CxRP2 may improve T cell killing of tumors.
Presenter: Tanaka, Motoyuki

**Fcγ receptor ligation triggers human monocyte differentiation into CD1b+ cells**
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In the innate immune response against mycobacteria infections, Fcγ receptors play an important role for monocyte functions such as the up-take of opsonized pathogens and the induction of pro-inflammatory cytokines. We previously reported that CD1b+ dendritic cells are abundant in the resistant, tuberculoid leprosy lesion but are absent in the progressive, lepromatous leprosy lesion. These data suggest that CD1b+ cells play a crucial role in the immune response against mycobacteria. Here we demonstrate that Fcγ receptor ligation by human IgG triggers the differentiation of monocytes into CD1b+ cells. The CD1b+ population increased in a dose-dependent manner, and the differentiation was blocked by anti-CD64 monoclonal antibodies. Furthermore, Fcγ receptor ligation triggered transcription of GM-CSF mRNA as measured by real time PCR. It is known that GM-CSF can induce CD1b expression on monocytes. These findings predict that the stimulation of Fcγ receptors induces GM-CSF production that in turn induces CD1b expression on monocytes. In conclusion, these results provide insight into the cross-talk between the humoral and innate immune response to pathogens.
Presenter: Tarasenko, Tatyana

The inositol phosphatase SHIP is required for an effective Th2 response
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Per Tatyana Tarasenko, abstract not available on the website.
Presenter: Treml, John

Ligand Independent Signaling drives development of B-1 B cells from fetal liver precursors
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Several factors are thought to control the development of B cell precursors into the known mature B cell niches. B cell antigen receptor (BCR) signal is well appreciated as a primary determinant in this process, indeed without a functional BCR, B cell development is blocked at the pro B stage. Previous work from this laboratory has established the role of ligand independent, "tonic", signaling using a membrane associated chimeric molecule expressing the immunoregulatory tyrosine activation motifs (ITAMs) of Igα and Igβ referred to as MAHB. Transduction of whole bone marrow with this construct is sufficient to overcome this developmental block in μMT mice and result in the production of immature/mature peripheral B cells. However, there was no evidence for the rescue of B-1 B cells in these mice. The absence of B-1 B cells in these mice can be attributed to a number of factors. Either signal strength from MAHB was insufficient to drive B-1 development or bone marrow precursors are innately incapable of producing a significant number of B-1 B cells. To address this question, we transduced day15 fetal liver cells from μMT donor animals with the MAHB construct. After a period of reconstitution, both CD5+ B-1a and CD5- B-1b B cells were found in the peritoneal cavity of these mice. These data argue that the source of hematopoietic precursors rather than signal strength is the primary determinant of B-1 B cell production. Further, tonic signal from the MAHB construct is sufficient to produce both B-1 and B-2 B cells when expressed in appropriate precursors. Future work includes analysis of transgenic animals with B cells expressing the MAHB construct in a competitive environment.
Autoimmune T cells Display Evidence of Activation-induced Split Anergy: A Mechanism of Persistence of Autoimmune T Cells
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Background and Objectives: Patients with autoimmune diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), have accumulation of activated T cells in their lymphoid organs and peripheral circulation. Normally activated T cells are eliminated via activation-induced cell death or kept silent via anergy. It is unclear how self-reactive T cells emerge and remain activated in autoimmune-prone individuals.

Methods: We have investigated this issue using T cells from animal models with SLE, including MRL-Fas-lpr/lpr (MRL-lpr), MRL-Fas+/- (MRL-fas) and NZB/NZW F1 (BWF1) mice, and from their MHC, age and sex-matched controls. Calcium flux, activation markers, and apoptosis in these T cells were analyzed by multi-parameter flow cytometry. Cytokine responses were analyzed by ELISA, intracellular staining and flow cytometry, and real-time quantitative PCR. Cell signaling molecules were analyzed by Western blot and flow cytometry.

Results: We first show here that T cells from lupus-susceptible strains are indeed activated, as demonstrated by increased expression of activation markers CD25, CD44, and CD69, and reduced expression of CD62L. More in vivo activated T cells secrete proinflammatory cytokine IFN-gamma in lupus strains than in normal strains. Furthermore, autoimmune T cells exhibit constitutive phosphorylation of multiple proximal and distal T cell signaling molecules, including ZAP-70, ERK1/2 and p38 MAP kinase, again suggesting in vivo activation of these T cells. Surprisingly, however, these activated autoimmune T cells display features of hyporesponsiveness when further stimulated through the T cell receptor (TCR), as shown by reduced calcium flux, IL-2 production, proliferation, and activation of T cell signaling molecules ZAP-70, ERK and JNK upon anti-CD3 stimulation. More importantly, whereas normal T cells undergo activation-induced cell death (apoptosis), T cells from lupus-prone MRL-lpr, MRL-fas and BWF1 mice display resistance to activation-induced cell death when stimulated through the TCR but not when T cell stimulation bypasses TCR signaling pathway.

Conclusions: Autoimmune T cells undergo chronic in vivo TCR signaling. Repeated or continuous activation of these T cells in vivo results in ‘activation-induced hyporesponsiveness’ and block in further TCR signaling, with ensuing resistance to activation-induced cell death. Our data provide a new model to explain in vivo persistence of autoimmune T cells in lupus-prone subjects. Our future goal is to explore whether in vivo blocking of the key TCR signaling molecules in autoimmune T cells revert the autoimmunity and prevent or suppress disease.
A regulatory role of L-plastin, an actin filament-bundling protein, in T cell function
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Per Chen Wang, abstract not available on the website.
TRAIL-R is an important regulator of T cell responses
Allen Wensky and Astar Winoto

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Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) is a member of the tumor necrosis factor (TNF) superfamily. Numerous studies have shown that TRAIL can induce apoptosis in many tumor cells but not nontransformed cells. TRAIL exists in both membrane and soluble forms and can signal through itself (reverse signaling) as well as initiate signaling through the TRAIL receptors. In mouse, there is one TRAIL signaling receptor. To define the physiological role of the signaling receptor for TRAIL (TRAIL-R), our lab generated TRAIL-R deficient mice and demonstrated a non-apoptotic role for TRAIL-R in inhibition of the innate immune response. However, the role of TRAIL-R in T cell mediated immunity is still poorly understood. One of the obstacles so far has been the fact that both TRAIL-R and membrane bound TRAIL can signal in T cells. Most of the studies have focused on TRAIL-mediated signals which have been shown to be involved in proliferation and cytokine production after suboptimal activation. We wished to address the function of TRAIL-R in T cell responses to help define the roles this molecule plays in a productive immune response and to resolve the function of TRAIL versus its receptor in T cell function. TRAIL-R mRNA is expressed at low levels in naïve and recently activated T cells and is upregulated significantly 24 hours after activation. Surface expression of TRAIL-R on T cells is observed at 24-48 hours post-activation. TRAIL-R does not play a noticeable role in apoptosis or proliferation after activation but does influence IFN-γ expression levels as early as 48 hours post-activation. T cells that lack TRAIL-R expression consistently produces less IFN-gamma compared to heterozygous littermates at 48-72 hours after activation. However, IL-4 and activated TGF-β expression is similar between wildtype and knockout T cells. Early IFN-γ production however (16-24 hrs) is similar for both groups, indicating that differences occur at later time points, shortly after TRAIL-R upregulation. The deviation in IFN-γ production levels however is not related to cell proliferation, apoptosis or Th2 skewing.

Ουρ δατα, ιν χον υγκτιον ωπη ςτερ πυβλιςεδ δατα, δεμονςτραει ηιω TRAIL Λ–Ρ αεχσ ισ α χρντρολ μεκςηιαυ φορ ιμμυνε μεςπνςεσ. Ωε εμποψεζει τηι συςτανεδ ανβ προπχτε ε εξπρεσιον ωφ της Τ1 χυτννε ΙΦΝ–γ can be maintained by TRAIL-R to potentially aid in the resolution of an immune insult.
Interleukin-2 rescues helpless CD8 T cells by modification of their program
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CD4 T cell help is required during priming for the development of durable CD8 T cell memory. While primary CD8 T cell responses can proceed normally in the absence of T cell help, antigen specific recall responses are severely compromised via a transcriptional program that results in TNF-related apoptosis inducing ligand (TRAIL)-mediated apoptosis. Here, we test a broad array of inflammatory and growth cytokines in their capacity to reverse the helpless program during secondary antigen encounter, and demonstrate that helpless CD8 T cells only fully regain their proliferative and functional capacity in the presence of exogenous IL-2. We show that IL-2 partly modifies the TRAIL/DR5 apoptotic pathway, providing a molecular basis for restoration of CD8 T cell effector function of helpless CD8 T cells. These results highlight an important role for IL-2 in regulating CD8 T cell memory and may have significant implications for prime-boost vaccination strategies.
iNKT Cells Regulate MZ-B cells
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Autoimmunity and Tolerance Laboratory, UCLA

Studies from ours and other laboratories suggest that in vivo activation of invariant natural killer T (iNKT) cells protect against the development of autoimmune disease, such as systemic lupus erythematosus (SLE), at least during early stages of disease development. However, the mechanisms of protection remain poorly understood. Several laboratories have also described that activated iNKT cells can trans-activate other immune cells including B, T and dendritic cells. Here, we show that although iNKT cells can trans-activate B cells to increase expression of activation markers and production of normal Ig, they selectively suppress IgG autoantibody production in a variety of in vitro as well as in vivo experimental systems including adoptive transfers in SCID and iNKT cell-deficient (Ja18−/−) mice. Whereas increased expression of B cell activation markers occurs via cytokines secreted by iNKT cells, iNKT cell-mediated suppression of IgG autoantibody production requires contact between iNKT cells and B cells. Furthermore, although iNKT cells increase production of cytokines by other immune cells, they specifically inhibit IL-10 production by B220+ cells in a contact-dependent manner. We then investigated whether iNKT cells affect autoantibody production through their effects on B cell differentiation. In particular, we have focused on marginal zone B (MZ-B) cells, since we found an expansion of activated MZ-B cells in autoimmune lupus-prone BWF1 mice. We found that the proportion of MZ-B cells is reduced when whole spleen cells are cultured in the presence of activated iNKT cells. Further support for the role of iNKT cells in regulating MZ-B cells came from iNKT cell transgenic (Vα14Tg) and deficient (Ja18−/−) mice. Whereas the proportion of MZ-B cells are increased in Ja18−/− mice as compared to wild type BALB/c mice, these cells are decreased in Vα14Tg mice. Thus, our results show that iNKT cells suppress autoreactive B cells and prevent systemic autoimmunity. They do so via regulating MZ-B cells. How activated iNKT cells regulate MZ-B cells and whether iNKT cells influence B cell development and differentiation in the bone marrow and periphery are subjects of our ongoing investigations.
Characterization of CD8+ T cells in the Islets of NOD mice
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NOD mice spontaneously develop Type 1 diabetes. The insulin producing cells of the islets are specifically targeted by the CD8+ T cells. We found two populations of CD8+ T cells in the islets. One population has the expected activated phenotype of CD62L\textsuperscript{lo} CD44\textsuperscript{hi}, while the second population has the unexpected unactivated phenotype of CD62L\textsuperscript{hi} CD44\textsuperscript{lo}. It has previously been thought that only memory or activated T cells traffic into the tissue and these cells should be CD44\textsuperscript{hi}. We further characterized the islet CD8+ T cells for their activation state by measuring their binding to NRP-V7 Kd tetramer, LAMP1 & 2 on their cell surface and Granzyme B intracellularly. We found that the CD8+ T cells with a low level of binding of NRP-V7 K\textsuperscript{d} express higher amounts of LAMP 1 & 2 on their surface and higher amounts of Granzyme B intracellularly than the CD8+ T cells with a high binding for NRP-V7 K\textsuperscript{d}. This data indicates that the CD8+ T cells with a low level of binding to NRP-V7 K\textsuperscript{d} may play a larger role in driving the disease than was previously thought.
Presenter: Zhifang, Zhang

Circulating Cytokines/Chemokines and Neutrophils Function in Patients with Fibromyalgia Syndrome (FMS)
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Per Zhang Zhifang, abstract not available on the website.