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J. Immunol. (156)

Corbaz, A., T. ten Hove, et al. (2002). "IL-18-Binding Protein Expression by Endothelial Cells and Macrophages Is Up-Regulated During Active Crohn's Disease." J. Immunol. **168**(7): 3608-3616.
http://www.jimmunol.org/cgi/content/abstract/168/7/3608

The pathogenesis of Crohn's disease (CD) remains under intense investigation. Increasing evidence suggests a role for mature IL-18 in the induction of proinflammatory cytokines and Th1 polarization in CD lesions. The aim of this study was to investigate the contribution of the IL-18-neutralizing (a and c) and non-neutralizing (b and d) isoforms of IL-18-binding protein (IL-18BP) during active CD. Intestinal endothelial cells and macrophages were the major source of IL-18BP within the submucosa, and this IL-18BP production was also found to be relevant to other types of endothelial cells (HUVEC) and macrophages (peripheral monocytes). IL-18BP messenger transcript and protein were significantly increased in surgically resected specimens from active CD compared with control patients, correlating with an up-regulation of IL-18. Analysis of the expression of the four IL-18BP isoforms as well as being free or bound to IL-18 was reported and revealed that unbound IL-18BP isoforms a and c and inactive isoform d were present in specimens from active CD and control patients while isoform b was not detected. IL-18/IL-18BP complex was also detected. Interestingly, although most was complexed, free mature IL-18 could still be detected in active CD specimens even in the presence of the IL-18BP isoform a/c. These results demonstrate that the appropriate neutralizing isoforms are present in the intestinal tissue of patients with active CD and highlights the complexity of IL-18/IL-18BP biology.

Egwuagu, C. E., C.-R. Yu, et al. (2002). "Suppressors of Cytokine Signaling Proteins Are Differentially Expressed in Th1 and Th2 Cells: Implications for Th Cell Lineage Commitment and Maintenance." J. Immunol. **168**(7): 3181-3187.

http://www.jimmunol.org/cgi/content/abstract/168/7/3181

Positive regulatory factors induced by IL-12/STAT4 and IL-4/STAT6 signaling during T cell development contribute to polarized patterns of cytokine expression manifested by differentiated Th cells. These two critical and antagonistic signaling pathways are under negative feedback regulation by a multimember family of intracellular proteins called suppressor of cytokine signaling (SOCS). However, it is not known whether these negative regulatory factors also modulate Th1/Th2 lineage commitment and maintenance. We show here that CD4+ naive T cells constitutively express low levels of SOCS1, SOCS2, and SOCS3 mRNAs. These mRNAs and their proteins increase significantly in nonpolarized Th cells after activation by TCR signaling. We further show that differentiation into Th1 or Th2 phenotype is accompanied by preferential expression of distinct SOCS mRNA transcripts and proteins. SOCS1 expression is 5-fold higher in Th1 than in Th2 cells, whereas Th2 cells contain 23-fold higher levels of SOCS3. We also

demonstrate that IL-12-induced STAT4 activation is inhibited in Th2 cells that express high levels of SOCS3 whereas IL-4/STAT6 signaling is constitutively activated in Th2 cells, but not Th1 cells, with high SOCS1 expression. These results suggest that mutually exclusive use of STAT4 and STAT6 signaling pathways by differentiated Th cells may derive in part, from SOCS3- or SOCS1-mediated repression of IL-12/STAT4- or IL-4/STAT6 signaling in Th2 and Th1 cells, respectively. Given the strong correlation between distinct patterns of SOCS expression and differentiation into the Th1 or Th2 phenotype, SOCS1 and SOCS3 proteins are therefore Th lineage markers that can serve as therapeutic targets for immune modulation therapy.

Fontenot, A. P., L. A. Maier, et al. (2002). "Beryllium Skin Patch Testing to Analyze T Cell Stimulation and Granulomatous Inflammation in the Lung." J. Immunol. **168**(7): 3627-3634.

http://www.jimmunol.org/cgi/content/abstract/168/7/3627

Chronic beryllium disease (CBD) is characterized by granulomatous inflammation and the accumulation of CD4+ T cells in the lung. Patch testing of CBD patients with beryllium sulfate results in granulomatous inflammation in the skin. We investigated whether the T cell clonal populations present in the lung of CBD patients would also be present in the involved skin of a positive beryllium patch test and thus mirror the granulomatous process in the lung. CBD patients with clonal TCR expansions in bronchoalveolar lavage (BAL) were selected for study. All three CBD patients studied had a positive response to beryllium sulfate application and a negative patch test to normal saline. Immunohistochemistry showed extensive infiltration with CD4+ T cells and few, if any, CD8+ T cells both at 3 days and at later times when granulomas were apparent. T cell infiltration early after skin testing appeared to be nonspecific with the TCR repertoire of infiltrating T cells being distinct from that present in BAL. At later times when granulomas were present, T cell clones in skin overlapped with those in BAL in all patients tested. Total TCR matches in skin and BAL were as high as 40% in selected V{beta} T cell subsets. Studies of peripheral blood T cells before and after patch testing provided evidence for mobilization of large numbers of pathogenic beryllium-reactive T cells into the circulating pool. These studies using skin patch testing provide new insight into the dynamics of T cell influx and mobilization during granulomatous inflammation.

Huang, C.-Y., R. Golub, et al. (2002). "Superantigen-Induced TCR {alpha} Locus Secondary Rearrangement: Role in Tolerance Induction." J. Immunol. **168**(7): 3259-3265.

http://www.jimmunol.org/cgi/content/abstract/168/7/3259

Immunization with superantigen in vivo induces transient activation of superantigen-specific T cells, followed by a superantigen-nonresponsive state. In this study, using a TCR {alpha} knock-in mouse in which the knock-in {alpha}-chain can be replaced with endogenous {alpha}-chain through secondary rearrangement, we show that immunization of superantigen changes the TCR {alpha}-chain expression on peripheral superantigen-specific T cells, induces expression of recombination-activating genes, and generates DNA double-strand breaks at the TCR {alpha}-chain locus. These results suggest that viral superantigens are capable of inducing peripheral TCR revision. Our findings thus provide a new perspective on pathogen-immune system interaction.

Johansson-Lindbom, B. and C. A. K. Borrebaeck (2002). "Germinal Center B Cells Constitute a Predominant Physiological Source of IL-4: Implication for Th2 Development In Vivo." J. Immunol.

168(7): 3165-3172.

http://www.jimmunol.org/cgi/content/abstract/168/7/3165

Protective immunity depends upon the capability of the immune system to properly adapt the response to the nature of an infectious agent. CD4+ Th cells are implicated in this orchestration by secreting a polarized pattern of cytokines. Although Th2 development in animal models and in human cells in vitro to a large extent depends on IL-4, the nature of the cells that provide the initial IL-4 in vivo is still elusive. In this report, we describe the anatomical localization as well as the identity of IL-4-producing cells in human tonsil, a representative secondary lymphoid organ. We demonstrate that IL-4 production is a normal and intrinsic feature of germinal center (GC) B cells. We also show that expression of IL-4 is highly confined to the GCs, in which the B cells constitute the prevalent cellular source. Furthermore, immunofluorescence analysis of colon mucosa reveals a strikingly similar pattern of IL-4-expressing cells compared with tonsils, demonstrating that IL-4 production from GC B cells is not a unique feature of the upper respiratory tract. Our results show that GCs provide the most appropriate microenvironment for IL-4-dependent Th2 polarization in vivo and imply a critical role for GC B cells in this differentiation process.

Locati, M., U. Deuschle, et al. (2002). "Analysis of the Gene Expression Profile Activated by the CC Chemokine Ligand 5/RANTES and by Lipopolysaccharide in Human Monocytes." J. Immunol. **168**(7): 3557-3562.

http://www.jimmunol.org/cgi/content/abstract/168/7/3557

The gene expression profile induced by the CC chemokine ligand (CCL) 5/RANTES in human monocytes was examined using the oligonucleotide array technology. Of 5600 transcripts examined, 42 were consistently induced by CCL5, and none were suppressed. Chemokine-inducible transcripts could be clustered in functional groups, including selected cytokines and receptors (e.g., IL-1{beta}, CCL2/monocyte chemotactic protein-1, and the CCL5 receptor CCR1) and molecules involved in extracellular matrix recognition and digestion (e.g., CD44 splice transcripts, urokinase-type plasminogen activator receptor, matrix metalloprotease (MMP)-9, and MMP-19). Transcript expression, confirmed by quantitative real-time PCR analysis for selected genes, was associated with protein induction for some (e.g., CCL2), but not all (e.g., IL-1{beta}), transcripts examined. The chemokine-induced gene profile was distinct from that activated by LPS, a prototypic phagocyte activator. Although certain transcripts were stimulated by both agonists (e.g., IL-1{beta} and CCL2), others were induced only by either LPS (e.g., TNF-{alpha} and IL-6) or CCL5 (e.g., MMP-19) or were divergently regulated (e.g., CCR1). Thus, CCL5, a prototypic CC inflammatory chemokine, activates a restricted transcriptional program in monocytes distinct from that induced by the prototypic pathogen-derived proinflammatory stimulant LPS. Chemokine-induced chemokines production could represent a novel amplification loop of leukocyte recruitment, while a subset of chemokine-inducible transcripts could be involved in monocyte extravasation and tissue invasion.

Oberholzer, A., C. Oberholzer, et al. (2002). "Increased Survival in Sepsis by In Vivo Adenovirus-Induced Expression of IL-10 in Dendritic Cells." J. Immunol. **168**(7): 3412-3418.

http://www.jimmunol.org/cgi/content/abstract/168/7/3412

The dendritic cell (DC) is the most potent APC of the immune system, capable of stimulating naive T cells to proliferate and differentiate into effector T cells. Recombinant adenovirus (Adv)

readily transduces DCs in vitro allowing directed delivery of transgenes that modify DC function and immune responses. In this study we demonstrate that footpad injection of a recombinant Adv readily targets transduction of myeloid and lymphoid DCs in the draining popliteal lymph node, but not in other lymphoid organs. Popliteal DCs transduced with an empty recombinant Adv undergo maturation, as determined by high MHC class II and CD86 expression. However, transduction with vectors expressing human IL-10 limit DC maturation and associated T cell activation in the draining lymph node. The extent of IL-10 expression is dose dependent; transduction with low particle numbers (105) yields only local expression, while transduction with higher particle numbers (107 and 1010) leads additionally to IL-10 appearance in the circulation. Furthermore, local DC expression of human IL-10 following in vivo transduction with low particle numbers (105) significantly improves survival following cecal ligation and puncture, suggesting that compartmental modulation of DC function profoundly alters the sepsis-induced immune response.

Sugita, S., T. F. Ng, et al. (2004). "CTLA-4+CD8+ T Cells That Encounter B7-2+ Iris Pigment Epithelial Cells Express Their Own B7-2 to Achieve Global Suppression of T Cell Activation." J. Immunol. **172**(7): 4184-4194.

http://www.jimmunol.org/cgi/content/abstract/172/7/4184

Pigment epithelial (PE) cells cultured from the eye possess the novel property of suppressing TCR-dependent activation of T cells in vitro. Iris PE (IPE) cells accomplish this suppression by a direct cell contact mechanism in which B7-2 expressed by the PE cells interacts with CTLA-4 on responding T cells. Because CTLA-4 expression is constitutively expressed on a very small proportion of naive splenic T cells and since exposure of splenic T cells to IPE leads to global T cell suppression, we have inquired into the mechanism by which suppression is achieved. Using splenic T cells and IPE from donor mice with disrupted genes for CD80 (B7-1), CD86 (B7-2), CTLA-4, and/or CD28, we report that B7-2+ IPE in the presence of anti-CD3 supported selectively the activation of CTLA-4+ CD8+ T cells that express their own B7-2 and secrete enhanced amounts of active TGF{beta}. By contrast, activation of CTLA-4-negative T cells, especially CD4+ cells, in these cultures was profoundly suppressed. Because global suppression of T cell activation in these cultures was obtained only when both IPE and T cells possessed B7-2 genes and expressed the costimulators as surface molecules, we propose that T cells activated in the presence of parenchymal cells from the eye (an immune privileged site) express B7-2 in a manner that equips them to suppress bystander T cells. Thus, B7-2 expression on T cells participates in their eventual ability to function as regulators in vitro.

Inoue, T., D. Hammaker, et al. (2005). "Regulation of p38 MAPK by MAPK Kinases 3 and 6 in Fibroblast-Like Synoviocytes." J. Immunol. **174**(7): 4301-4306.

http://www.jimmunol.org/cgi/content/abstract/174/7/4301

The p38 MAPK signal transduction pathway is a key regulator of IL-1 and TNF-{alpha} production in rheumatoid arthritis. Previous studies demonstrated that upstream MAPK kinases (MKK3 and MKK6) that regulate p38 are activated in rheumatoid arthritis synovium. However, their functional relevance in fibroblast-like synoviocytes (FLS) has not been determined. To investigate the relative contribution of MKK3 and MKK6 to p38 activation, the effect of dominant-negative (DN) MKK3 and MKK6 constructs on cultured FLS was evaluated. Cultured FLS were stimulated with medium or IL-1{beta}, and immunoblotting was performed. In some experiments, cells were lysed and immunoprecipitated with anti-p38 Ab, followed by in vitro kinase assay with [{gamma}-32P]ATP and GST-activating transcription factor-2 as substrate. IL-1{beta} rapidly induced p38 phosphorylation in cells transfected with empty vector (pcDNA3.1), but was inhibited by 25% in

cells expressing DN MKK3 or DN MKK6. Cotransfection with both DN plasmids decreased phospho-p38 by almost 75%. In vitro kinase assays on IL-1-stimulated FLS also showed that the combination of DN MKK3 and DN MKK6 markedly decreased kinase activity compared with empty vector or the individual DN plasmids. Furthermore, IL-1{beta}-induced IL-8, IL-6, and matrix metalloproteinase-3 protein production was significantly inhibited in DN MKK3/DN MKK6-transfected cells. The constructs had no effect on the respective mediator mRNA levels. These data demonstrate that MKK3 and MKK6 make individual contributions to p38 activation in FLS after cytokine stimulation, but that both must be blocked for maximum inhibition.

Ortega-Cava, C. F., S. Ishihara, et al. (2003). "Strategic Compartmentalization of Toll-Like Receptor 4 in the Mouse Gut." J. Immunol. **170**(8): 3977-3985.

http://www.jimmunol.org/cgi/content/abstract/170/8/3977

Pattern recognition receptors (PRRs), which include the Toll-like receptors (TLRs), are involved in the innate immune response to infection. TLR4 is a model for the TLR family and is the main LPS receptor. We wanted to determine the expression of TLR4 and compare it with that of TLR2 and CD14 along the gastrointestinal mucosa of normal and colitic BALB/c mice. Colitis was induced with 2.5% dextran sodium sulfate (DSS). Mucosa from seven segments of the digestive tract (stomach, small intestine in three parts, and colon in three parts) was isolated by two different methods. Mucosal TLR4, CD14, TLR2, MyD88, and IL-1{beta} mRNA were semiquantified by Northern blotting. TLR4 protein was determined by Western blotting. TLR4/MD-2 complex and CD14 were evaluated by immunohistochemistry. PRR genes were constitutively expressed and were especially stronger in colon. TLR4 and CD14 mRNA were increased in the distal colon, but TLR2 mRNA was expressed more strongly in the proximal colon, and MyD88 had a uniform expression throughout the gut. Accordingly, TLR4 and CD14 protein levels were higher in the distal colon. TLR4/MD-2 and CD14 were localized at crypt bottom epithelial cells. TLR4/MD2, but not CD14, was found in mucosal mononuclear cells. Finally, DSS-induced inflammation was localized in the distal colon. All genes studied were up-regulated during DSS-induced inflammation, but the normal colon-stressed gut distribution was preserved. Our findings demonstrate that TLR4, CD14, and TLR2 are expressed in a compartmentalized manner in the mouse gut and provide novel information about the in vivo localization of PRRs.

Petersson, K., H. Pettersson, et al. (2003). "Staphylococcal Enterotoxin H Induces V{alpha}-Specific Expansion of T Cells." J. Immunol. **170**(8): 4148-4154.

http://www.jimmunol.org/cgi/content/abstract/170/8/4148

Staphylococcal enterotoxin H (SEH) is a bacterial superantigen secreted by Staphylococcus aureus. Superantigens are presented on the MHC class II and activate large amounts of T cells by cross-linking APC and T cells. In this study, RT-PCR was used to show that SEH stimulates human T cells via the V{alpha} domain of TCR, in particular V{alpha}10 (TRAV27), while no TCR V{beta}-specific expansion was seen. This is in sharp contrast to all other studied bacterial superantigens, which are highly specific for TCR V{beta}. It was further confirmed by flow cytometry that SEH stimulation does not alter the levels of certain TCR V{beta}. In a functional assay addressing cross-reactivity, V{beta} binding superantigens were found to form one group, whereas SEH has different properties that fit well with V{alpha} reactivity. As SEH binds on top of MHC class II, an interaction between MHC and TCR upon SEH binding is not likely. This concludes that the specific expansion of TCR V{alpha} is not due to contacts between MHC and TCR, instead we suggest that SEH directly interacts with the TCR V{alpha} domain.

Dogusan, Z., E. Montecino-Rodriguez, et al. (2004). "Macrophages and Stromal Cells Phagocytose Apoptotic Bone Marrow-Derived B Lineage Cells." J. Immunol. **172**(8): 4717-4723.

http://www.jimmunol.org/cgi/content/abstract/172/8/4717

It has been hypothesized that B cell precursors that undergo programmed cell death due to nonproductive Ig gene rearrangements are cleared from the bone marrow by macrophages. However, a role for macrophages in this process is supported only by micrographs showing their association with apoptotic-appearing, B lineage cells. Functional data demonstrating phagocytosis of apoptotic, bone marrow lymphocytes by macrophages have not been presented, nor have receptors potentially involved in that process been identified. The data in this report demonstrate that macrophages isolated from murine bone marrow efficiently phagocytose apoptotic murine B lineage cells using multiple receptors that include CD14, integrins, class A scavenger receptor, and CD31 (PECAM-1). In addition, the results further reveal a new role for the hemopoietic microenvironment in B cell development in view of data demonstrating that murine bone marrow stromal cells are also capable of clearing apoptotic cells via an integrin-dependent mechanism.

Maaser, C., J. Heidemann, et al. (2004). "Human Intestinal Microvascular Endothelial Cells Express Toll-Like Receptor 5: A Binding Partner for Bacterial Flagellin." J. Immunol. **172**(8): 5056-5062.

http://www.jimmunol.org/cgi/content/abstract/172/8/5056

Bacterial flagellin has recently been identified as a ligand for Toll-like receptor 5 (TLR5). Human sites known to specifically express TLR5 include macrophages and gastric and intestinal epithelium. Because infection of intestinal epithelial cells with Salmonella leads to an active transport of flagellin to the subepithelial compartment in proximity to microvessels, we hypothesized that human intestinal endothelial cells functionally express TLR5, thus enabling an active inflammatory response upon binding of translocated flagellin. Endothelial expression of TLR5 in human macro- and microvascular endothelial cells was examined by RT-PCR, immunoblot analysis, and immunofluorescence. Endothelial expression of TLR5 in vivo was verified by immunohistochemistry. Endothelial modulation of ICAM-1 expression was quantitated using flow cytometry, and leukocyte transmigration in vitro was assessed by an endothelial transmigration assay. Epithelial-endothelial cellular interactions upon infection with viable Salmonella were investigated using a coculture system in vitro. We found that Salmonella-infected intestinal epithelial cells induce endothelial ICAM-1 expression in cocultured human endothelial cells. Both macro- (HUVEC) and microvascular endothelial cells derived from human skin (human dermal microvascular endothelial cell 1) and human colon (human intestinal microvascular endothelial cells) were found to express high constitutive amounts of TLR5 mRNA and protein. These findings were paralleled by strong immunoreactivity for TLR5 of normal human colonic microvessels in vivo. Furthermore, incubation of human dermal microvascular endothelial cells with flagellin from clinical isolates of Escherichia and Salmonella strains led to a marked up-regulation of ICAM-1, as well as to an enhanced leukocyte transendothelial cell migration. These results suggest that endothelially expressed TLR5 might play a previously unrecognized role in the innate immune response toward bacterial Ags.

Li, J. S.-y., F. Chu, et al. (2002). "Antibodies Highly Effective in SCID Mice During Infection by the Intracellular Bacterium Ehrlichia chaffeensis Are of Picomolar Affinity and Exhibit Preferential Epitope and Isotype Utilization." J. Immunol. **169**(3): 1419-1425.

http://www.jimmunol.org/cgi/content/abstract/169/3/1419

Although often considered to be ineffective against intracellular bacteria, Abs, in the absence of lymphocytes, have been shown previously to protect SCID mice from lethal infection by the obligate intracellular bacterium Ehrlichia chaffeensis, even when administered well after infection has been established. To identify characteristics of Abs that are critical for host defense during this intracellular infection, a panel of Ehrlichia-specific mAbs was generated and analyzed. Among 100 Abs recovered, 39 recognized an amino-terminal hypervariable region of an outer membrane protein (OMP), demonstrating that the OMPs are both antigenically variable and immunodominant. A subset of 16 representative OMP-specific Abs was further examined to identify characteristics that were essential for in vivo efficacy. The highly effective Abs recognized a linear epitope within the first hypervariable region of OMP-1g. Only IgG were found to be effective, and among the effective IgG, the following hierarchy was observed: IgG2a > IgG3 = IgG2b. The most striking characteristics of the highly effective Abs were their picomolar binding affinities and long binding t1/2. Thus, although epitope recognition and isotype use may contribute to efficacy, high affinity may be a critical characteristic of Abs that can act effectively during this intracellular bacterial infection.

Austin, A. S., K. M. Haas, et al. (2003). "Identification and Characterization of a Novel Regulatory Factor: IgA-Inducing Protein." J. Immunol. **171**(3): 1336-1342.

http://www.jimmunol.org/cgi/content/abstract/171/3/1336

IgA is the predominant Ig isotype in mucosal secretions and thus plays a pivotal role in host defense. The mechanisms by which IgA expression is regulated may differ among species and involve multiple pathways. Various cytokines and costimulators have been identified which regulate expression of this isotype, including IL-10, IL-2, vasoactive intestinal peptide, and TGF-{beta}. We have tested a wide array of known factors, but only under very limited conditions do these factors mediate substantial IgA production in vitro from bovine B cells. In response to these findings, we generated a cDNA library in a mammalian expression vector from activated cells derived from bovine gut-associated lymphoid tissues (Peyer's patch and mesenteric lymph node cells) as a source of soluble factor(s) that may regulate IgA production. We have identified a novel factor, IgA-inducing protein, which stimulates relatively high levels of IgA production in vitro following CD40 stimulation in coculture with IL-2. Our data suggest that IgA-inducing protein regulates IgA by acting as a switch or differentiation factor and is expressed in a variety of lymphoid and nonlymphoid tissues.

Jensen, L. E. and A. S. Whitehead (2003). "Pellino3, a Novel Member of the Pellino Protein Family, Promotes Activation of c-Jun and Elk-1 and May Act as a Scaffolding Protein." J. Immunol. **171**(3): 1500-1506.

http://www.jimmunol.org/cgi/content/abstract/171/3/1500

Toll-like receptors and the IL-1R are part of the innate immune response aimed at mobilizing defense mechanisms in response to infections or injury. These receptors can initiate common intracellular signaling cascades. One intermediate component in these signaling cascades is Pellino, which was first identified in Drosophila and shown to interact with IL-1R-associated kinase. Two homologues, Pellino1 and Pellino2, have been identified in mammals. A novel member of the Pellino protein family has been identified and named Pellino3. Pellino3 shares 84 and 85% amino acid identity with Pellino1 and Pellino2, respectively. Two alternatively spliced Pellino3 mRNAs, Pellino3a and Pellino3b, are widely expressed. Pellino3 physically interacts with

IL-1R-associated kinase-1, TNF receptor-associated factor-6, TGF-{beta}-activated kinase-1, and NF-{kappa}B-inducing kinase in an IL-1-dependent manner, suggesting that it plays a role as a scaffolding protein. In reporter assays Pellino3 leads to activation of c-Jun and Elk-1, but not NF-{kappa}B. Pellino3 also leads to activation of c-Jun N-terminal kinase. These data suggest that Pellino3 plays an important role in the innate immune response.

Christiansen, S. C., J. Eddleston, et al. (2002). "Up-Regulation of Functional Kinin B1 Receptors in Allergic Airway Inflammation." J. Immunol. **169**(4): 2054-2060.

http://www.jimmunol.org/cgi/content/abstract/169/4/2054

B1 receptors are known to be induced during allergic airway inflammation in animal models. However, little is known regarding in vivo B1 receptor expression in humans. We examined B1 receptor mRNA expression in nasal tissue samples from allergic rhinitis and normal subjects. Allergic rhinitis subjects displayed significantly higher expression of B1 receptor mRNA than did the normal subjects, and nasal allergen challenge increased B1 receptor mRNA expression at 8 to 24 h time points in allergic rhinitis subjects. No significant difference was found in B2 receptor expression. To confirm B2 and B1 receptor functional activity, subjects were challenged with kinin agonists. Nasal challenge with the B1 receptor ligand, Lys-des-Arg-bradykinin (BK), activated extracellular signal-regulated kinase in allergic rhinitis, but not normal, subjects. Nasal challenge with the B2 receptor ligand, BK, activated extracellular signal-regulated kinase in both allergic rhinitis and normal subjects. The consequences of B1 receptor activation were investigated using the human airway epithelial cell lines A549 and BEAS-2B. We demonstrated that Lys-des-Arg-BK activates the transcription factor AP-1. Taken together, these results show that functional B1 receptors are induced in the airway during allergic inflammation and suggest that they participate in the regulation of gene expression.

Holtmeier, W., J. Kaller, et al. (2002). "Development and Compartmentalization of the Porcine TCR {delta} Repertoire at Mucosal and Extraintestinal Sites: The Pig as a Model for Analyzing the Effects of Age and Microbial Factors." J. Immunol. **169**(4): 1993-2002.

http://www.jimmunol.org/cgi/content/abstract/169/4/1993

{gamma}{delta} T cells are an important component of the mucosal immune system. Previously, we have shown that the TCR {delta} repertoire in human intestine is polyclonal at birth and becomes increasingly restricted with age. In this study, we expand those studies to the pig which allows more extensive experiments including several organs. Tissues from different mucosal sites like the stomach, duodenum, ileum, Peyer's patches, jejunum, and colon, and also extraintestinal sites like the lung, spleen, thymus and mesenteric lymph nodes, were obtained from conventionally reared pigs aged 2 wk to 5.5 years. In addition, tissues were also obtained from 10-wk-old specified pathogen- and germ-free pigs. TCRDV1-DV5 transcripts were amplified by RT-PCR after which complementarity-determining region 3 spectratyping was performed. Individual bands were excised from the gels and directly sequenced. The intestinal TCR {delta} repertoire showed increasing restriction with age and was highly oligoclonal in the adult 2- to 5.5-year-old pigs. In old pigs, we observed a striking compartmentalization. Different TCR {delta} repertoires were present between the lungs and the intestinal mucosa but also within different parts of the gastrointestinal tract. However, occasionally we observed identical TCR {delta} transcripts in the intestine and the lungs and shared clones could be detected also along the entire gastrointestinal tract. Thus, subsets of {gamma}{delta} T cells are likely to transport immunological information between different compartments of the immune system. Furthermore, these data support the hypothesis that in each mucosal site, different Ags are responsible for selecting and maintaining the {gamma}{delta} TCR over time.

Ishida, I., H. Kubo, et al. (2002). "Hypoxia Diminishes Toll-Like Receptor 4 Expression Through Reactive Oxygen Species Generated by Mitochondria in Endothelial Cells." J. Immunol. **169**(4): 2069-2075.

http://www.jimmunol.org/cgi/content/abstract/169/4/2069

Hypoxia and inflammation often occur simultaneously due to prevention of adequate gas exchange. Understanding the influence of hypoxia on the inflammatory response is important because hypoxia directly regulates expression of many genes, including those regulating inflammation, and plays a role in modulating the resolution of an inflammatory response. LPS is a major mediator of cellular injury and inflammation that induces its effects through Toll-like receptor 4 (TLR4). The aim of this study was to evaluate the effect of hypoxia on TLR4 expression. Hypoxia decreased TLR4 expression on cultured endothelial cells. Furthermore, LPS-induced ICAM-1 up-regulation was decreased by hypoxia. Because reactive oxygen species (ROS) generated from mitochondria are one of the signaling molecules induced by hypoxia, the role of ROS in hypoxia-induced TLR4 down-regulation was evaluated. Our data showed that hypoxia increased ROS generation and that hypoxia-induced TLR4 down-regulation was inhibited by myxothiazol, a mitochondrial site III electron transport inhibitor. Hypoxia also inhibited AP-1 translocation. Since the TLR4 promoter has a binding site for AP-1, hypoxia-induced TLR4 down-regulation may be due to an ROS-mediated decrease in AP-1-binding activity. We conclude that hypoxia decreases TLR4 expression in endothelial cells and that this change is mediated by mitochondrial ROS leading to attenuation of AP-1 transcriptional activity.

Khan, N., N. Shariff, et al. (2002). "Cytomegalovirus Seropositivity Drives the CD8 T Cell Repertoire Toward Greater Clonality in Healthy Elderly Individuals." J. Immunol. **169**(4): 1984-1992.

http://www.jimmunol.org/cgi/content/abstract/169/4/1984

The deterioration in immune function with aging is thought to make a major contribution to the increased morbidity and mortality from infectious disease in old age. One aspect of immune senescence is the reduction in CD8 T cell repertoire as due to the accumulation of oligoclonal, memory T cells and a reduction in the naive T cell pool. CD8 T cell clonal expansions accumulate with age, but their antigenic specificity remains unknown. In this study, we show that in elderly individuals seropositivity for human CMV leads to the development of oligoclonal populations of CMV-specific CTL that can constitute up to one-quarter of the total CD8 T cell population. Furthermore, CMV-specific CTL have a highly polarized membrane phenotype that is typical of effector memory cells (CD28-, CD57+, CCR7-). TCR analyses show that CMV-specific CTL have highly restricted clonality with greater restriction in the larger expansions. Clonal analysis of the total CD8 T cell repertoire was compared between CMV-seropositive and CMV-seronegative donors. Thirty-three percent more clonal expansions were observed in CMV-seropositive donors in comparison with seronegative individuals. These data implicate CMV as a major factor in driving oligoclonal expansions in old age. Such a dramatic accumulation of virus-specific effector CTL might impair the ability to respond to heterologous infection and may underlie the negative influence of CMV seropositivity on survival in the very elderly.

Kondo, E., M. S. Topp, et al. (2002). "Efficient Generation of Antigen-Specific Cytotoxic T Cells Using Retrovirally Transduced CD40-Activated B Cells." J. Immunol. **169**(4): 2164-2171.

http://www.jimmunol.org/cgi/content/abstract/169/4/2164

The development of rapid, efficient, and safe methods for generating Ag-specific T cells is necessary for the clinical application of adoptive immunotherapy. We show that B cells stimulated with CD40 ligand and IL-4 (CD40-B cells) can be efficiently transduced with retroviral vectors encoding a model Ag, CMV tegument protein pp65 gene, and maintain high levels of costimulatory molecules after gene transfer. CTL lines specific for pp65 were readily generated in all four healthy CMV-seropositive donors by stimulating autologous CD8+ T cells with these transduced CD40-B cells, both of which were derived from 10 ml peripheral blood. ELISPOT assays revealed that the CTL lines used multiple HLA alleles as restricting elements. Thus, CD40-B cells transduced retrovirally with Ag-encoding cDNA can be potent APC and facilitate to generate Ag-specific CTL in vitro.

Santangelo, S., D. J. Cousins, et al. (2002). "DNA Methylation Changes at Human Th2 Cytokine Genes Coincide with DNase I Hypersensitive Site Formation During CD4+ T Cell Differentiation." J. Immunol. **169**(4): 1893-1903.

http://www.jimmunol.org/cgi/content/abstract/169/4/1893

The differentiation of naive CD4+ T lymphocytes into Th1 and Th2 lineages generates either cellular or humoral immune responses. Th2 cells express the cytokines IL-4, -5, and -13, which are implicated in asthma and atopy. Much has been published about the regulation of murine Th2 cytokine expression, but studies in human primary T cells are less common. We have developed a method for differentiating human CD45RA+ (naive) T cells into Th1 and Th2 populations that display distinct cytokine expression profiles. We examined both CpG methylation, using bisulfite DNA modification and sequencing, and chromatin structure around the IL-4 and IL-13 genes before and after human T cell differentiation and in normal human skin fibroblasts. In naive cells, the DNA was predominantly methylated. After Th2 differentiation, DNase I hypersensitive sites (DHS) appeared at IL-4 and IL-13 and CpG demethylation occurred only around the Th2-specific DHS. Both DHS and CpG demethylation coincided with consensus binding sites for the Th2-specific transcription factor GATA-3. Although fibroblasts, like naive and Th1 cells, did not express IL-4 or IL-13, DHS and unmethylated CpG sites that were distinct from the Th2-specific sites were observed, suggesting that chromatin structure in this cluster not only varies in T cells according to IL-4/IL-13 expression but is also tissue specific.

Jinquan, T., H. H. Jacobi, et al. (2003). "CCR3 Expression Induced by IL-2 and IL-4 Functioning as a Death Receptor for B Cells." J. Immunol. **171**(4): 1722-1731.

http://www.jimmunol.org/cgi/content/abstract/171/4/1722

We report that CCR3 is not expressed on freshly isolated peripheral and germinal B cells, but is up-regulated after stimulation with IL-2 and IL-4 ([~]98% CCR3+). Ligation of CCR3 by eotaxin/chemokine ligand (CCL) 11 induces apoptosis in IL-2- and IL-4-stimulated primary CD19+ ([~]40% apoptotic cells) B cell cultures as well as B cell lines, but has no effect on chemotaxis or cell adhesion. Freshly isolated B cells express low levels of CD95 and CD95 ligand (CD95L) (19 and 21%, respectively). Expression is up-regulated on culture in the presence of a combination of IL-2, IL-4, and eotaxin/CCL11 (88% CD95 and 84% CD95L). We therefore propose that ligation of such newly induced CCR3 on peripheral and germinal B cells by eotaxin/CCL11 leads to the enhanced levels of CD95 and CD95L expression. Ligation of CD95 by its CD95L expressed on neigboring B cells triggers relevant death signaling pathways, which include an increase in levels of Bcl-2 expression, its functional activity, and the release of cytochrome c from the mitochondria into the cytosol. These events initiate a cascade of enzymatic processes of the caspase family, culminating in programmed cell death. Interaction between CCR3 and eotaxin/CCL11 may, besides promoting allergic reactions, drive activated B cells to apoptosis, thereby reducing levels

of Ig production, including IgE, and consequently limit the development of the humoral immune response. The apoptotic action of eotaxin/CCL11 suggests a therapeutic modality in the treatment of B cell lymphoma.

Gounni, A. S., Q. Hamid, et al. (2004). "IL-9-Mediated Induction of Eotaxin1/CCL11 in Human Airway Smooth Muscle Cells." J. Immunol. **173**(4): 2771-2779.

http://www.jimmunol.org/cgi/content/abstract/173/4/2771

Recent work has shown the potential importance of IL-9 in allergic diseases. The development of transgenic mice overexpressing IL-9 has suggested a key role for this cytokine in the development of the asthmatic phenotype including airway eosinophilia. In this study, we evaluated the expression of the IL-9R and the effects of IL-9 on human ASM cells by examining the release of Th2-associated chemokines (eotaxin1/CCL11 and thymus- and activation-regulated chemokine (TARC)/CCL17). IL-9R {alpha}-chain mRNA and surface expression were detected in cultured human airway smooth muscle (ASM) cells. In addition, primary cultured ASM cells, as well as bronchial smooth muscle cells within biopsies of asthmatics and not control subjects, revealed IL-9R protein expression. IL-9 stimulation of human ASM cells resulted in release of eotaxin1/CCL11, but had no effect on the release of TARC/CCL17, in time- and dose-dependent manner. Moreover, in vitro chemotaxis assay demonstrated that conditioned medium from IL-9-stimulated ASM cells attracted human eosinophils. Neutralizing Abs to IL-9, but not to IL-4 or IL-13, reduced significantly IL-9-induced production of eotaxin1/CCL11 from ASM cells. Interestingly, real-time RT-PCR showed that IL-9 up-regulated eotaxin1/CCL11 mRNA expression, but had no effect on TARC/CCL17. Treatment with Act D abrogates IL-9-induced eotaxin1/CCL11 mRNA and protein release by ASM cells. Finally, transfection study using eotaxin1/CCL11 promoter luciferase construct confirmed that IL-9 induced eotaxin1/CCL11 at the transcriptional level. Taken together, these data provide new evidence demonstrating that IL-9-dependent activation of ASM cells contributes to eosinophilic inflammation observed in asthma.

Eddleston, J., S. C. Christiansen, et al. (2002). "Functional Expression of the C-X-C Chemokine Receptor CXCR4 by Human Bronchial Epithelial Cells: Regulation by Proinflammatory Mediators." J. Immunol. **169**(11): 6445-6451.

http://www.jimmunol.org/cgi/content/abstract/169/11/6445

CXCR4 and its ligand stromal cell-derived factor 1{alpha} (SDF-1{alpha}) have recently been implicated in the development of airway inflammation in a mouse model of allergic airway disease. Here we report, for the first time, the expression of a functional CXCR4 in primary human normal bronchial epithelial cells and the regulation of CXCR4 gene expression by proinflammatory mediators. Both bradykinin (BK) and IL-1{beta} induced an accumulation of CXCR4 mRNA in normal bronchial epithelial cells in a time-dependent manner, with peak levels of CXCR4 mRNA reached between 4 and 24 h after stimulation. Ligand activation of CXCR4 in airway epithelial cells resulted in the activation of the extracellular signal-regulated kinase and stress-activated protein kinase/c-Jun amino-terminal kinase signaling pathways and calcium mobilization. Pretreatment of airway epithelial cells with BK or IL-1{beta} enhanced SDF-1{alpha} induced phospho-extracellular signal-regulated kinase and calcium mobilization, in addition to increasing the level of CXCR4 protein. Finally, we describe the expression of CXCR4 mRNA and its regulation by BK in vivo in human nasal tissue. CXCR4 mRNA levels are significantly higher in the nasal tissue of symptomatic allergic rhinitis subjects compared with normal subjects. Moreover, BK challenge significantly increased CXCR4 mRNA levels in nasal tissue of mild allergic rhinitis subjects in vivo, but not normal controls. In conclusion, this study demonstrates that human airway epithelial cells respond to proinflammatory mediators by up-regulating the

chemokine receptor CXCR4, thus enabling the cells to respond more effectively to constitutively expressed SDF-1{alpha}. This may lead to enhanced activation of intracellular signaling pathways resulting in the release of mediators involved in inflammatory allergic airway disease.

Stehlik, C., S. H. Lee, et al. (2003). "Apoptosis-Associated Speck-Like Protein Containing a Caspase Recruitment Domain Is a Regulator of Procaspase-1 Activation." J. Immunol. **171**(11): 6154-6163.

http://www.jimmunol.org/cgi/content/abstract/171/11/6154

Apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC)/target of methylation-induced silencing/PYCARD represents one of only two proteins encoded in the human genome that contains a caspase recruitment domain (CARD) together with a pyrin, AIM, ASC, and death domain-like (PAAD)/PYRIN/DAPIN domain. CARDs regulate caspase family proteases. We show here that ASC binds by its CARD to procaspase-1 and to adapter proteins involved in caspase-1 activation, thereby regulating cytokine pro-IL-1{beta} activation by this protease in THP-1 monocytes. ASC enhances IL-1{beta} secretion into the cell culture supernatants, at low concentrations, while suppressing at high concentrations. When expressed in HEK293 cells, ASC interferes with Cardiak/Rip2/Rick-mediated oligomerization of procaspase-1 and suppresses activation this protease, as measured by protease activity assays. Moreover, ASC also recruits procaspase-1 into ASC-formed cytosolic specks, separating it from Cardiak. We also show that expression of the PAAD/PYRIN family proteins pyrin or cryopyrin/PYPAF1/NALP3 individually inhibits IL-1{beta} secretion but that coexpression of ASC with these proteins results in enhanced IL-1{beta} secretion. However, expression of ASC uniformly interferes with caspase-1 activation and IL-1{beta} secretion induced by proinflammatory stimuli such as LPS and TNF, suggesting pathway competition. Moreover, LPS and TNF induce increases in ASC mRNA and protein expression in cells of myeloid/monocytic origin, revealing another level of cross-talk of cytokine-signaling pathways with the ASC-controlled pathway. Thus, our results suggest a complex interplay of the bipartite adapter protein ASC with PAAD/PYRIN family proteins, LPS (Toll family receptors), and TNF in the regulation of procaspase-1 activation, cytokine production, and control of inflammatory responses.

Horowitz, M. C., Y. Xi, et al. (2004). "Pax5-Deficient Mice Exhibit Early Onset Osteopenia with Increased Osteoclast Progenitors." J. Immunol. **173**(11): 6583-6591.

http://www.jimmunol.org/cgi/content/abstract/173/11/6583

Pax5 encodes BSAP, a member of the paired box domain transcription factors, whose expression is restricted to B lymphocyte lineage cells. Pax5-/- mice have a developmental arrest of the B cell lineage at the pro-B cell stage. We show here that Pax5-/- mice are severely osteopenic, missing 60% of their bone mass. The osteopenia can be accounted for by a >100% increase in the number of osteoclasts in bone measured histomorphometrically. This is not due to a lack of B cells, because other strains of B cell-deficient mice do not exhibit this phenotype. There was no difference in the number of osteoclasts produced in vitro by wild-type and Pax5-/- bone marrow cells. In contrast, spleen cells from Pax5-/- mice produce as much as five times the number of osteoclasts as control spleen cells. Culture of Pax5-/- spleen cells yields a population of adherent cells that grow spontaneously in culture without added growth factors for >4 wk. These cells have a monocyte phenotype, produce large numbers of osteoclasts when induced in vitro, and therefore are highly enriched in osteoclast precursors. These data demonstrate a previously unsuspected connection between B cell and osteoclast development and a key role for Pax5 in the control of osteoclast development.

Torikai, H., Y. Akatsuka, et al. (2004). "A Novel HLA-A*3303-Restricted Minor Histocompatibility Antigen Encoded by an Unconventional Open Reading Frame of Human TMSB4Y Gene." J. Immunol. **173**(11): 7046-7054.

http://www.jimmunol.org/cgi/content/abstract/173/11/7046

Female-to-male hemopoietic stem cell transplantation (HSCT) elicits T cell responses against male-specific minor histocompatibility (H-Y) Ags encoded by the Y chromosome. All previously identified H-Y Ags are encoded by conventional open reading frames, but we report in this study the identification of a novel H-Y Ag encoded in the 5'-untranslated region of the TMSB4Y gene. An HLA-A*3303-restricted CD8+ CTL clone was isolated from a male patient after an HSCT from his HLA-identical sister. Using a panel of cell lines carrying Y chromosome terminal deletions, a narrow region controlling the susceptibility of these target cells to CTL recognition was localized. Minigene transfection and epitope reconstitution assays identified an 11-mer peptide, EVLLRPGLHFR, designated TMSB4Y/A33, whose first amino acid was located 405 bp upstream of the TMSB4Y initiation codon. Analysis of the precursor frequency of CTL specific for recipient minor histocompatibility Ags in post-HSCT peripheral blood T cells revealed that a significant fraction of the total donor CTL response in this patient was directed against the TMSB4Y epitope. Tetramer analysis continued to detect TMSB4Y/A33-specific CD8+ T cells at least up to 700 days post-HSCT. This finding underscores the in vivo immunological relevance of minor histocompatibility Ags derived from unconventional open reading frame products.

Kanayama, N., T. Kimoto, et al. (2002). "B Cell Selection and Affinity Maturation During an Antibody Response in the Mouse with Limited B Cell Diversity." J. Immunol. **169**(12): 6865-6874.

http://www.jimmunol.org/cgi/content/abstract/169/12/6865

The quasi-monoclonal mouse has limited B cell diversity, whose major ([~]80%) B cell Ag receptors are comprised of the knockin VH 17.2.25 (VHT)-encoded H chain and the {lambda}1 or {lambda}2 L chain, thereby being specific for 4-hydroxy-3-nitrophenylacetyl. The p-nitrophenylacetyl (pNP) was found to be a low affinity analog of nitrophenylacetyl. We examined affinity maturation of anti-pNP IgG by analyzing mAbs obtained from quasi-monoclonal mice that were immunized with this low affinity Ag. The results are: 1) Although VHT/{lambda}1 and VHT/{lambda}2 IgM were equally produced, VHT/{lambda}2 IgG almost exclusively underwent affinity maturation toward pNP. 2) A common mutation in complementarity-determining region 3 of VHT (T313A) mainly contributed to generating the specificity for pNP. 3) Because mutated VHT-encoded {gamma}-chains could form {lambda}1-bearing IgG in Chinese hamster ovary cells, apparent absence of VHT/{lambda}1 anti-pNP IgG may not be due to the incompatibility between the {gamma}-chains and the {lambda}1-chain, but may be explained by the fact that VHT/{lambda}1 B cells showed 50- to 100-fold lower affinity for pNP than VHT/{lambda}2 B cells. 4) Interestingly, a pNP-specific IgM mAb that shared common mutations including T313A with high affinity anti-pNP IgG was isolated, suggesting that a part of hypermutation coupled with positive selection can occur before isotype switching. Thus, even weak B cell receptor engagement can elicit an IgM response, whereas only B cells that received signals stronger than a threshold may be committed to an affinity maturation process.

Uemura, K., M. Saka, et al. (2002). "L-MBP Is Expressed in Epithelial Cells of Mouse Small Intestine." J. Immunol. **169**(12): 6945-6950.

http://www.jimmunol.org/cgi/content/abstract/169/12/6945

The mannan-binding proteins (L-MBP and S-MBP, also denoted MBL-C and MBL-A), mainly produced in liver and existing in liver and serum, play important roles in the innate immunity against a variety of pathogens. Total RNA from mouse tissues were screened for MBP mRNA by RT-PCR. In addition to liver, S-MBP mRNA was detected in lung, kidney, and testis, and L-MBP mRNA was detected in kidney, thymus, and small intestine. Quantitative RT-PCR revealed that the small intestine is a predominant site of extrahepatic expression of L-MBP. Western blotting with polyclonal Abs against rat L-MBP demonstrated this protein in Triton X-100 extracts of the small intestine obtained from mice that had undergone systemic perfusion. Immunohistochemical staining with an mAb against mouse L-MBP and in situ hybridization revealed that L-MBP is selectively expressed in some villous epithelial cells of the small intestine. These findings suggest that L-MBP plays a role in mucosal innate immunity.

Yasuoka, H., H. Ihn, et al. (2003). "A Novel Protein Highly Expressed in Testis Is Overexpressed in Systemic Sclerosis Fibroblasts and Targeted by Autoantibodies." J. Immunol. **171**(12): 6883-6890.

http://www.jimmunol.org/cgi/content/abstract/171/12/6883

Nearly all autoantibody specificities in sera from patients with systemic sclerosis (SSc) target proteins distributed ubiquitously, and Abs against proteins whose expression is restricted to the affected sites have not been identified. In this study we describe SSc-specific autoantibody to a novel testicular Ag, termed protein highly expressed in testis (PHET), which is ectopically overexpressed in SSc dermal fibroblasts. A partial cDNA encoding PHET was isolated by immunoscreening of a HepG2 cDNA library with an SSc serum. PHET appeared to be a member of the UniGene cluster Hs.129872, but had a unique exon composition and a characteristic mRNA expression profile restricted to the testis. Serum Abs to a recombinant PHET fragment were detected in nine (8.4%) of 107 SSc patients, but in none of 50 systemic lupus erythematosus patients or 77 healthy controls. In SSc patients, the presence of anti-PHET Abs was associated with diffuse cutaneous SSc and lung involvement ($p = 0.02$ and 0.01, respectively). PCR-based quantitative analysis of PHET mRNA expression in cultured dermal fibroblasts showed increased expression of PHET mRNA in SSc fibroblasts compared with control fibroblasts. PHET-reactive Abs purified from SSc sera stained the cytoplasm of SSc dermal fibroblasts, and the staining intensity tended to be more prominent on SSc compared with control fibroblasts. These findings suggest that the autoantibody response to PHET can be induced by ectopic overexpression of PHET in dermal fibroblasts in SSc patients.

Johnson, E. N., E. R. Appelbaum, et al. (2004). "Neuromedin Elicits Cytokine Release in Murine Th2-Type T Cell Clone D10.G4.1." J. Immunol. **173**(12): 7230-7238.

http://www.jimmunol.org/cgi/content/abstract/173/12/7230

Neuromedin U (NmU), originally isolated from porcine spinal cord and later from other species, is a novel peptide that potently contracts smooth muscle. NmU interacts with two G protein-coupled receptors designated as NmU-1R and NmU-2R. This study demonstrates a potential proinflammatory role for NmU. In a mouse Th2 cell line (D10.G4.1), a single class of high affinity saturable binding sites for 125I-labeled NmU (KD 364 pM and Bmax 1114 fmol/mg protein) was identified, and mRNA encoding NmU-1R, but not NmU-2R, was present. Competition binding analysis revealed equipotent, high affinity binding of NmU isopeptides to membranes prepared from D10.G4.1 cells. Exposure of these cells to NmU isopeptides resulted in an increase in

intracellular Ca2+ concentration (EC50 4.8 nM for human NmU). In addition, NmU also significantly increased the synthesis and release of cytokines including IL-4, IL-5, IL-6, IL-10, and IL-13. Studies using pharmacological inhibitors indicated that maximal NmU-evoked cytokine release required functional phospholipase C, calcineurin, MEK, and PI3K pathways. These data suggest a role for NmU in inflammation by stimulating cytokine production by T cells.

Combriato, G. and H. G. Klobeck (2002). "Regulation of Human Ig{lambda} Light Chain Gene Expression by NF-{kappa}B." J. Immunol. **168**(3): 1259-1266.

http://www.jimmunol.org/cgi/content/abstract/168/3/1259

The human Ig{lambda} enhancer consists of three separated sequence elements that we identified previously by mapping DNase I-hypersensitive regions (HSS) downstream of the C region of the Ig{lambda} L chain genes (HSS-1, HSS-2, and HSS-3). It has been shown by several laboratories that expression of the H chain genes as well as the {kappa} genes, but not the {lambda} genes, is dependent on constitutive NF-{kappa}B proteins present in the nucleus. In this study we show by band-shift experiments, in vivo footprinting, and transient transfection assays that all three hypersensitive sites of the human Ig{lambda} enhancer contain functional NF-{kappa}B sites that act synergistically on expression. We further show that the chicken {lambda} enhancer also contains a functional NF-{kappa}B site but the mouse {lambda} enhancer contains a mutated, nonfunctional NF-{kappa}B site that is responsible for its low enhancer activity. It is possible that the inactivating mutation in the mouse Ig{lambda} enhancer was compensated for by an expansion of the Ig{kappa} L chain locus, followed by a contraction of the Ig{lambda} locus in this species.

Yamanishi, Y., D. L. Boyle, et al. (2002). "Expression and Regulation of Aggrecanase in Arthritis: The Role of TGF-{beta}." J. Immunol. **168**(3): 1405-1412.

http://www.jimmunol.org/cgi/content/abstract/168/3/1405

Aggrecanases are key matrix-degrading enzymes that act by cleaving aggrecan at the Glu373-Ala374 site. While these fragments have been detected in osteoarthritis (OA) and rheumatoid arthritis (RA) cartilage and synovial fluid, no information is available on the regulation or expression of the two key aggrecanases (aggrecanase-1 and aggrecanase-2) in synovial tissue (ST) or fibroblast-like synoviocytes (FLS). The aggrecanase-1 gene was constitutively expressed by both RA and OA FLS. Real-time PCR demonstrated that TGF-{beta} significantly increased aggrecanase-1 gene expression in FLS. Aggrecanase-1 induction peaked after 24 h of TGF-{beta} stimulation. The expression of aggrecanase-1 mRNA was significantly greater in RA ST than in OA or nonarthritis ST. Aggrecanase-2 mRNA and protein were constitutively produced by nonarthritis, OA, and RA FLS but were not increased by IL-1, TNF-{alpha}, or TGF-{beta}. Furthermore, OA, RA, and nonarthritis ST contained similar amounts of immunoreactive aggrecanase-2. The major form of the aggrecanase-2 enzyme was 70 kDa in nonarthritis ST, whereas a processed 53-kDa form was abundant in RA ST. Therefore, aggrecanase-1 and -2 are differentially regulated in FLS. Both are constitutively expressed, but aggrecanase-1 is induced by cytokines, especially TGF-{beta}. In contrast, aggrecanase-2 protein may be regulated by a post-translational mechanism in OA and RA ST. Synovial and FLS production of aggrecanase can contribute to cartilage degradation in RA and OA.

Jiankuo, M., W. Xingbing, et al. (2003). "Peptide Nucleic Acid Antisense Prolongs Skin Allograft Survival

by Means of Blockade of CXCR3 Expression Directing T Cells into Graft." J. Immunol. **170**(3): 1556-1565.

http://www.jimmunol.org/cgi/content/abstract/170/3/1556

CXCR3, predominantly expressed on memory/activated T cells, is a receptor for both IFN-{gamma}-inducible protein 10/CXC chemokine ligand (CXCL)10 and monokine induced by IFN-{gamma}/CXCL9. It was reported that CXC chemokines IFN-{gamma}-inducible protein 10/CXCL10 and monokine induced by IFN-{gamma}/CXCL9 play a critical role in the allograft rejection. We report that CXCR3 is a dominant factor directing T cells into mouse skin allograft, and that peptide nucleic acid (PNA) CXCR3 antisense significantly prolongs skin allograft survival by means of blockade of CXCR3 expression directing T cells into allografts in mice. We found that CXCR3 is highly up-regulated in spleen T cells and allografts from BALB/c recipients by day 7 of receiving transplantation, whereas CCR5 expression is moderately increased. We designed PNA CCR5 and PNA CXCR3 antisenses, and i.v. treated mice that received skin allograft transplantations. The PNA CXCR3 at a dosage of 10 mg/kg/day significantly prolonged mouse skin allograft survival (17.1 {+/-} 2.4 days) compared with physiological saline treatment (7.5 {+/-} 0.7 days), whereas PNA CCR5 (10 mg/kg/day) marginally prolonged skin allograft survival (10.7 {+/-} 1.1 days). The mechanism of prolongation of skin allograft survival is that PNA CXCR3 directly blocks the CXCR3 expression in T cells, which is responsible for directing T cells into skin allograft to induce acute rejection, without interfering with other functions of the T cells. These results were obtained at mRNA and protein levels by flow cytometry and real-time quantitative RT-PCR technique, and confirmed by chemotaxis, Northern and Western blot assays, and histological evaluation of skin grafts. The present study indicates the therapeutic potential of PNA CXCR3 to prevent acute transplantation rejection.

Katavic, V., I. K. Lukic, et al. (2003). "Increased Bone Mass Is a Part of the Generalized Lymphoproliferative Disorder Phenotype in the Mouse." J. Immunol. **170**(3): 1540-1547.

http://www.jimmunol.org/cgi/content/abstract/170/3/1540

We investigated the bone phenotype of mice with generalized lymphoproliferative disorder (gld) due to a defect in the Fas ligand-mediated apoptotic pathway. C57BL/6-gld mice had greater whole body bone mineral density and greater trabecular bone volume than their wild-type controls. gld mice lost 5-fold less trabecular bone and had less osteoclasts on bone surfaces after ovariectomy-induced bone resorption. They also formed more bone in a model of osteogenic regeneration after bone marrow ablation, had less osteoclasts on bone surfaces and less apoptotic osteoblasts. gld and wild-type mice had similar numbers of osteoclasts in bone marrow cultures, but marrow stromal fibroblasts from gld mice formed more alkaline phosphatase-positive colonies. Bone diaphyseal shafts and bone marrow stromal fibroblasts produced more osteoprotegerin mRNA and protein than wild-type mice. These findings provide evidence that the disturbance of the bone system is a part of generalized lymphoproliferative syndrome and indicates the possible role of osteoprotegerin as a regulatory link between the bone and immune system.

Guillonneau, C., C. Louvet, et al. (2004). "The Role of TNF-Related Activation-Induced Cytokine-Receptor Activating NF-{kappa}B Interaction in Acute Allograft Rejection and CD40L-Independent Chronic Allograft Rejection." J. Immunol. **172**(3): 1619-1629.

http://www.jimmunol.org/cgi/content/abstract/172/3/1619

We analyzed the role of TNF-related activation-induced cytokine (TRANCE), a member of the TNF family expressed on activated T cells that shares functional properties with CD40L, and its receptor-activating NF-{kappa}B (RANK) which is mostly expressed on mature dendritic cells, during allogenic responses in vivo using a rodent heart allograft model. TRANCE mRNA was strongly up-regulated in acutely rejected allografts on days 4 and 5 posttransplantation whereas RANK was detected as early as day 1 but did not show further up-regulation during the first week. Immunofluoresence analyses of heart allografts showed that 80 and 100% of TRANCE and RANK-expressing cells were T cells and APCs, respectively. We show for the first time that short-term TRANCE blockade using a mouse RANKIg fusion molecule can significantly prolong heart allograft survival in both rat and mouse models. Similarly, rat heart allografts transduced with a RANKIg encoding recombinant adenovirus exhibited a significant prolongation of survival (14.3 vs 7.6 days, $p < 0.0001$). However, TRANCE blockade using RANKIg did not appear to inhibit allogeneic T and B cell priming humoral responses against RANKIg. Interestingly, TRANCE blockade induced strong up-regulation of CD40 ligand (CD40L) mRNA in allografts. Combined CD40L and TRANCE blockade resulted in significantly decreased chronic allograft rejection lesions as well as allogeneic humoral responses compared with CD40L blockade alone. We conclude that TRANCE-RANK interactions play an important role during acute allograft rejection and that CD40L-independent allogeneic immune responses can be, at least in part, dependent on the TRANCE pathway of costimulation.

Kirkiles-Smith, N. C., K. Mahboubi, et al. (2004). "IL-11 Protects Human Microvascular Endothelium from Alloinjury In Vivo by Induction of Survivin Expression." J. Immunol. **172**(3): 1391-1396.

http://www.jimmunol.org/cgi/content/abstract/172/3/1391

IL-11 can reduce tissue injury in animal models of inflammation but the mechanism(s) is unknown. When C.B-17 SCID/beige mice bearing human skin grafts are injected i.p. with human PBMC allogeneic to the donor skin, infiltrating T cells destroy human microvessels by day 21. Intradermal injection of human IL-11 (500 ng/day) delays the time course of graft microvessel loss without reducing the extent of T cell infiltration. Protective actions of IL-11 are most pronounced on day 15. IL-11 has no effect on T cell activation marker, effector molecule, cytokine expression, or endothelial ICAM-1 expression. IL-11 up-regulates the expression of survivin, a cytoprotective protein, in graft keratinocytes and endothelial cells. Topical application of survivin antisense oligonucleotide down-regulates survivin expression in both cell types and largely abrogates the protective effect of IL-11. We conclude that in this human transplant model, IL-11 exerts a cytoprotective rather than anti-inflammatory or immunomodulatory effect mediated through induction of survivin.

Nakamura, Y., S. Esnault, et al. (2004). "Ets-1 Regulates TNF-{alpha}-Induced Matrix Metalloproteinase-9 and Tenascin Expression in Primary Bronchial Fibroblasts." J. Immunol. **172**(3): 1945-1952.

http://www.jimmunol.org/cgi/content/abstract/172/3/1945

Increased subepithelial deposition of extracellular matrix proteins is a key feature in bronchial asthma. Matrix metalloproteinase-9 (MMP-9) is a proteolytic enzyme that degrades the extracellular matrix. Tenascin is an extracellular matrix glycoprotein that is abundant in thickened asthmatic subbasement membrane. The expression of MMP-9 and tenascin reflects disease activity in asthma and airway remodeling. The molecular mechanisms regulating the expression of these proteins remain unknown. Both MMP-9 and tenascin promoters contain an Ets binding site, suggesting control by Ets-1. Thus, we hypothesized that Ets-1 expression is increased in asthma and that it contributed to enhanced MMP-9 and tenascin expression. To test this hypothesis, we determined the expression of Ets-1 in bronchial biopsies obtained from asthmatic

subjects and determined the expression of Ets-1, MMP-9, and tenascin by bronchial fibroblasts activated ex vivo. We observed that nuclear extracts from TNF-{alpha}-activated fibroblasts showed increased Ets-binding activity. In addition, TNF-{alpha}-activated fibroblasts had increased expression of Ets-1 mRNA and protein, which preceded an increase in MMP-9 and tenascin mRNA. Furthermore, treatment of fibroblasts with Ets-1 antisense oligonucleotides down-regulated TNF-{alpha}-induced Ets-1, MMP-9, and, to a lesser extent, tenascin protein expression or activity. Taken together, these data demonstrate that TNF-{alpha} increases MMP-9 and tenascin expression in bronchial fibroblasts via the transcription factor Ets-1, and suggest a role for Ets-1 in airway remodeling in asthma.

Diaz, L. S., H. Foster, et al. (2005). "VCAM-1 Expression on CD8+ Cells Correlates with Enhanced Anti-HIV Suppressing Activity." J. Immunol. **174**(3): 1574-1579.

http://www.jimmunol.org/cgi/content/abstract/174/3/1574

CD8+ cells from HIV-infected individuals showing the CD8+ cell noncytotoxic antiviral response unexpectedly revealed mRNA for VCAM-1, a cell surface molecule found on endothelial cells. Uninfected subjects had undetectable levels of VCAM-1 mRNA in their CD8+ cells. Flow cytometry analysis showed that up to 12% of the CD8+ cells from HIV-positive individuals expressed VCAM-1 compared with 0.8% of the CD8+ cells of HIV-negative individuals. Enrichment of the CD8+VCAM-1+ cell population and subsequent coculture with CD4+ cells acutely infected with HIV-1 showed that the VCAM-1+CD8+ cells were able to suppress viral replication with 50% less input cells than the unseparated CD8+ cell population. This study demonstrates, for the first time, the expression of VCAM-1 on CD8+ cells. Moreover, the CD8+VCAM-1+ cells show enhanced CD8+ cell noncytotoxic antiviral response activity that could have clinical importance in HIV infection.

Sacedon, R., B. Diez, et al. (2005). "Sonic Hedgehog Is Produced by Follicular Dendritic Cells and Protects Germinal Center B Cells from Apoptosis." J. Immunol. **174**(3): 1456-1461.

http://www.jimmunol.org/cgi/content/abstract/174/3/1456

The Hedgehog (Hh) signaling pathway is involved in the development of many tissues during embryogenesis, but has also been described to function in adult self-renewing tissues. In the immune system, Sonic Hedgehog (Shh) regulates intrathymic T cell development and modulates the effector functions of peripheral CD4+ T cells. In this study we investigate whether Shh signaling is involved in peripheral B cell differentiation in mice. Shh is produced by follicular dendritic cells, mainly in germinal centers (GCs), and GC B cells express both components of the Hh receptor, Patched and Smoothened. Blockade of the Hh signaling pathway reduces the survival, and consequently the proliferation and Ab secretion, of GC B cells. Furthermore, Shh rescues GC B cells from apoptosis induced by Fas ligation. Taken together, our data suggest that Shh is one of the survival signals provided by follicular dendritic cells to prevent apoptosis in GC B cells.

Tao, D., L. Shangwu, et al. (2005). "CD226 Expression Deficiency Causes High Sensitivity to Apoptosis in NK T Cells from Patients with Systemic Lupus Erythematosus." J. Immunol. **174**(3): 1281-1290.

http://www.jimmunol.org/cgi/content/abstract/174/3/1281

Humans and mice with systemic lupus erythematosus (SLE) and related autoimmune diseases have reduced numbers of NK T cells. An association between NK T cell deficiency and autoimmune disease has been identified. However, the mechanisms for reduction of NK T cell number in patients with SLE are unknown. In the present study we report that NK T cells from active SLE patients are highly sensitive to anti-CD95-induced apoptosis compared with those from normal subjects and inactive SLE patients. CD226 expression is deficient on NK T cells from active SLE patients. The expression of one antiapoptotic member protein, survivin, is found to be selectively deficient in freshly isolated NK T cells from active SLE patients. CD226 preactivation significantly up-regulates survivin expression and activation, which can rescue active SLE NK T cells from anti-CD95-induced apoptosis. In transfected COS7 cells, we confirm that anti-CD95-mediated death signals are inhibited by activation of the CD226 pathway through stabilization of caspase-8 and caspase-3 and through activation of survivin. We therefore conclude that deficient expression of CD226 and survivin in NK T cells from active SLE is a molecular base of high sensitivity of the cells to anti-CD95-induced apoptosis. These observations offer a potential explanation for high apoptotic sensitivity of NK T cells from active SLE, and provide a new insight into the mechanism of reduction of NK T cell number in SLE and understanding the association between NK T cell deficiency and autoimmune diseases.

Attur, M. G., M. N. Dave, et al. (2002). "Functional Genomic Analysis of Type II IL-1{beta} Decoy Receptor: Potential for Gene Therapy in Human Arthritis and Inflammation." J. Immunol. **168**(4): 2001-2010.

http://www.jimmunol.org/cgi/content/abstract/168/4/2001

Gene expression arrays show that human epithelial cells and human arthritis-affected cartilage lack detectable amounts of mRNA for IL-1 antagonizing molecules: IL-1Ra and IL-1RII, but constitutively express IL-1. Functional genomic analysis was performed by reconstituting human IL-1RII expression in various IL-1RII-deficient cell types to examine its antagonist role using gene therapy approaches. Adenovirus-expressing IL-1RII when transduced into human and bovine chondrocytes, human and rabbit synovial cells, human epithelial cells, and rodent fibroblasts expressed membrane IL-1RII and spontaneously released functional soluble IL-1RII. The IL-1RII+ (but not IL-1RII-) cells were resistant to IL-1{beta}-induced, NO, PGE2, IL-6, and IL-8 production or decreased proteoglycan synthesis. IL-1RII inhibited the function of IL-1 in chondrocytes and IL-1- and TNF-{alpha}-induced inflammatory mediators in human synovial and epithelial cells. IL-1RII+ chondrocytes were more resistant to induction of NO and PGE2 by IL-1{beta} compared with IL-1RII- cells incubated with a 10-fold (weight) excess of soluble type II IL-1R (sIL-1RII) protein. In cocultures, IL-1RII+ synovial cells released sIL-1RII, which in a paracrine fashion protected chondrocytes from the effects of IL-1{beta}. Furthermore, IL-1RII+ (but not IL-1RII-) chondrocytes when transplanted onto human osteoarthritis-affected cartilage in vitro, which showed spontaneous release of sIL-1RII for 20 days, inhibited the spontaneous production of NO and PGE2 in cartilage in ex vivo. In summary, reconstitution of IL-1RII in IL-1RII- cells using gene therapy approaches significantly protects cells against the autocrine and paracrine effects of IL-1 at the signaling and transcriptional levels.

Lu, G., B. M. Janjic, et al. (2002). "Innate Direct Anticancer Effector Function of Human Immature Dendritic Cells. II. Role of TNF, Lymphotoxin-{alpha}1{beta}2, Fas Ligand, and TNF-Related Apoptosis-Inducing Ligand." J. Immunol. **168**(4): 1831-1839.

http://www.jimmunol.org/cgi/content/abstract/168/4/1831

Our recent studies have demonstrated that human immature dendritic cells (DCs) are able to directly and effectively mediate apoptotic killing against a wide array of cultured and freshly-

isolated cancer cells without harming normal cells. In the present study, we demonstrate that this tumoricidal activity is mediated by multiple cytotoxic TNF family ligands. We determine that human immature DCs express on their cell surface four different cytotoxic TNF family ligands: TNF, lymphotoxin-{alpha}1{beta}2, Fas ligand, and TNF-related apoptosis inducing ligand; while cancer cells express the corresponding death receptors. Disruptions of interactions between the four ligands expressed on DCs and corresponding death-signaling receptors expressed on cancer cells using specific Abs or R:Fc fusion proteins block the cytotoxic activity of DCs directed against cancer cells. The novel findings suggest that DC killing of cancer cells is mediated by the concerted engagement of four TNF family ligands of DCs with corresponding death receptors of cancer cells. Overall, our data demonstrate that DCs are fully equipped for an efficient direct apoptotic killing of cancer cells and suggest that this mechanism may play a critical role in both afferent and efferent anti-tumor immunity.

Johnson, D. R. (2003). "Locus-Specific Constitutive and Cytokine-Induced HLA Class I Gene Expression." J. Immunol. **170**(4): 1894-1902.

http://www.jimmunol.org/cgi/content/abstract/170/4/1894

Cytokine induction of the MHC class I genes increases the nascent molecules available for binding potentially antigenic peptides. The human H chain loci, HLA-A, -B, and -C, encode highly homologous and polymorphic mRNAs. Here, these transcripts were resolved and measured by competitive PCR of cDNA using locus-specific primers. Endothelial cells expressed many HLA-A and -B, but fewer HLA-C, transcripts. In contrast, HeLa cells expressed many HLA-A and -C, but fewer HLA-B, transcripts. The inflammatory cytokines TNF-{alpha}, IFN-{beta}, and IFN-{gamma} induced HLA-B strongly, but HLA-A and -C weakly in both cell types. Combined treatment with IFNs and TNF further increased HLA-A and -B, but not HLA-C transcripts. The constitutive and inducible activities of transfected promoters correlated well with mRNA levels. The weak IFN response of the HLA-A2 promoter was not due to variations in the IFN consensus sequence, the site {alpha}, or a 3-bp insertion between them. The HLA-Cw6 promoter was less TNF responsive due to a variant {kappa}B enhancer, which also reduced the IFN responses. The NF-{kappa}B subunit RelA strongly activated the HLA-A2 and -B7 promoters but only weakly activated the HLA-Cw6 promoter due to the variant {kappa}B. Cotransfecting NF-{kappa}B1 with RelA further increased activity of the HLA-A2 and -B7, but not HLA-Cw6, promoters. All three promoters were activated by MHC class II trans-activator, but not CREB-binding protein, whereas IFN regulatory factor-1 and -2 weakly activated the HLA-B7 and -Cw6, but not HLA-A2, promoters. These studies illustrate common and locus-specific mechanisms that may be targeted to modulate immune reactions.

Kwok, L.-Y., S. Lutjen, et al. (2003). "The Induction and Kinetics of Antigen-Specific CD8 T Cells Are Defined by the Stage Specificity and Compartmentalization of the Antigen in Murine Toxoplasmosis." J. Immunol. **170**(4): 1949-1957.

http://www.jimmunol.org/cgi/content/abstract/170/4/1949

Toxoplasma gondii forms different life stages, fast-replicating tachyzoites and slow-growing bradyzoites, in mammalian hosts. CD8 T cells are of crucial importance in toxoplasmosis, but it is unknown which parasite stage is recognized by CD8 T cells. To analyze stage-specific CD8 T cell responses, we generated various recombinant Toxoplasma gondii expressing the heterologous Ag {beta}-galactosidase ({beta}-gal) and studied whether 1) secreted or cytoplasmic Ags and 2) tachyzoites or bradyzoites, which persist intracerebrally, induce CD8 T cells. We monitored the frequencies and kinetics of {beta}-gal-specific CD8 T cells in infected mice by MHC class I tetramer staining. Upon oral infection of B6C (H-2bxd) mice, only {beta}-gal-secreting tachyzoites

induced {beta}-gal-specific CD8 T cells. However, upon secondary infection of mice that had received a primary infection with tachyzoites secreting {beta}-gal, {beta}-gal-secreting tachyzoites and bradyzoites transiently increased the frequency of intracerebral {beta}-gal-specific CD8 T cells. Frequencies of splenic and cerebral {beta}-gal-specific CD8 T cells peaked at day 23 after infection, thereafter persisting at high levels in the brain but declining in the spleen. Splenic and cerebral {beta}-gal-specific CD8 T cells produced IFN-{gamma} and were cytolytic upon specific restimulation. Thus, compartmentalization and stage specificity of an Ag determine the induction of CD8 T cells in toxoplasmosis.

Blois, S., M. Tometten, et al. (2005). "Intercellular Adhesion Molecule-1/LFA-1 Cross Talk Is a Proximate Mediator Capable of Disrupting Immune Integration and Tolerance Mechanism at the Feto-Maternal Interface in Murine Pregnancies." J. Immunol. **174**(4): 1820-1829.

http://www.jimmunol.org/cgi/content/abstract/174/4/1820

Our understanding why a woman's immune system does not reject her histoincompatible fetus is still very limited. Distinct insights into the mechanisms involved in pregnancy maintenance may help us to prevent pregnancy complications, e.g., miscarriages or pre-eclampsia. Immune integration and tolerance at the feto-maternal interface appear to be indispensable for successful pregnancy maintenance. Little is known about the cross talk between ICAM-1, expressed on epithelium, endothelium, and APC, and its ligand, LFA-1, at the feto-maternal interface. However, based on the role of ICAM-1/LFA-1 in allograft acceptance or rejection upon transplantation, adhesion molecules are likely to interfere with successful pregnancy outcome. In this study, we tested the hypothesis that ICAM-1/LFA-1 pathways may be involved in pregnancy rejection in murine models. By blocking ICAM-1/LFA-1-mediated intercellular adhesion events, we show that fetal immune acceptance is restored in challenged pregnancies (e.g., upon exposure to sound stress), and adoptive transfer of LFA-1 cells into pregnant mice induces rejection only in abortion-prone mouse models. ICAM-1/LFA-1 cross talk leads to increased recruitment of proinflammatory cells to the implantation site, promotes dendritic cell maturation in the decidua, and subsequently induces additional local Th1 polarization via mature dendritic cells. Furthermore, our observations clearly point out that mechanisms of fetal tolerance, e.g., indoleamine 2,3-dioxygenase expression, presence of CD4+CD25bright regulatory T cells, and synthesis of asymmetric Abs, are ICAM-1/LFA-1 dependent. Hence, our data shed light on a hierarchical network of immune integration at the feto-maternal interface, in which ICAM-1/LFA-1 cross talk is clearly a proximate mediator capable of disrupting successful pregnancy maintenance.

Nonaka, S., T. Naito, et al. (2005). "Intestinal {gamma}{delta} T Cells Develop in Mice Lacking Thymus, All Lymph Nodes, Peyer's Patches, and Isolated Lymphoid Follicles." J. Immunol. **174**(4): 1906-1912.

http://www.jimmunol.org/cgi/content/abstract/174/4/1906

Through analysis of athymic (nu/nu) mice carrying a transgenic gene encoding GFP instead of RAG-2 product, it has recently been reported that, in the absence of thymopoiesis, mesenteric lymph nodes and Peyer's patches (PP) but not gut cryptopatches are pivotal birthplace of mature T cells such as the thymus-independent intestinal intraepithelial T cells (IEL). To explore and evaluate this important issue, we generated nu/nu mice lacking all lymph nodes (LN) and PP by administration of lymphotoxin-{beta} receptor-Ig and TNF receptor 55-Ig fusion proteins into the timed pregnant nu/+ mice that had been mated with male nu/nu mice (nu/nu LNP- mice). We also generated nu/nu aly/aly (aly, alymphoplasia) double-mutant mice that inherently lacked all LN, PP, and isolated lymphoid follicles. Although {gamma}{delta}-IEL were slightly smaller in number than those in nu/nu mice, substantial colonization of {gamma}{delta}-IEL was found to take place

in the intestinal epithelia of nu/nu LNP- and nu/nu aly/aly mice. Notably, the population size of a major CD8{alpha}{alpha}+ {gamma}{delta}-IEL subset was maintained, the use of TCR-{gamma}-chain variable gene segments by these {gamma}{delta}-IEL was unaltered, and the development of cryptopatches remained intact in these nu/nu LNP- and nu/nu aly/aly mice. These findings indicate that all LN, including mesenteric LN, PP, and isolated lymphoid follicles, are not an absolute requirement for the development of {gamma}{delta}-IEL in athymic nu/nu mice.

Aoyagi, K., J. M. Dijkstra, et al. (2002). "Classical MHC Class I Genes Composed of Highly Divergent Sequence Lineages Share a Single Locus in Rainbow Trout (Oncorhynchus mykiss)." J. Immunol. **168**(1): 260-273.

http://www.jimmunol.org/cgi/content/abstract/168/1/260

The classical MHC class I genes have been known to be highly polymorphic in various vertebrates. To date, putative allelic sequences of the classical MHC class I genes in teleost fish have been reported in several studies. However, the establishment of their allelic status has been hampered in most cases by the lack of appropriate genomic information. In the present study, using heterozygous and homozygous fish, we obtained classical-type MHC class I sequences of rainbow trout (Oncorhynchus mykiss) and investigated their allelic relationship by gene amplification and Southern and Northern hybridization analyses. The results indicated that all MHC class I sequences we obtained were derived from a single locus. Based on this, a unique polymorphic nature of the MHC class I locus of rainbow trout has been revealed. The mosaic combination of highly divergent ancient sequences in the peptide-binding domains is notable, and the variable nature around the boundary between the {alpha}3 and transmembrane domains is unprecedented.

Lauw, F. N., J. Branger, et al. (2002). "IL-18 Improves the Early Antimicrobial Host Response to Pneumococcal Pneumonia." J. Immunol. **168**(1): 372-378.

http://www.jimmunol.org/cgi/content/abstract/168/1/372

To determine the role of endogenous IL-18 during pneumonia, IL-18 gene-deficient (IL-18-/-) mice and wild-type (WT) mice were intranasally inoculated with Streptococcus pneumoniae, the most common causative agent of community-acquired pneumonia. Infection with S. pneumoniae increased the expression of IL-18 mRNA and was associated with elevated concentrations of both precursor and mature IL-18 protein within the lungs. IL-18-/- mice had significantly more bacteria in their lungs and were more susceptible for progressing to systemic infection at 24 and 48 h postinoculation. Similarly, treatment of WT mice with anti-IL-18 was associated with enhanced outgrowth of pneumococci. In contrast, the clearance of pneumococci from lungs of IL-12-/- mice was unaltered when compared with WT mice. Furthermore, anti-IL-12 did not influence bacterial clearance in either IL-18-/- or WT mice. These data suggest that endogenous IL-18, but not IL-12, plays an important role in the early antibacterial host response during pneumococcal pneumonia.

McKay, P. F., J. E. Schmitz, et al. (2002). "Vaccine Protection Against Functional CTL Abnormalities in Simian Human Immunodeficiency Virus-Infected Rhesus Monkeys." J. Immunol. **168**(1): 332-337.

http://www.jimmunol.org/cgi/content/abstract/168/1/332

Accumulating evidence suggests that HIV-specific CD8+ CTL are dysfunctional in HIV-infected individuals with progressive clinical disease. In the present studies, cytokine production by virus-specific CTL was assessed in the rhesus monkey model for AIDS to determine its contribution to the functional impairment of CTL. CTL from monkeys infected with nonpathogenic isolates of simian and simian-human immunodeficiency virus expressed high levels of IFN-{gamma}, TNF-{alpha}, and IL-2 after in vitro exposure to a nonspecific mitogen or the optimal peptide representing a dominant virus-specific CTL epitope. However, similarly performed studies assessing these capabilities in CTL from monkeys infected with pathogenic immunodeficiency virus isolates demonstrated a significant dysfunction in the ability of the CTL to produce IL-2 and TNF-{alpha}. Importantly, CTL from vaccinated monkeys that effectively controlled the replication of a highly pathogenic simian-human immunodeficiency virus isolate following challenge demonstrated a preserved capacity to produce these cytokines. These experiments suggest that defects in cytokine production may contribute to CTL dysfunction in chronic HIV or SIV infection. Moreover, an AIDS vaccine that confers protection against clinical disease evolution in this experimental model also preserves the functional capacity of these CTL to produce both IL-2 and TNF-{alpha}.

Shum, B. P., L. R. Flodin, et al. (2002). "Conservation and Variation in Human and Common Chimpanzee CD94 and NKG2 Genes." J. Immunol. **168**(1): 240-252.

http://www.jimmunol.org/cgi/content/abstract/168/1/240

To assess polymorphism and variation in human and chimpanzee NK complex genes, we determined the coding-region sequences for CD94 and NKG2A, C, D, E, and F from several human (Homo sapiens) donors and common chimpanzees (Pan troglodytes). CD94 is highly conserved, while the NKG2 genes exhibit some polymorphism. For all the genes, alternative mRNA splicing variants were frequent among the clones obtained by RT-PCR. Alternative splicing acts similarly in human and chimpanzee to produce the CD94B variant from the CD94 gene and the NKG2B variant from the NKG2A gene. Whereas single chimpanzee orthologs for CD94, NKG2A, NKG2E, and NKG2F were identified, two chimpanzee paralogs of the human NKG2C gene were defined. The chimpanzee Pt-NKG2CI gene encodes a protein similar to human NKG2C, whereas in the chimpanzee Pt-NKG2CII gene the translation frame changes near the beginning of the carbohydrate recognition domain, causing premature termination. Analysis of a panel of chimpanzee NK cell clones showed that Pt-NKG2CI and Pt-NKG2CII are independently and clonally expressed. Pt-NKG2CI and Pt-NKG2CII are equally diverged from human NKG2C, indicating that they arose by gene duplication subsequent to the divergence of chimpanzee and human ancestors. Genomic DNA from 80 individuals representing six primate species were typed for the presence of CD94 and NKG2. Each species gave distinctive typing patterns, with NKG2A and CD94 being most conserved. Seven different NK complex genotypes within the panel of 48 common chimpanzees were due to differences in Pt-NKG2C and Pt-NKG2D genes.

Langdon, C., C. Kerr, et al. (2003). "Oncostatin M Regulates Eotaxin Expression in Fibroblasts and Eosinophilic Inflammation in C57BL/6 Mice." J. Immunol. **170**(1): 548-555.

http://www.jimmunol.org/cgi/content/abstract/170/1/548

Oncostatin M (OSM) is a member of the IL-6/LIF (or gp130) cytokine family, and its potential role in inflammation is supported by a number of activities identified in vitro. In this study, we investigate the action of murine OSM on expression of the CC chemokine eotaxin by fibroblasts in vitro and on mouse lung tissue in vivo. Recombinant murine OSM stimulated eotaxin protein production and mRNA levels in the NIH 3T3 fibroblast cell line. IL-6 could regulate a small

induction of eotaxin in NIH 3T3 cells, but other IL-6/LIF cytokines (LIF, cardiotrophin-1 (CT-1)) had no effect. Cell signaling studies showed that murine OSM, LIF, IL-6, and CT-1 stimulated the tyrosine phosphorylation of STAT-3, suggesting STAT-3 activation is not sufficient for eotaxin induction in NIH 3T3 cells. OSM induced ERK-1,2 and p38 mitogen-activated protein kinase phosphorylation in NIH 3T3 cells, and inhibitors of ERK (PD98059) or p38 (SB203580) could partially reduce OSM-induced eotaxin production, suggesting partial dependence on mitogen-activated protein kinase signaling. OSM (but not LIF, IL-6, or CT-1) also induced eotaxin release by mouse lung fibroblast cultures derived from C57BL/6 mice. Overexpression of murine OSM in lungs of C57BL/6 mice using an adenovirus vector encoding murine OSM resulted in a vigorous inflammatory response by day 7 after intranasal administration, including marked extracellular matrix accumulation and eosinophil infiltration. Elevated levels of eotaxin mRNA in whole lung were detected at days 4 and 5. These data strongly support a role of OSM in lung inflammatory responses that involve eosinophil infiltration.

Workalemahu, G., M. Foerster, et al. (2003). "Human {gamma}{delta}-T Lymphocytes Express and Synthesize Connective Tissue Growth Factor: Effect of IL-15 and TGF-{beta}1 and Comparison with {alpha}{beta}-T Lymphocytes." J. Immunol. **170**(1): 153-157.

http://www.jimmunol.org/cgi/content/abstract/170/1/153

T lymphocytes bearing the {gamma}{delta}-TCR accumulate during wound healing and inflammation. However, the role of {gamma}{delta}-T lymphocytes in fibrogenic tissue reactions is not well understood. Therefore, we addressed the question of whether human {gamma}{delta}-T cells express and synthesize connective tissue growth factor (CTGF), a factor known to regulate fibrogenesis and wound healing. In addition, the lymphoblastic leukemia T cell line (Loucy) that possesses characteristics typical of {gamma}{delta}-T cells was used as a model to evaluate the regulation of CTGF gene expression. Blood {gamma}{delta}-T cells isolated from healthy donors were grown in the presence of IL-15/TGF-{beta}1 for 48 h and assessed for the expression and synthesis of CTGF. Nonstimulated human blood {gamma}{delta}-T cells and Loucy {gamma}{delta}-T cells expressed low levels of CTGF mRNA. Costimulation of the cells with IL-15 and TGF-{beta}1 resulted in a substantially increased level of CTGF mRNA expression within 4-8 h, and it remained elevated for at least 48 h. In contrast, no CTGF mRNA was detected when nonstimulated and stimulated human CD4+ {alpha}{beta}-T cells were analyzed. In addition, Western blot analysis of human {gamma}{delta}-T cell lysates prepared 4 days following stimulation with IL-15 and TGF-{beta}1 revealed a 38-kDa CTGF protein in cell lysates of human {gamma}{delta}-T cells. Detection was confirmed using Colo 849 fibroblasts, which can constitutively express high levels of CTGF. In conclusion, we herein present novel evidence that in contrast to CD4+ {alpha}{beta}-T cells human {gamma}{delta}-T cells are capable of expressing CTGF mRNA and synthesizing its corresponding protein, which supports the concept that {gamma}{delta}-T cells may contribute to wound healing or tissue fibrotic processes.

Carlson, K. A., G. Leisman, et al. (2004). "Molecular Characterization of a Putative Antiretroviral Transcriptional Factor, OTK18." J. Immunol. **172**(1): 381-391.

http://www.jimmunol.org/cgi/content/abstract/172/1/381

Elucidation of the factors involved in host defense against human immunodeficiency viral infection remains pivotal if viral control may be achieved. Toward these ends, we investigated the function of a putative antiretroviral factor, OTK18, isolated by differential display of mRNA from HIV type 1-infected primary human monocyte-derived macrophages. Molecular and immunohistochemical analyses showed that the OTK18 nucleotide sequence contains 13 adjacent C2H2-type zinc finger motifs, a Kruppel-associated box, and is localized to both cytosol and nucleus. Mutational

analyses revealed that both the Kruppel-associated box and zinc finger regions of OTK18 are responsible for the transcriptional suppressive activities of this gene. OTK18 was copiously expressed in macrophages following HIV type I infection and diminished progeny virion production. A mechanism for this antiretroviral activity was by suppression of HIV type 1 Tat-induced viral long terminal repeat promoter activity. Our findings suggest that one possible function of OTK18 is as a HIV type 1-inducible transcriptional suppresser.

Galietta, L. J. V., P. Pagesy, et al. (2002). "IL-4 Is a Potent Modulator of Ion Transport in the Human Bronchial Epithelium In Vitro." J. Immunol. **168**(2): 839-845.

http://www.jimmunol.org/cgi/content/abstract/168/2/839

Recent data show that proinflammatory stimuli may modify significantly ion transport in the airway epithelium and therefore the properties of the airway surface fluid. We have studied the effect of IL-4, a cytokine involved in the pathogenesis of asthma, on transepithelial ion transport in the human bronchial epithelium in vitro. Incubation of polarized bronchial epithelial cells with IL-4 for 6-48 h causes a marked inhibition of the amiloride-sensitive Na+ channel as measured in short circuit current experiments. On the other hand, IL-4 evokes a 2-fold increase in the current activated by a cAMP analog, which reflects the activity of the cystic fibrosis transmembrane conductance regulator (CFTR). Similarly, IL-4 enhances the response to apical UTP, an agonist that activates Ca2+-dependent Cl- channels. These effects are mimicked by IL-13 and blocked by an antagonist of IL-4R{alpha}. RT-PCR experiments show that IL-4 elicits a 7-fold decrease in the level of the {gamma} amiloride-sensitive Na+ channel mRNA, one of the subunits of the amiloride-sensitive Na+ channel, and an increase in CFTR mRNA. Our data suggest that IL-4 may favor the hydration of the airway surface by decreasing Na+ absorption and increasing Cl- secretion. This could be required to fluidify the mucus, which is hypersecreted during inflammatory conditions. On the other hand, the modifications of ion transport could also affect the ion composition of airway surface fluid.

Holling, T. M., N. van der Stoep, et al. (2002). "Activated Human T Cells Accomplish MHC Class II Expression Through T Cell-Specific Occupation of Class II Transactivator Promoter III." J. Immunol. **168**(2): 763-770.

http://www.jimmunol.org/cgi/content/abstract/168/2/763

Activated human T cells express HLA-DR, HLA-DQ, and HLA-DP on their surface, but the regulation and functioning of MHC class II molecules in T lymphocytes are poorly understood. Because the MHC class II transactivator (CIITA) is essential for MHC class II expression, we have investigated transcriptional activation of CIITA in activated T cells. In this study, we show that in human activated CD4+ T cells, CIITA promoter III (CIITA-PIII) drives the expression of CIITA. The in vivo genomic footprint analysis revealed activated T cell-specific occupation of CIITA-PIII. Subsequent EMSA analysis of several promoter regions showed differences in banding pattern among activated T cells, naive T cells, primary B cells, and Raji B cells. Activating response element (ARE)-1 is shown to interact with the acute myeloid leukemia 2 transcription factor in nuclear extracts derived from both T and B cells. Interestingly, the acute myeloid leukemia 3 transcription factor was bound in nuclear extracts of T cells only. The ARE-2 sequence is able to bind CREB/activating transcription factor family members in both T and B cells. In addition, a yet unidentified Ets family member was found to interact with site C in activated T cells, whereas in B cells site C was bound by PU.1 and Pip/IFN regulatory factor 4/IFN consensus sequence binding protein for activated T cells. In Jurkat T cells, both ARE-1 and ARE-2 are crucial for CIITA-PIII activity, similar to Raji B cells. The differential banding pattern in in vivo genomic footprinting and transcription factor binding at the ARE-1 and site C between T

cells and B cells probably reflects differences in CIITA-PIII activation pathways employed by these cell types.

Michalec, L., B. K. Choudhury, et al. (2002). "CCL7 and CXCL10 Orchestrate Oxidative Stress-Induced Neutrophilic Lung Inflammation." J. Immunol. **168**(2): 846-852.

http://www.jimmunol.org/cgi/content/abstract/168/2/846

Oxidative stress from ozone (O3) exposure augments airway neutrophil recruitment and chemokine production. We and others have shown that severe and sudden asthma is associated with airway neutrophilia, and that O3 oxidative stress is likely to augment neutrophilic airway inflammation in severe asthma. However, very little is known about chemokines that orchestrate oxidative stress-induced neutrophilic airway inflammation in vivo. To identify these chemokines, three groups of BALB/c mice were exposed to sham air, 0.2 ppm O3, or 0.8 ppm O3 for 6 h. Compared with sham air, 0.8 ppm O3, but not 0.2 ppm O3, induced pronounced neutrophilic airway inflammation that peaked at 18 h postexposure. The 0.8 ppm O3 up-regulated lung mRNA of CXCL1,2,3 (mouse growth-related oncogene-{alpha} and macrophage-inflammatory protein-2), CXCL10 (IFN-{gamma}-inducible protein-10), CCL3 (macrophage-inflammatory protein-1{alpha}), CCL7 (monocyte chemoattractant protein-3), and CCL11 (eotaxin) at 0 h postexposure, and expression of CXCL10, CCL3, and CCL7 mRNA was sustained 18 h postexposure. O3 increased lung protein levels of CXCL10, CCL7, and CCR3 (CCL7R). The airway epithelium was identified as a source of CCL7. The role of up-regulated chemokines was determined by administering control IgG or IgG Abs against six murine chemokines before O3 exposure. As expected, anti-mouse growth-related oncogene-{alpha} inhibited neutrophil recruitment. Surprisingly, Abs to CCL7 and CXCL10 also decreased neutrophil recruitment by 63 and 72%, respectively. These findings indicate that CCL7 and CXCL10, two chemokines not previously reported to orchestrate neutrophilic inflammation, play a critical role in mediating oxidative stress-induced neutrophilic airway inflammation. These observations may have relevance in induction of neutrophilia in severe asthma.

Zarember, K. A. and P. J. Godowski (2002). "Tissue Expression of Human Toll-Like Receptors and Differential Regulation of Toll-Like Receptor mRNAs in Leukocytes in Response to Microbes, Their Products, and Cytokines." J. Immunol. **168**(2): 554-561.

http://www.jimmunol.org/cgi/content/abstract/168/2/554

Members of the Toll-like receptor (TLR) family mediate dorsoventral patterning and cellular adhesion in insects as well as immune responses to microbial products in both insects and mammals. TLRs are characterized by extracellular leucine-rich repeat domains and an intracellular signaling domain that shares homology with cytoplasmic sequences of the mammalian IL-1 receptor and plant disease resistance genes. Ten human TLRs have been cloned as well as RP105, a protein similar to TLR4 but lacking the intracellular signaling domain. However, only five TLRs have described functions as receptors for bacterial products (e.g., LPS, lipoproteins). To identify potential sites of action, we used quantitative real-time RT-PCR to examine systematically the expression of mRNAs encoding all known human TLRs, RP105, and several other proteins important in TLR functions (e.g., MD-1, MD-2, CD14, MyD88). Most tissues tested expressed at least one TLR, and several expressed all (spleen, peripheral blood leukocytes). Analysis of TLR expression in fractionated primary human leukocytes (CD4+, CD8+, CD19+, monocytes, and granulocytes) indicates that professional phagocytes express the greatest variety of TLR mRNAs although several TLRs appear more restricted to B cells, suggesting additional roles for TLRs in adaptive immunity. Monocyte-like THP-1 cells regulate TLR mRNA levels in response to a variety of stimuli including phorbol esters, LPS, bacterial

lipoproteins, live bacteria, and cytokines. Furthermore, addition of Escherichia coli to human blood ex vivo caused distinct changes in TLR expression, suggesting that important roles exist for these receptors in the establishment and resolution of infections and inflammation.

Bennett, F., D. Luxenberg, et al. (2003). "Program Death-1 Engagement Upon TCR Activation Has Distinct Effects on Costimulation and Cytokine-Driven Proliferation: Attenuation of ICOS, IL-4, and IL-21, But Not CD28, IL-7, and IL-15 Responses." J. Immunol. **170**(2): 711-718.

http://www.jimmunol.org/cgi/content/abstract/170/2/711

The program death 1 (PD-1) receptor and its ligands, PD-1 ligand (PD-L)1 and PD-L2, define a novel regulatory pathway with potential inhibitory effects on T, B, and monocyte responses. In the present study, we show that human CD4+ T cells express PD-1, PD-L1, and PD-L2 upon activation, and Abs to the receptor can be agonists or antagonists of the pathway. Under optimal conditions of stimulation, ICOS but not CD28 costimulation can be prevented by PD-1 engagement. IL-2 levels induced by costimulation are critical in determining the outcome of the PD-1 engagement. Thus, low to marginal IL-2 levels produced upon ICOS costimulation account for the greater sensitivity of this pathway to PD-1-mediated inhibition. Interestingly, exogenous IL-2, IL-7, and IL-15 but not IL-4 and IL-21 can rescue PD-1 inhibition, suggesting that among these cytokines only those that activate STAT5 can rescue PD-1 inhibition. As STAT5 has been implicated in the maintenance of IL-2R{alpha} expression, these results suggest that IL-7 and IL-15 restore proliferation under conditions of PD-1 engagement by enhancing high-affinity IL-2R expression and hence, IL-2 responsiveness.

Thorn, C. F. and A. S. Whitehead (2002). "Differential Glucocorticoid Enhancement of the Cytokine-Driven Transcriptional Activation of the Human Acute Phase Serum Amyloid A Genes, SAA1 and SAA2." J. Immunol. **169**(1): 399-406.

http://www.jimmunol.org/cgi/content/abstract/169/1/399

The human acute phase serum amyloid A (A-SAA) genes, SAA1 and SAA2, have a high degree of sequence identity that extends [~]450 bp upstream of their transcription start sites. Each promoter contains analogously positioned functional binding sites for the transcription factors NF-{kappa}B and NF-IL6. In human HepG2 hepatoma cells transfected with SAA promoter luciferase reporter constructs, administration of IL-1 and IL-6, singly or in combination, induced SAA1 and SAA2 transcriptional readouts that were qualitatively indistinguishable. However, under induced conditions, the SAA2 promoter had a significant quantitative transcriptional advantage over the SAA1 promoter. The application of the synthetic glucocorticoid dexamethasone in the context of cytokine stimulation enhanced the transcriptional activity of the SAA1, but not the SAA2, promoter such that readout from the former became equivalent to that from the latter. A putative glucocorticoid response element (GRE) is present (between residues -208 and -194) only in the SAA1 gene; a similar sequence in the corresponding region of the SAA2 gene is disrupted by a nine-residue insertion. The SAA1 GRE was shown to be functionally active and the SAA2 disrupted GRE was shown to be functionally inactive in experiments using reporter constructs carrying SAA1 and SAA2 promoters that had been modified by site-specific mutagenesis. Quantitative analysis of transcript-specific RT-PCR products, derived from SAA1 and SAA2 mRNAs after treatment of HepG2 cells with cytokines in the presence or absence of dexamethasone, confirmed that the endogenous SAA1 gene has a cytokine-driven transcriptional disadvantage that is superseded by a marginal transcriptional advantage when glucocorticoids are present.

Gonzalez-Munoz, A. L., A.-F. Le Rolle, et al. (2003). "A Novel Instance of Class I Modification (cim) Affecting Two of Three Rat Class I RT1-A Molecules Within One MHC Haplotype." J. Immunol. **171**(1): 274-284.

http://www.jimmunol.org/cgi/content/abstract/171/1/274

MHC class I expression by rats of the RT1o, RT1d, and RT1m MHC haplotypes was investigated. Identical, functional cDNAs were obtained from RT1o and BDIX (RT1dv1) rats for three MHC class I molecules. RT1-A1o/d and -A2o/d are closely related in sequence to other cloned rat class Ia genes that have been shown to map to the RT1-A region, while RT1-A3{degrees} is highly homologous to a class I gene identified by sequencing an RT1-An genomic contig and is named A3n. Detailed analysis of the three molecules was undertaken using serology with mAbs, two-dimensional gel analysis of immunoprecipitates, and killing assays using cytotoxic T cells. Arguments are presented suggesting that A1{degrees} is the principal MHC class Ia (classical) restricting element of this haplotype. A2{degrees}, which is highly cross-reactive with A1{degrees}, and A3{degrees} probably play more minor or distinct roles in Ag presentation. Unexpectedly, cDNAs encoding exactly the same three molecules were cloned from rats of the RT1m haplotype, an MHC that until now was thought to possess unique class Ia genes. RT1m contains the TAP-B allele of the TAP transporter, and we present evidence that functional polymorphism in rat TAP has an even greater impact on the expression of RT1-A1{degrees} and -A2{degrees} than it does on RT1-Aa in the established case of class I modification (cim). Historically, this led to the misclassification of RT1m class Ia molecules as separate and distinct.

Hug, A., M. Korporal, et al. (2003). "Thymic Export Function and T Cell Homeostasis in Patients with Relapsing Remitting Multiple Sclerosis." J. Immunol. **171**(1): 432-437.

http://www.jimmunol.org/cgi/content/abstract/171/1/432

Multiple sclerosis (MS) is an inflammatory and possibly autoimmune mediated demyelinating disease of the CNS. Autoimmunity within the CNS may be triggered by dysfunction of peripheral immune tolerance mechanisms via changes in the homeostatic composition of peripheral T cells. We have assessed the release of naive T lymphocytes from the thymus in patients with relapsing remitting MS (RRMS) to identify alterations in the equilibrium of the peripheral T cell compartment. Thymic T cell production was estimated by measuring TCR excision circles (TRECs) as a traceable molecular marker in recent thymic emigrants. A total of 46 treatment-naive patients with active RRMS and 49 gender- and age-matched healthy persons were included in the study. The levels of TREC-expressing CD4+ and CD8+ T lymphocytes were significantly decreased in MS patients, and TREC quantities overall matched those of 30 years older healthy individuals. The average concentrations of TRECs/106 CD4+ and CD8+ T lymphocytes derived from MS patients and healthy donors were 26 x 103/106 and 28 x 103/106 vs 217 x 103/106 and 169 x 103/106, respectively. To account for any influence of T cell proliferation on TREC levels, we assayed T lymphocytes from additional patients with MS and normal individuals for telomere length (n = 20) and telomerase activity (8 MS patients, 16 controls), respectively. There were no significant differences between CD4+ and CD8+ T cells from MS patients and controls. Altogether, our findings suggest that an impaired thymic export function and, as a consequence, altered ability to maintain T cell homeostasis and immune tolerance may play an important pathogenic role in RRMS.

Teusch, N., E. Lombardo, et al. (2004). "The Low Molecular Weight GTPase RhoA and Atypical Protein

Kinase C{zeta} Are Required for TLR2-Mediated Gene Transcription." J. Immunol. **173**(1): 507-514.

http://www.jimmunol.org/cgi/content/abstract/173/1/507

The Rho GTPases are molecular switches that regulate many essential cellular processes, including actin dynamics, gene transcription, cell cycle progression, cell adhesion, and motility. In this study, we report that stimulation of TLR2 in human epithelial and monocytic cells leads to rapid and transient activation of RhoA. RhoA cooperated with the canonical I-{kappa}B kinase-mediated pathway that induces the release of NF-{kappa}B, in regulating the trans activation of the NF-{kappa}B subunit p65/RelA by affecting Ser311 phosphorylation, and subsequent cytokine production. Another consequence of TLR2 stimulation by bacterial derived products was the activation of atypical protein kinase C (PKC) {zeta} and association of this protein kinase with RhoA. Inhibition of PKC{zeta} decreased NF-{kappa}B activation and p65/RelA trans activation without affecting I-{kappa}B{alpha} degradation. The observation of a transient, stimulus-dependent association of RhoA with PKC{zeta} suggests that RhoA mediates at least partially its effect on gene transcription through atypical PKC. In contrast to previous studies, identifying Rac1-PI3K as an upstream element in TLR2-initiated response to NF-{kappa}B, PI3K signaling was not required for RhoA or PKC{zeta} activity. These results indicate that multiple GTPase-regulated pathways emerge from stimulated Toll receptors, controlling different aspects of NF-{kappa}B-mediated gene transcription.

Wicker, L. S., G. Chamberlain, et al. (2004). "Fine Mapping, Gene Content, Comparative Sequencing, and Expression Analyses Support Ctla4 and Nramp1 as Candidates for Idd5.1 and Idd5.2 in the Nonobese Diabetic Mouse." J. Immunol. **173**(1): 164-173.

http://www.jimmunol.org/cgi/content/abstract/173/1/164

At least two loci that determine susceptibility to type 1 diabetes in the NOD mouse have been mapped to chromosome 1, Idd5.1 (insulin-dependent diabetes 5.1) and Idd5.2. In this study, using a series of novel NOD.B10 congenic strains, Idd5.1 has been defined to a 2.1-Mb region containing only four genes, Ctla4, Icos, Als2cr19, and Nrp2 (neuropilin-2), thereby excluding a major candidate gene, Cd28. Genomic sequence comparison of the two functional candidate genes, Ctla4 and Icos, from the B6 (resistant at Idd5.1) and the NOD (susceptible at Idd5.1) strains revealed 62 single nucleotide polymorphisms (SNPs), only two of which were in coding regions. One of these coding SNPs, base 77 of Ctla4 exon 2, is a synonymous SNP and has been correlated previously with type 1 diabetes susceptibility and differential expression of a CTLA-4 isoform. Additional expression studies in this work support the hypothesis that this SNP in exon 2 is the genetic variation causing the biological effects of Idd5.1. Analysis of additional congenic strains has also localized Idd5.2 to a small region (1.52 Mb) of chromosome 1, but in contrast to the Idd5.1 interval, Idd5.2 contains at least 45 genes. Notably, the Idd5.2 region still includes the functionally polymorphic Nramp1 gene. Future experiments to test the identity of Idd5.1 and Idd5.2 as Ctla4 and Nramp1, respectively, can now be justified using approaches to specifically alter or mimic the candidate causative SNPs.

Zegarra-Moran, O., C. Folli, et al. (2004). "Double Mechanism for Apical Tryptophan Depletion in Polarized Human Bronchial Epithelium." J. Immunol. **173**(1): 542-549.

http://www.jimmunol.org/cgi/content/abstract/173/1/542

Indoleamine 2,3-dioxygenase is an enzyme that catabolizes tryptophan to kynurenine. We

investigated the consequences of IDO induction by IFN-{gamma} in polarized human bronchial epithelium. IDO mRNA expression was undetectable in resting conditions, but strongly induced by IFN-{gamma}. We determined the concentration of tryptophan and kynurenine in the extracellular medium, and we found that apical tryptophan concentration was lower than the basolateral in resting cells. IFN-{gamma} caused a decrease in tryptophan concentration on both sides of the epithelium. Kynurenine was absent in control conditions, but increased in the basolateral medium after IFN-{gamma} treatment. The asymmetric distribution of tryptophan and kynurenine suggested the presence of a transepithelial amino acid transport. Uptake experiments with radiolabeled amino acids demonstrated the presence of a Na+-dependent amino acid transporter with broad specificity that was responsible for the tryptophan/kynurenine transport. We confirmed these data by measuring the short-circuit currents elicited by direct application of tryptophan or kynurenine to the apical surface. The rate of amino acid transport was dependent on the transepithelial potential, and we established that in cystic fibrosis epithelia, in which the transepithelial potential is significantly more negative than in noncystic fibrosis epithelia, amino acid uptake was reduced. This work suggests that human airway epithelial cells maintain low apical tryptophan concentrations by two mechanisms, a removal through a Na+-dependent amino acid transporter and an IFN-{gamma}-inducible degradation by IDO.

Ryan, A. F., R. L. Grendell, et al. (2002). "A Soluble Isoform of the Rhesus Monkey Nonclassical MHC Class I Molecule Mamu-AG Is Expressed in the Placenta and the Testis." J. Immunol. **169**(2): 673-683.

http://www.jimmunol.org/cgi/content/abstract/169/2/673

The nonclassical MHC class I locus HLA-G is expressed primarily in the placenta, although other sites of expression have been noted in normal and pathological situations. In addition, soluble HLA-G isoforms have been detected in the serum of pregnant and nonpregnant women as well as men. The rhesus monkey placenta expresses a novel nonclassical MHC class I molecule Mamu-AG, which has features remarkably similar to those of HLA-G. We determined that the rhesus placenta expresses Mamu-AG mRNA (Mamu-AG5), retaining intron 4 as previously noted in HLA-G5. Immunostaining experiments with Ab 16G1 against the soluble HLA-G5 intron 4 peptide demonstrated that an immunoreactive protein(s) was present in the syncytiotrophoblasts of the chorionic villi of the rhesus placenta, within villous cytotrophoblasts, and occasionally within cells of the villous stroma. The Mamu-AG5 mRNA was readily detected in rhesus testis (although not in ejaculated sperm). Whereas an Ab against membrane-bound Mamu-AG stained few cells, primarily in the interstitium of the testis, there was consistent immunostaining for Mamu-AG5 in cells within the seminiferous tubules, which was corroborated by localization of Mamu-AG mRNA by in situ hybridization. While primary spermatocytes were negative, Sertoli cells, spermatocytes, and spermatids were consistently positive for 16G1 immunostaining. The specific recognition of the soluble Mamu-AG isoform was confirmed by Western blotting of Mamu-AG5 expressed in heterologous cells. The results demonstrate that a soluble nonclassical MHC class I molecule is expressed in the rhesus monkey placenta and testis, and confirm and extend the unique homology between HLA-G and the rhesus nonclassical molecule Mamu-AG.

Ioannidis, V., B. Kunz, et al. (2003). "Initiation and Limitation of Ly-49A NK Cell Receptor Acquisition by T Cell Factor-1." J. Immunol. **171**(2): 769-775.

http://www.jimmunol.org/cgi/content/abstract/171/2/769

The establishment of clonally variable expression of MHC class I-specific receptors by NK cells is not well understood. The Ly-49A receptor is used by {approx}20% of NK cells, whereby most cells express either the maternal or paternal allele and few express simultaneously both alleles.

We have previously shown that NK cells expressing Ly-49A were reduced or almost absent in mice harboring a single or no functional allele of the transcription factor T cell factor-1 (TCF-1), respectively. In this study, we show that enforced expression of TCF-1 in transgenic mice yields an expanded Ly-49A subset. Even though the frequencies of Ly-49A+ NK cells varied as a function of the TCF-1 dosage, the relative abundance of mono- and biallelic Ly-49A cells was maintained. Mono- and biallelic Ly-49A NK cells were also observed in mice expressing exclusively a transgenic TCF-1, i.e., expressing a fixed amount of TCF-1 in all NK cells. These findings suggest that Ly-49A acquisition is a stochastic event due to limiting TCF-1 availability, rather than the consequence of clonally variable expression of the endogenous TCF-1 locus. Efficient Ly-49A acquisition depended on the expression of a TCF-1 isoform, which included a domain known to associate with the TCF-1 coactivator {beta}-catenin. Indeed, the proximal Ly-49A promoter was {beta}-catenin responsive in reporter gene assays. We thus propose that Ly-49A receptor expression is induced from a single allele in occasional NK cells due to a limitation in the amount of a transcription factor complex requiring TCF-1.

Kim, H., J. M. Suh, et al. (2003). "Thyrotropin-Mediated Repression of Class II Trans-Activator Expression in Thyroid Cells: Involvement of STAT3 and Suppressor of Cytokine Signaling." J. Immunol. **171**(2): 616-627.

http://www.jimmunol.org/cgi/content/abstract/171/2/616

It has been suggested that class I and class II MHC are contributing factors for numerous diseases including autoimmune thyroid diseases, type 1 diabetes, rheumatoid arthritis, Alzheimer's disease, and multiple sclerosis. The class II trans-activator (CIITA), which is a non-DNA-binding regulator of class II MHC transcription, regulates the constitutive and inducible expression of the class I and class II genes. FRTL-5 thyroid cells incubated in the presence of IFN-{gamma} have a significantly higher level of cell surface rat MHC class II RTI.B. However, the IFN-{gamma}-induced RT1.B expression was suppressed significantly in cells incubated in the presence of thyrotropin. Thyrotropin (TSH) represses IFN-{gamma}-induced CIITA expression by inhibiting type IV CIITA promoter activity through the suppression of STAT1 activation and IFN regulatory factor 1 induction. This study found that TSH induces transcriptional activation of the STAT3 gene through the phosphorylation of STAT3 and CREB activation. TSH induces SOCS-1 and SOCS-3, and TSH-mediated SOCS-3 induction was dependent on STAT3. The cell line stably expressing the wild-type STAT3 showed a higher CIITA induction in response to IFN-{gamma} and also exhibited TSH repression of the IFN-{gamma}-mediated induction of CIITA. However, TSH repression of the IFN-{gamma}-induced CIITA expression was not observed in FRTL-5 thyroid cells, which stably expresses the dominant negative forms of STAT3, STAT3-Y705F, and STAT3-S727A. This report suggests that TSH is also engaged in immunomodulation through signal cross-talk with the cytokines in thyroid cells.

Lafont, B. A. P., A. Buckler-White, et al. (2003). "Characterization of Pig-Tailed Macaque Classical MHC Class I Genes: Implications for MHC Evolution and Antigen Presentation in Macaques." J. Immunol. **171**(2): 875-885.

http://www.jimmunol.org/cgi/content/abstract/171/2/875

MHC-dependent CD8+ T cell responses have been associated with control of viral replication and slower disease progression during lentiviral infections. Pig-tailed macaques (Macaca nemestrina) and rhesus monkeys (Macaca mulatta), two nonhuman primate species commonly used to model HIV infection, can exhibit distinct clinical courses after infection with different primate lentiviruses. As an initial step in assessing the role of MHC class I restricted immune responses to these infections, we have cloned and characterized classical MHC class I genes of pig-tailed macaques

and have identified 19 MHC class I alleles (Mane) orthologous to rhesus macaque MHC-A, -B, and -I genes. Both Mane-A and Mane-B loci were found to be duplicated, and no MHC-C locus was detected. Pig-tailed and rhesus macaque MHC-A alleles form two groups, as defined by 14 polymorphisms affecting mainly their B peptide-binding pockets. Furthermore, an analysis of multiple pig-tailed monkeys revealed the existence of three MHC-A haplotypes. The distribution of these haplotypes in various Old World monkeys provides new insights about MHC-A evolution in nonhuman primates. An examination of B and F peptide-binding pockets in rhesus and pig-tailed macaques suggests that their MHC-B molecules present few common peptides to their respective CTLs.

Chen, Y., S. S. Yan, et al. (2004). "Blockade of Late Stages of Autoimmune Diabetes by Inhibition of the Receptor for Advanced Glycation End Products." J. Immunol. **173**(2): 1399-1405.

http://www.jimmunol.org/cgi/content/abstract/173/2/1399

Ligation of the receptor for advanced glycation end products (RAGE) occurs during inflammation. Engagement of RAGE results in enhanced expression of addressins and it is therefore, not surprising that previous studies have shown a role of RAGE/ligand interactions in immune responses including cell/cell contact but the role of RAGE in spontaneous autoimmunity has not been clearly defined. To study the role of RAGE/ligand interactions in autoimmune diabetes, we tested the ability of soluble RAGE, a scavenger of RAGE ligands, in late stages of diabetes development in the NOD mouse-disease transferred with diabetogenic T cells and recurrent disease in NOD/scid recipients of syngeneic islet transplants. RAGE expression was detected on CD4+, CD8+, and B cells from diabetic mice and transferred to NOD/scid recipients. RAGE and its ligand, S100B, were found in the islets of NOD/scid mice that developed diabetes. Treatment of recipient NOD/scid mice with soluble RAGE prevented transfer of diabetes and delayed recurrent disease in syngeneic islet transplants. RAGE blockade was associated with increased expression of IL-10 and TGF-{beta} in the islets from protected mice. RAGE blockade reduced the transfer of disease with enriched T cells, but had no effect when diabetes was transferred with the activated CD4+ T cell clone, BDC2.5. We conclude that RAGE/ligand interactions are involved in the differentiation of T cells to a mature pathogenic phenotype during the late stages of the development of diabetes.

Yu, C.-R., R. M. Mahdi, et al. (2004). "Cell Proliferation and STAT6 Pathways Are Negatively Regulated in T Cells by STAT1 and Suppressors of Cytokine Signaling." J. Immunol. **173**(2): 737-746.

http://www.jimmunol.org/cgi/content/abstract/173/2/737

Suppressor of cytokine signaling (SOCS) proteins have emerged as important regulators of cytokine signals in lymphocytes. In this study, we have investigated regulation of SOCS expression and their role in Th cell growth and differentiation. We show that SOCS genes are constitutively expressed in naive Th cells, albeit at low levels, and are differentially induced by Ag and Th-polarizing cytokines. Whereas cytokines up-regulate expression of SOCS1, SOCS2, SOCS3, and cytokine-induced Src homology 2 protein, Ags induce down-regulation of SOCS3 within 48 h of Th cell activation and concomitantly up-regulate SOCS1, SOCS2, and cytokine-induced Src homology 2 protein expression. We further show that STAT1 signals play major roles in inducing SOCS expression in Th cells and that induction of SOCS expression by IL-4, IL-12, or IFN-{gamma} is compromised in STAT1-deficient primary Th cells. Surprisingly, IL-4 is a potent inducer of STAT1 activation in Th2 but not Th1 cells, and SOCS1 or SOCS3 expression is dramatically reduced in STAT1-/- Th2 cells. To our knowledge, this is the first report of IL-4-induced STAT1 activation in Th cells, and suggests that its induction of SOCS, may in part, regulate IL-4 functions in Th2 cells. In fact, overexpression of SOCS1 in Th2 cells represses

STAT6 activation and profoundly inhibits IL-4-induced proliferation, while depletion of SOCS1 by an anti-sense SOCS1 cDNA construct enhances cell proliferation and induces constitutive activation of STAT6 in Th2 cells. These results are consistent with a model where IL-4 has dual effects on differentiating T cells: it simulates proliferation/differentiation through STAT6 and autoregulates its effects on Th2 growth and effector functions via STAT1-dependent up-regulation of SOCS proteins.

Jaatinen, T., M. Eholuoto, et al. (2002). "Characterization of a De Novo Conversion in Human Complement C4 Gene Producing a C4B5-Like Protein." J. Immunol. **168**(11): 5652-5658.

http://www.jimmunol.org/cgi/content/abstract/168/11/5652

Complement C4 is a highly polymorphic protein essential for the activation of the classical complement pathway. Most of the allelic variation of C4 resides in the C4d region. Four polymorphic amino acid residues specify the isotype and an additional four specify the Rodgers and Chido determinants of the protein. Rare C4 allotypes have been postulated to originate from recombination between highly homologous C4 genes through gene conversions. Here we describe the development of a de novo C4 hybrid protein with allotypic and antigenic diversity resulting from nonhomologous intra or interchromosomal recombination of the maternal chromosomes. A conversion was observed between maternal C4A3a and C4B1b genes producing a functional hybrid gene in one of the children. The codons determining the isotype, Asp1054, Leu1101, Ser1102, Ile1105 and His1106, were characteristic of C4B gene, whereas the polymorphic sites in exon and intron 28 were indicative of C4A3a sequence. The protein produced by this hybrid gene was electrophoretically similar to C4B5 allotype. It also possesses reversed antigenicity being Rodgers 1, 2, 3 and Chido-1, -2, -3, 4, -5, and -6. Our case describes the development of a rare bimodular C4B-C4B haplotype containing a functional de novo C4 hybrid gene arisen through gene conversion from C4A to C4B. Overall the data supports the hypothesis of gene conversions as an ongoing process increasing allelic diversity in the C4 locus.

Abraham, E., M. R. Gyetko, et al. (2003). "Urokinase-Type Plasminogen Activator Potentiates Lipopolysaccharide-Induced Neutrophil Activation." J. Immunol. **170**(11): 5644-5651.

http://www.jimmunol.org/cgi/content/abstract/170/11/5644

Urokinase plasminogen activator (uPA) is a serine protease that catalyzes the conversion of plasminogen to plasmin. Although increased circulating levels of uPA are present in endotoxemia and sepsis, conditions in which activated neutrophils contribute to the development of acute organ dysfunction, the ability of uPA to participate directly in LPS-induced neutrophil activation has not been examined. In the present experiments, we show that uPA can enhance activation of neutrophils exposed to submaximal stimulatory doses of LPS. In particular, uPA increased LPS-induced activation of intracellular signaling pathways, including Akt and c-Jun N-terminal kinase, nuclear translocation of the transcriptional regulatory factor NF-{kappa}B, and expression of proinflammatory cytokines, including IL-1{beta}, macrophage-inflammatory protein-2, and TNF-{alpha}. There was no effect of uPA on LPS-induced activation of p38 mitogen-activated protein kinase in neutrophils. Transgenic mice unable to produce uPA (uPA-/-) were protected from endotoxemia-induced lung injury, as determined by development of lung edema, pulmonary neutrophil accumulation, lung IL-1{beta}, macrophage-inflammatory protein-2, and TNF-{alpha} cytokine levels. These results demonstrate that uPA can potentiate LPS-induced neutrophil responses and also suggest that such effects are sufficiently important in vivo to play a major contributory role in neutrophil-mediated inflammatory responses, such as the development of acute lung injury.

Anders, H.-J., M. Frink, et al. (2003). "CC Chemokine Ligand 5/RANTES Chemokine Antagonists Aggravate Glomerulonephritis Despite Reduction of Glomerular Leukocyte Infiltration." J. Immunol. **170**(11): 5658-5666.

http://www.jimmunol.org/cgi/content/abstract/170/11/5658

The chemokine CC chemokine ligand (CCL)5/RANTES as well as its respective receptor CCR5 mediate leukocyte infiltration during inflammation and are up-regulated early during the course of glomerulonephritis (GN). We tested the effects of the two CCL5/RANTES blocking analogs, Met-RANTES and amino-oxypentane-RANTES, on the course of horse apoferritin (HAF)-induced GN. HAF-injected control mice had proliferative GN with mesangial immune complex deposits of IgG and HAF. Daily i.p. injections of Met-RANTES or amino-oxypentane-RANTES markedly reduced glomerular cell proliferation and glomerular macrophage infiltration, which is usually associated with less glomerular injury and proteinuria in HAF-GN. Surprisingly, however, HAF-GN mice treated with both analogs showed worse disease with mesangiolysis, capillary obstruction, and nephrotic range albuminuria. These findings were associated with an enhancing effect of the CCL5/RANTES analogs on the macrophage activation state, characterized by a distinct morphology and increased inducible NO synthetase expression in vitro and in vivo, but a reduced uptake of apoptotic cells in vivo. The humoral response and the Th1/Th2 balance in HAF-GN and mesangial cell proliferation in vitro were not affected by the CCL5/RANTES analogs. We conclude that, despite blocking local leukocyte recruitment, chemokine analogs can aggravate some specific disease models, most likely due to interactions with systemic immune reactions, including the removal of apoptotic cells and inducible NO synthetase expression.

Gasche, C., P. Grundtner, et al. (2003). "Novel Variants of the IL-10 Receptor 1 Affect Inhibition of Monocyte TNF-{alpha} Production." J. Immunol. **170**(11): 5578-5582.

http://www.jimmunol.org/cgi/content/abstract/170/11/5578

IL-10-deficient mice exhibit spontaneous enterocolitis and other symptoms akin to Crohn's disease, indicating that IL-10 might regulate normal physiology in the gut. However, clinical trials with IL-10 in Crohn's disease were disappointing, although some patients showed healing of intestinal mucosa. This study searched for genetic polymorphisms within the IL-10 pathway. We decided to screen for mutations of the IL-10R1 cDNA in healthy volunteers and Crohn's disease patients and identified two novel variants: a serine 138-to-glycine (S138G) and a glycine 330-to-arginine (G330R) substitution. The allelic frequency in a European cohort was relatively high (16% for the S138G and 33% for the G330R), and S138G was in strong linkage disequilibrium with G330R. A similar allele frequency was found in a group of Crohn's patients. In IL-10R1 G330R-expressing monocytes, the inhibitory effect of IL-10 on TNF-{alpha} production was diminished, indicating that this variant may be a loss-of-function allele. No such difference was observed between haplotypes 4 (G330R only) and 7 (S138G and G330R). In addition, these IL-10R1 variants had no influence on the IL-10R1 expression density. Structural analysis of the S138G variant revealed that the substitution of S138G may interfere with binding of IL-10 to IL-10R1.

Hewison, M., L. Freeman, et al. (2003). "Differential Regulation of Vitamin D Receptor and Its Ligand in Human Monocyte-Derived Dendritic Cells." J. Immunol. **170**(11): 5382-5390.

http://www.jimmunol.org/cgi/content/abstract/170/11/5382

The functions of dendritic cells (DCs) are tightly regulated such that protective immune responses are elicited and unwanted immune responses are prevented. 1{alpha}25-dihydroxyvitamin D3 (1{alpha}25(OH)2D3) has been identified as a major factor that inhibits the differentiation and maturation of DCs, an effect dependent upon its binding to the nuclear vitamin D receptor (VDR). Physiological control of 1{alpha}25(OH)2D3 levels is critically dependent upon 25-hydroxyvitamin D3-1{alpha}-hydroxylase (1{alpha}OHase), a mitochondrial cytochrome P450 enzyme that catalyzes the conversion of inactive precursor 25-hydroxyvitamin D3 (25(OH)D3) to the active metabolite 1{alpha}25(OH)2D3. Using a human monocyte-derived DC (moDC) model, we have examined the relationship between DC VDR expression and the impact of exposure to its ligand, 1{alpha}25(OH)2D3. We show for the first time that moDCs are able to synthesize 1{alpha}25(OH)2D3 in vitro as a consequence of increased 1{alpha}OHase expression. Following terminal differentiation induced by a diverse set of maturation stimuli, there is marked transcriptional up-regulation of 1{alpha}OHase leading to increased 1{alpha}OHase enzyme activity. Consistent with this finding is the observation that the development and function of moDCs is inhibited at physiological concentrations of the inactive metabolite 25(OH)D3. In contrast to 1{alpha}OHase, VDR expression is down-regulated as monocytes differentiate into immature DCs. Addition of 1{alpha}25(OH)2D3 to moDC cultures at different time points indicates that its inhibitory effects are greater in monocyte precursors than in immature DCs. In conclusion, differential regulation of endogenous 1{alpha}25(OH)2D3 ligand and its nuclear receptor appear to be important regulators of DC biology and represent potential targets for the manipulation of DC function.

LaBonte, M. L., E. I. Choi, et al. (2004). "Molecular Determinants Regulating the Pairing of NKG2 Molecules with CD94 for Cell Surface Heterodimer Expression." J. Immunol. **172**(11): 6902-6912.

http://www.jimmunol.org/cgi/content/abstract/172/11/6902

The lytic capacity of a NK cell is regulated, in part, by the balance in cell surface expression between inhibitory CD94/NKG2A and activating CD94/NKG2C heterodimers. We demonstrate that, in the absence of DAP12, rhesus monkey NKG2A is preferentially expressed at the cell surface with CD94 due to a single amino acid difference in the transmembrane of NKG2A and NKG2C. Furthermore, in the context of an NKG2A transmembrane, the stalk domain of NKG2C was found to enhance heterodimer formation with CD94 compared with the stalk domain of NKG2A. In the presence of DAP12, the ability of NKG2C to compete for cell surface CD94 heterodimerization is enhanced and approaches that of NKG2A. Finally, allelic differences that affect the ability of rhesus NKG2A to reach the cell surface with CD94 could also be mapped to the transmembrane. These differences in the ability of inhibitory and activating NKG2 molecules to reach the cell surface provide a mechanism for the regulation of NK cell activity.

Shiina, T., S. Shimizu, et al. (2004). "Comparative Genomic Analysis of Two Avian (Quail and Chicken) MHC Regions." J. Immunol. **172**(11): 6751-6763.

http://www.jimmunol.org/cgi/content/abstract/172/11/6751

We mapped two different quail Mhc haplotypes and sequenced one of them (haplotype A) for comparative genomic analysis with a previously sequenced haplotype of the chicken Mhc. The quail haplotype A spans 180 kb of genomic sequence, encoding a total of 41 genes compared with only 19 genes within the 92-kb chicken Mhc. Except for two gene families (B30 and tRNA), both species have the same basic set of gene family members that were previously described in the chicken "minimal essential" Mhc. The two Mhc regions have a similar overall organization but differ markedly in that the quail has an expanded number of duplicated genes with 7 class I, 10 class IIB, 4 NK, 6 lectin, and 8 B-G genes. Comparisons between the quail and chicken Mhc

class I and class II gene sequences by phylogenetic analysis showed that they were more closely related within species than between species, suggesting that the quail Mhc genes were duplicated after the separation of these two species from their common ancestor. The proteins encoded by the NK and class I genes are known to interact as ligands and receptors, but unlike in the quail and the chicken, the genes encoding these proteins in mammals are found on different chromosomes. The finding of NK-like genes in the quail Mhc strongly suggests an evolutionary connection between the NK C-type lectin-like superfamily and the Mhc, providing support for future studies on the NK, lectin, class I, and class II interaction in birds.

Swanson, P. J., S. L. Kuslak, et al. (2004). "Fatal Acute Lymphoblastic Leukemia in Mice Transgenic for B Cell-Restricted bcl-xL and c-myc." J. Immunol. **172**(11): 6684-6691.

http://www.jimmunol.org/cgi/content/abstract/172/11/6684

Expression of the c-myc gene is frequently dysregulated in malignant tumors and translocations of c-myc into the Ig H chain locus are associated with Burkitt's-type lymphoma. There is indirect evidence that bcl-x, an anti-apoptotic member of the bcl-2 gene family, may also contribute to a variety of B lymphoid tumors. In this study, we show that mice transgenic for both B cell-restricted c-myc and bcl-xL developed aggressive, acute leukemias expressing early B lineage and stem cell surface markers. Of interest, the tumor cells proliferated and differentiated down the B cell developmental pathway following in vitro treatment with IL-7. Analysis of sorted leukemic cells from spleen indicated constitutive expression of sterile {micro} and {kappa} transcripts in combination with evidence for D-JH DNA rearrangements. Several B cell-specific genes were either not expressed or were expressed at low levels in primary tumor cells and were induced following culture with IL-7. IL-7 also increased V-J{kappa} and V-DJH rearrangements. These data demonstrate oncogenic synergy between c-myc and bcl-xL in a new mouse model for acute lymphoblastic leukemia. Tumors in these animals target an early stage in B cell development characterized by the expression of both B lineage and stem cell genes.

De Creus, A., K. Van Beneden, et al. (2002). "Developmental and Functional Defects of Thymic and Epidermal V{gamma}3 Cells in IL-15-Deficient and IFN Regulatory Factor-1-Deficient Mice." J. Immunol. **168**(12): 6486-6493.

http://www.jimmunol.org/cgi/content/abstract/168/12/6486

In this study, the role of IL-15 and its regulation by the transcription factor IFN regulatory factor-1 (IRF-1) in murine V{gamma}3 T cell development and activity is assessed. Compared with wild-type (WT) mice, reduced numbers of mature V{gamma}3 cells were found in the fetal thymus of IL-15-/- mice, while IRF-1-/- mice displayed normal frequencies. V{gamma}3+ dendritic epidermal T cells (DETCs) were absent in IL-15-/- mice but present in IRF-1-/- mice. DETCs from IRF-1-/- mice displayed morphologically a less mature phenotype and showed different emergence kinetics during ontogeny. This corresponded with lower IL-15 mRNA levels in the skin epidermis. Comparable levels of IL-7 were found in the skin of WT and IL-15-/- mice. Adoptive transfer experiments of WT fetal thymocytes into IL-15-/- mice did not result in the development of V{gamma}3+ DETCs, confirming the nonredundant role of IL-15 in the skin during DETC development. In vitro, cytolytic activity of IL-15-/- V{gamma}3 cells was normal after stimulation with IL-15 and was further enhanced by addition of IL-12. In contrast, cytolytic activity of IRF-1-/- V{gamma}3 cells remained defective after stimulation with IL-15 in combination with IL-12. These data suggest that IL-15 is redundant for the development and/or survival of mature V{gamma}3 cells in the fetal thymus, whereas it is essential for the localization of V{gamma}3 cells in the skin. Furthermore, a possible role for IRF-1 in inducing morphological maturation of DETCs and cytolytic capacity of V{gamma}3 cells is suggested.

Meusel, T. R., K. E. Kehoe, et al. (2002). "Protein Kinase R Regulates Double-Stranded RNA Induction of TNF-{alpha} But Not IL-1{beta} mRNA in Human Epithelial Cells." J. Immunol. **168**(12): 6429-6435.

http://www.jimmunol.org/cgi/content/abstract/168/12/6429

Epithelial cells represent the initial site of respiratory viral entry and the first line of defense against such infections. This early antiviral response is characterized by an increase in the production of proinflammatory cytokines such as TNF-{alpha} and IL-1{beta}. dsRNA, which is a common factor present during the life cycle of both DNA and RNA viruses, is known to induce TNF-{alpha} and IL-1{beta} in a variety of cells. In this work we provide data showing that dsRNA treatment induces TNF-{alpha} and IL-1{beta} in human lung epithelial cells via two different mechanisms. Our data show that dsRNA activation of dsRNA-activated protein kinase (PKR) is associated with induction of TNF-{alpha} but not IL-1{beta} expression. An inhibitor of PKR activation blocked the dsRNA-induced elevations in TNF-{alpha} but not IL-1{beta} mRNA in epithelial cells. Data obtained from infection of epithelial cells with a vaccinia virus lacking the PKR inhibitory polypeptide, E3L, revealed that PKR activation was essential for TNF-{alpha} but not for IL-1{beta} expression. In this report, we provide experimental support for the differential regulation of proinflammatory cytokine expression by dsRNA and viral infections in human airway epithelial cells.

Seiffert, K., J. Hosoi, et al. (2002). "Catecholamines Inhibit the Antigen-Presenting Capability of Epidermal Langerhans Cells." J. Immunol. **168**(12): 6128-6135.

http://www.jimmunol.org/cgi/content/abstract/168/12/6128

The sympathetic nervous system modulates immune function at a number of levels. Within the epidermis, APCs (Langerhans cells (LC)) are frequently anatomically associated with peripheral nerves. Furthermore, some neuropeptides have been shown to regulate LC Ag-presenting function. We explored the expression of adrenergic receptors (AR) in murine LC and assessed their functional role on Ag presentation and modulation of cutaneous immune responses. Both purified LC and the LC-like cell lines XS52-4D and XS106 expressed mRNA for the ARs {alpha}1A and {beta}2. XS106 cells and purified LC also expressed {beta}1-AR mRNA. Treatment of murine epidermal cell preparations with epinephrine (EPI) or norepinephrine inhibited Ag presentation in vitro. Furthermore, pretreatment of epidermal cells with EPI or norepinephrine in vitro suppressed the ability of these cells to present Ag for elicitation of delayed-type hypersensitivity in previously immunized mice. This effect was blocked by use of the {beta}2-adrenergic antagonist ICI 118,551 but not by the {alpha}-antagonist phentolamine. Local intradermal injection of EPI inhibited the induction of contact hypersensitivity to epicutaneously administered haptens. Surprisingly, injection of EPI at a distant site also suppressed induction of contact hypersensitivity. Thus, catecholamines may have both local and systemic effects. We conclude that specific ARs are expressed on LC and that signaling through these receptors can decrease epidermal immune reactions.

Shin, J.-S., J. Yu, et al. (2002). "Peptide Mimotopes of Pneumococcal Capsular Polysaccharide of 6B Serotype: A Peptide Mimotope Can Bind to Two Unrelated Antibodies." J. Immunol. **168**(12): 6273-6278.

http://www.jimmunol.org/cgi/content/abstract/168/12/6273

Two groups of bacteriophage clones displaying the antigenic properties of serotype 6B pneumococcal capsular polysaccharide (PS) were obtained from different phage libraries expressing random heptameric peptides. One group, biopanned with a mouse mAb (Hyp6BM1), is comprised of 17 phage clones expressing 10 unique sequences of linear peptides. The other group, selected with another mAb (Hyp6BM8), contained six clones, all of which expressed the identical circular peptide. Phage clones expressing the linear peptides (e.g., PhaM1L3) bound only to Hyp6BM1, but not other 6B PS-specific mAb, and their binding could be inhibited with pneumococcal capsular type 6B PS only. In contrast, a phage clone expressing the circular peptide (PhaM8C1) cross-reacted with several other 6B PS-specific mAbs, and their binding could be inhibited with pneumococcal capsular PS of 6A and 6B serotypes. Two short peptides, PepM1L3 and PepM8C1, reflecting the peptide inserts of the corresponding phage clones, could inhibit the binding of the two clones to their respective mAb. Interestingly, the peptide insert in PhaM8C1 was identical to that in PhaB3C4, a previously reported mimotope of {alpha}(2[-&gt]8) polysialic acid, Neisseria meningitidis group B PS. Indeed, PhaM8C1 bound to HmenB3 (a meningococcal Ab), and their association could be inhibited with {alpha}(2-8) polysialic acid, but not with 6B PS. Conversely, {alpha}(2-8) polysialic acid could not inhibit the binding of PhaM8C1 to Hyp6BM8. The two-dimensional nuclear magnetic resonance studies indicate that PepM8C1 peptide can assume several conformations in solution. The ability of this peptide to assume multiple conformations might account for its ability to mimic more than one Ag type.

Kohler, R. E., A. C. Caon, et al. (2003). "A Role for Macrophage Inflammatory Protein-3{alpha}/CC Chemokine Ligand 20 in Immune Priming During T Cell-Mediated Inflammation of the Central Nervous System." J. Immunol. **170**(12): 6298-6306.

http://www.jimmunol.org/cgi/content/abstract/170/12/6298

Chemokines are a family of cytokines that exhibit selective chemoattractant properties for target leukocytes and play a significant role in leukocyte migration. In this study, we have investigated the role of the C-C chemokine, macrophage inflammatory protein (MIP)-3{alpha}/CC chemokine ligand 20, in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), a model of T cell-dependent inflammation. Expression in the CNS of MIP-3{alpha}, as determined by RT-PCR, increased in a time-dependent manner such that peak expression correlated with peak clinical disease. Similarly, levels of immunoreactive MIP-3{alpha} in the draining lymph nodes increased up to 10-fold 9 days postimmunization and remained elevated for up to 21 days postimmunization. The increased production of MIP-3{alpha} coincided with onset of clinical disease. Treatment of mice with specific neutralizing anti-MIP-3{alpha} Abs significantly reduced the severity of both clinical EAE and neuroinflammation by inhibiting the sensitization of lymphocytes to the specific Ag and release of lymphocytes from the draining lymph nodes. In contrast, adoptive transfer experiments indicated that MIP-3{alpha} was not essential for the effector phase of EAE. Together, these data demonstrate that MIP-3{alpha} plays a critical role in the sensitization phase of EAE.

Miyakoshi, A., W. K. Yoon, et al. (2003). "Characterization of the Antigen Specificity and TCR Repertoire, and TCR-Based DNA Vaccine Therapy in Myelin Basic Protein-Induced Autoimmune Encephalomyelitis in DA Rats." J. Immunol. **170**(12): 6371-6378.

http://www.jimmunol.org/cgi/content/abstract/170/12/6371

Like Lewis rats, DA rats are an experimental autoimmune encephalomyelitis (EAE)-susceptible strain and develop severe EAE upon immunization with myelin basic protein (MBP). However, there are several differences between the two strains. In the present study we induced acute EAE in DA rats by immunization with MBP and MBP peptides and examined the Ag specificity and

TCR repertoire of encephalitogenic T cells. It was found that although immunization with MBP and a peptide corresponding to its 62-75 sequence (MBP62-75) induced clinical EAE, the responses of lymph node T cells isolated from MBP-immunized rats to MBP62-75 was marginal, indicating that this peptide contains major encephalitogenic, but not immunodominant, epitopes. The TCR analysis by CDR3 spectratyping of spinal cord T cells revealed that V{beta}10 and V{beta}15 spectratype expansion was always found in MBP62-75-immunized symptomatic rats. On the basis of these findings, we examined the encephalitogenicity of V{beta}10- and V{beta}15-positive T cells. First, the adoptive transfer experiments revealed that V{beta}10-positive T line cells derived from MBP62-75-immunized rats induced clinical EAE in recipients. Second, administration of DNA vaccines encoding V{beta}10 and V{beta}15, alone or in combination, ameliorated MBP62-75-induced EAE. Collectively, it was strongly suggested that V{beta}10- and V{beta}15-positive T cells are encephalitogenic. Analyses of the Ag specificity and T cell repertoire of pathogenic T cells performed in this study provide useful information for designing specific immunotherapies against autoimmune diseases.

Brooks, S. A., J. E. Connolly, et al. (2004). "The Role of mRNA Turnover in the Regulation of Tristetraprolin Expression: Evidence for an Extracellular Signal-Regulated Kinase-Specific, AU-Rich Element-Dependent, Autoregulatory Pathway." J. Immunol. **172**(12): 7263-7271.

http://www.jimmunol.org/cgi/content/abstract/172/12/7263

Tristetraprolin (TTP) is a regulator of TNF-{alpha} mRNA stability and is the only trans-acting factor shown to be capable of regulating AU-rich element-dependent mRNA turnover at the level of the intact animal. Using the THP-1 myelomonocytic cell line, we demonstrated for the first time that TTP is encoded by an mRNA with a short half-life under resting conditions. Using pharmacologic inhibitors of the mitogen-activated protein kinase pathways, we show that the induction of TTP by LPS activation is mediated through changes in transcription, mRNA stability, and translation. A coordinate increase in both TTP and TNF-{alpha} mRNA stability occurs within 15 min of LPS treatment, but is transduced through different mitogen-activated protein kinase pathways. This regulation of TTP and TNF-{alpha} mRNA stability is associated with the finding that TTP binds these mRNA under both resting and LPS-activated conditions in vivo. Finally, we demonstrate that TTP can regulate reporter gene expression in a TTP 3' untranslated region-dependent manner and identify three distinct AU-rich elements necessary to mediate this effect. Thus, TTP regulates its own expression in a manner identical to that seen with the TNF-{alpha} 3' untranslated region, indicating that this autoregulation is mediated at the level of mRNA stability. In this manner, TTP is able to limit the production of its own proteins as well as that of TNF-{alpha} and thus limit the response of the cell to LPS.

Hubert, F.-X., C. Voisine, et al. (2004). "Rat Plasmacytoid Dendritic Cells Are an Abundant Subset of MHC Class II+ CD4+CD11b-OX62- and Type I IFN-Producing Cells That Exhibit Selective Expression of Toll-Like Receptors 7 and 9 and Strong Responsiveness to CpG." J. Immunol. **172**(12): 7485-7494.

http://www.jimmunol.org/cgi/content/abstract/172/12/7485

We have identified in the rat a new subset of MHC class II+ CD4+CD3-CD11b- leukocytes that produce high amounts of type I IFN upon viral stimulation and that appeared homologous to plasmacytoid DC (pDC) previously described in humans and mice. These cells exhibited the following phenotype: CD5+,CD90+,CD45R+,CD45RC+,CD11c-,CD161a+,CD200+,CD172a+,CD32+,CD86+. Rat pDC did not express the DC-specific marker OX62 and were more abundant in the spleen than the classical CD4+ and CD4- subsets of OX62+CD11b+ DC we previously described that produced very little, if any, type I IFN. Spleen

pDC exhibited an undifferentiated morphology and rapidly died in vitro, but showed extensive dendrite formation, survival, maturation, and moderate type I IFN production upon stimulation by oligonucleotides containing type B CpG motifs (CpG ODN). Type A CpG ODN and CD40 ligand induced pDC to produce large amounts of type I IFN, but did not promote maturation. CpG ODN and CD40 ligand, but not influenza virus, induced IL-12p40 and IL-6 secretion. Spleen pDC did not produce IL-12p70, TNF-{alpha}, IL-1{beta}, or IL-10 using these stimulation conditions. Correlating with their strong responsiveness to virus and CpG ODN, rat pDC specifically expressed Toll-like receptor 7 and 9 mRNA. Fresh spleen pDC were poor stimulators of allogenic CD4+ and CD8+ T cells, but became potent inducers of allogenic T cell proliferation as well as Th1 differentiation after stimulation by type B CpG. Therefore, rat pDC appear very similar to human pDC, indicating that the specific phenotype and functions of pDC have been highly conserved between species.

Braun, M., M. Wunderlin, et al. (2002). "Xenopus laevis Stromal Cell-Derived Factor 1: Conservation of Structure and Function During Vertebrate Development." J. Immunol. **168**(5): 2340-2347.

http://www.jimmunol.org/cgi/content/abstract/168/5/2340

Transmembrane signaling of the CXC chemokine stromal cell-derived factor-1 (SDF-1) is mediated by CXCR4, a G protein-coupled receptor initially identified in leukocytes and shown to serve as a coreceptor for the entry of HIV into lymphocytes. Characterization of SDF-1- and CXCR4-deficient mice has revealed that SDF-1 and CXCR4 are of vital developmental importance. To study the role of the SDF-1/CXCR4-chemokine/receptor system as a regulator of vertebrate development, we isolated and characterized a cDNA encoding SDF-1 of the lower vertebrate Xenopus laevis (xSDF-1). Recombinant xSDF-1 was produced in insect cells, purified, and functionally characterized. Although xSDF-1 is only 64-66% identical with its mammalian counterparts, it is indistinguishable from human (h)SDF-1{alpha} in terms of activating both X. laevis CXCR4 and hCXCR4. Thus, both xSDF-1 and hSDF-1{alpha} promoted CXCR4-mediated activation of heterotrimeric Gi2 in a cell-free system and induced release of intracellular calcium ions in and chemotaxis of intact lymphoblastic cells. Analysis of the time course of xSDF-1 mRNA expression during Xenopus embryogenesis revealed a tightly coordinated regulation of xSDF-1 and X. laevis CXCR4. xSDF-1 mRNA was specifically detected in the developing CNS, incipient sensory organs, and the embryonic heart. In Xenopus, CXCR4 mRNA appears to be absent from the heart anlage, but present in neural crest cells. This observation suggests that xSDF-1 expressed in the heart anlage may attract cardiac neural crest cells expressing CXCR4 to migrate to the primordial heart to regulate both septation of the cardiac outflow tract and differentiation of the myocardium during early heart development.

Raynard, S. J., L. R. Read, et al. (2002). "Evidence for the Murine IgH {micro} Locus Acting as a Hot Spot for Intrachromosomal Homologous Recombination." J. Immunol. **168**(5): 2332-2339.

http://www.jimmunol.org/cgi/content/abstract/168/5/2332

Homologous recombination accomplishes the exchange of genetic information between two similar or identical DNA duplexes. It can occur either by gene conversion, a process of unidirectional genetic exchange, or by reciprocal crossing over. Homologous recombination is well known for its role in generating genetic diversity in meiosis and, in mitosis, as a DNA repair mechanism. In the immune system, the evidence suggests a role for homologous recombination in Ig gene evolution and in the diversification of Ab function. Previously, we reported the occurrence of homologous recombination between repeated, donor and recipient alleles of the Ig H chain {micro} gene C (C{micro}) region residing at the Ig {micro} locus in mouse hybridoma cells. In this study, we constructed mouse hybridoma cell lines bearing C{micro} region

heteroalleles to learn more about the intrachromosomal homologous recombination process. A high frequency of homologous recombination (gene conversion) was observed for markers spanning the entire recipient C{micro} region, suggesting that recombination might initiate at random sites within the C{micro} region. The C{micro} region heteroalleles were equally proficient as either conversion donors or recipients. Remarkably, when the same C{micro} heteroalleles were tested for recombination in ectopic genomic positions, the mean frequency of gene conversion was reduced by at least 65-fold. These results are consistent with the murine IgH {micro} locus behaving as a hot spot for intrachromosomal homologous recombination.

Shilling, H. G., L. A. Guethlein, et al. (2002). "Allelic Polymorphism Synergizes with Variable Gene Content to Individualize Human KIR Genotype." J. Immunol. **168**(5): 2307-2315.

http://www.jimmunol.org/cgi/content/abstract/168/5/2307

Killer Ig-like receptor (KIR) genes are a multigene family on human chromosome 19. KIR genes occur in various combinations on different haplotypes. Additionally, KIR genes are polymorphic. To examine how allelic polymorphism diversifies KIR haplotypes with similar or identical combinations of KIR genes, we devised methods for discriminating alleles of KIR2DL1, -2DL3, -3DL1, and -3DL2. These methods were applied to 143 individuals from 34 families to define 98 independent KIR haplotypes at the allele level. Three novel 3DL2 alleles and a chimeric 3DL1/3DL2 sequence were also identified. Among the A group haplotypes were 22 different combinations of 2DL1, 2DL3, 3DL1, and 3DL2 alleles. Among the B group haplotypes that were unambiguously determined were 15 distinct haplotypes involving 9 different combinations of KIR genes. A and B haplotypes both exhibit strong linkage disequilibrium (LD) between 2DL1 and 2DL3 alleles, and between 3DL1 and 3DL2 alleles. In contrast, there was little LD between the 2DL1/2DL3 and 3DL1/3DL2 pairs that define the two halves of the KIR gene complex. The synergistic combination of allelic polymorphism and variable gene content individualize KIR genotype to an extent where unrelated individuals almost always have different KIR types. This level of diversity likely reflects strong pressure from pathogens on the human NK cell response.

Veenstra, K. G., Z. L. Jonak, et al. (2002). "IL-12 Induces Monocyte IL-18 Binding Protein Expression Via IFN-{gamma}." J. Immunol. **168**(5): 2282-2287.

http://www.jimmunol.org/cgi/content/abstract/168/5/2282

IL-18 is a Th1 cytokine that synergizes with IL-12 and IL-2 in the stimulation of lymphocyte IFN-{gamma} production. IL-18 binding protein (IL-18BP) is a recently discovered inhibitor of IL-18 that is distinct from the IL-1 and IL-18 receptor families. In this report we show that IL-18BPa, the IL-18BP isoform with the highest affinity for IL-18, was strongly induced by IL-12 in human PBMC. Other Th1 cytokines, including IFN-{gamma}, IL-2, IL-15, and IL-18, were also capable of augmenting IL-18BPa expression. In contrast, IL-1{alpha}, IL-1{beta}, TNF-{alpha}, IFN-{gamma}-inducible protein-10, and Th2 cytokines such as IL-4 and IL-10 did not induce IL-18BPa. Although monocytes were found to be the primary source of IL-18BPa, the induction of IL-18BPa by IL-12 was mediated through IFN-{gamma} derived predominantly from NK cells. IL-18BPa production was observed in cancer patients receiving recombinant human IL-12 and correlated with the magnitude of IFN-{gamma} production. The IFN-{gamma}/IL-18BPa negative feedback loop identified in this study may be capable of broadly controlling immune activation by cytokines that synergize with IL-18 to induce IFN-{gamma} and probably plays a key role in the modulation of both innate and adaptive immunity.

Precopio, M. L., J. L. Sullivan, et al. (2003). "Differential Kinetics and Specificity of EBV-Specific CD4+ and CD8+ T Cells During Primary Infection." J. Immunol. **170**(5): 2590-2598.

http://www.jimmunol.org/cgi/content/abstract/170/5/2590

The generation and maintenance of virus-specific CD4+ T cells in humans are not well understood. We used short in vitro stimulation assays followed by intracellular cytokine staining to characterize the timing, magnitude, and Ag specificity of CD4+ T cells over the course of primary EBV infection. Lytic and latent protein-specific CD4+ T cells were readily detected at presentation with acute infectious mononucleosis and declined rapidly thereafter. Responses to BZLF-1, BMLF-1, and Epstein-Barr nuclear Ag-3A were more commonly detected than responses to Epstein-Barr nuclear Ag-1. Concurrent analyses of BZLF-1-specific CD4+ and CD8+ T cells revealed differences in the expansion, specificity, and stability of CD4+ and CD8+ T cell-mediated responses over time. Peripheral blood EBV load directly correlated with the frequency of EBV-specific CD4+ T cell responses at presentation and over time, suggesting that EBV-specific CD4+ T cell responses are Ag-driven.

Zhou, H., E. Bengten, et al. (2003). "The T Cell Receptor {beta} Locus of the Channel Catfish, Ictalurus punctatus, Reveals Unique Features." J. Immunol. **170**(5): 2573-2581.

http://www.jimmunol.org/cgi/content/abstract/170/5/2573

Previously, a series of clonal alloantigen-dependent T cell lines established from the channel catfish revealed distinctly different TCR{beta} rearrangements. Here, a follow-up study of the junctional diversity of these TCR gene rearrangements focuses on characterization of the genomic organization of the TCRB locus. Surprisingly, a total of 29 JB genes and two substantially different CB genes were identified downstream of a single DB gene. This is in contrast to the situation in mammals, where two clusters of a DB gene, six or seven JB genes, and a CB gene are found in tandem. The catfish CB genes are [~]36% identical at the amino acid level. All 29 catfish JB gene segments appear functional. Thirteen were used in the 19 cDNAs analyzed, of these eight were used by the 11 catfish clonal alloantigen-dependent T cell lines. As might be expected, CDR3 diversity is enhanced by N-nucleotide additions as well as nucleotide deletions at the V-D and D-J junctions. Taken together, compared with that in mammals, genomic sequencing of the catfish TCR DB-JB-CB region reveals a unique locus containing a greater number of JB genes and two distinct CB genes.

He, B., A. Chadburn, et al. (2004). "Lymphoma B Cells Evade Apoptosis through the TNF Family Members BAFF/BLyS and APRIL." J. Immunol. **172**(5): 3268-3279.

http://www.jimmunol.org/cgi/content/abstract/172/5/3268

The mechanisms underlying the autonomous accumulation of malignant B cells remain elusive. We show in this study that non-Hodgkin's lymphoma (NHL) B cells express B cell-activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL), two powerful B cell-activating molecules usually expressed by myeloid cells. In addition, NHL B cells express BAFF receptor, which binds BAFF, as well as transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and B cell maturation Ag (BCMA), which bind both BAFF and APRIL. Neutralization of endogenous BAFF and APRIL by soluble TACI and BCMA decoy receptors attenuates the survival of NHL B cells, decreases activation of the prosurvival transcription factor NF-{kappa}B, down-regulates the antiapoptotic proteins Bcl-2 and Bcl-xL, and up-regulates the proapoptotic protein Bax. Conversely, exposure of NHL B cells to recombinant

or myeloid cell-derived BAFF and APRIL attenuates apoptosis, increases NF-{kappa}B activation, up-regulates Bcl-2 and Bcl-xL, and down-regulates Bax. In some NHLs, exogenous BAFF and APRIL up-regulate c-Myc, an inducer of cell proliferation; down-regulate p53, an inhibitor of cell proliferation; and increase Bcl-6, an inhibitor of B cell differentiation. By showing that nonmalignant B cells up-regulate BAFF and APRIL upon stimulation by T cell CD40 ligand, our findings indicate that NHL B cells deregulate an otherwise physiological autocrine survival pathway to evade apoptosis. Thus, neutralization of BAFF and APRIL by soluble TACI and BCMA decoy receptors could be useful to dampen the accumulation of malignant B cells in NHL patients.

Lausen, B. F., L. Hougs, et al. (2004). "Human Memory B Cells Transferred by Allogenic Bone Marrow Transplantation Contribute Significantly to the Antibody Repertoire of the Recipient." J. Immunol. **172**(5): 3305-3318.

http://www.jimmunol.org/cgi/content/abstract/172/5/3305

The bone marrow is an important source of Abs involved in long-term protection from recurrence of infections. Allogenic bone marrow transplantation (BMT) fails to restore this working memory. Attempts to overcome this immunodeficiency by immunization of the donor have not been very successful. More needs to be known about transfer of B cell memory by BMT. We tracked memory B cells from the donor to the recipient during BMT of a girl with leukocyte adhesion deficiency. Vaccination of her HLA-identical sibling donor 7 days before harvest induced Haemophilus influenzae type b (Hib) capsular polysaccharide (HibCP)-specific B cells readily detectable in marrow and blood. BMT did not lead to spontaneous production of HibCP Abs, but the recipient responded well to booster immunizations 9 and 11 mo after BMT. HibCP-specific B cells were obtained 7 days after the vaccinations, and their VH genes were sequenced and analyzed for rearrangements and unique patterns of somatic hypermutations identifying clonally related cells. Ninety (74%) of 121 sequences were derived from only 16 precursors. Twelve clones were identified in the donor, and representatives from all of them were detected in the recipient where they constituted 61 and 68% of the responding B cells after the first and second vaccinations, respectively. No evidence for re-entry of memory clones into the process of somatic hypermutation was seen in the recipient. Thus, memory B cells were transferred from the donor, persisted for at least 9 mo in the recipient, and constituted the major part of the HibCP-specific repertoire.

Pewe, L. L., J. M. Netland, et al. (2004). "Very Diverse CD8 T Cell Clonotypic Responses after Virus Infections." J. Immunol. **172**(5): 3151-3156.

http://www.jimmunol.org/cgi/content/abstract/172/5/3151

We measured CD8 T cell clonotypic diversity to three epitopes recognized in C57BL/6 mice infected with mouse hepatitis virus, strain JHM, or lymphocytic choriomeningitis virus. We isolated epitope-specific T cells with an IFN-{gamma} capture assay or MHC class I/peptide tetramers and identified different clonotypes by V{beta} chain sequence analysis. In agreement with our previous results, the number of different clonotypes responding to all three epitopes fit a log-series distribution. From these distributions, we estimated that >1000 different clonotypes responded to each immunodominant CD8 T cell epitope; the response to a subdominant CD8 T cell epitope was modestly less diverse. These results suggest that T cell response diversity is greater by 1-2 orders of magnitude than predicted previously.

Qiao, Y., S. Prabhakar, et al. (2004). "Posttranscriptional Inhibition of Gene Expression by Mycobacterium tuberculosis Offsets Transcriptional Synergism with IFN-{gamma} and Posttranscriptional Up-Regulation by IFN-{gamma}." J. Immunol. **172**(5): 2935-2943.

http://www.jimmunol.org/cgi/content/abstract/172/5/2935

Host defense against Mycobacterium tuberculosis requires the cytokine IFN-{gamma} and IFN regulatory factor 1 (IRF-1), a transcription factor that is induced to high levels by IFN-{gamma}. Therefore, we chose to study regulation of IRF-1 expression as a model for effects of M. tuberculosis on response to IFN-{gamma}. We found that IRF-1 mRNA abundance increased far more than transcription rate in human monocytic THP-1 cells stimulated by IFN-{gamma}, but less than transcription rate in cells infected by M. tuberculosis. IFN-{gamma} stimulation of infected cells caused a synergistic increase in IRF-1 transcription, yet IRF-1 mRNA abundance was similar in uninfected and infected cells stimulated by IFN-{gamma}, as was the IRF-1 protein level. Comparable infection by Mycobacterium bovis bacillus Calmette-Guerin failed to induce IRF-1 expression and had no effect on the response to IFN-{gamma}. We also examined the kinetics of transcription, the mRNA t1/2, and the distribution of IRF-1 transcripts among total nuclear RNA, poly(A) nuclear RNA, and poly(A) cytoplasmic RNA pools in cells that were infected by M. tuberculosis and/or stimulated by IFN-{gamma}. Our data suggest that infection by M. tuberculosis inhibits RNA export from the nucleus. Moreover, the results indicate that regulated entry of nascent transcripts into the pool of total nuclear RNA affects IRF-1 expression and that this process is stimulated by IFN-{gamma} and inhibited by M. tuberculosis. The ability of infection by M. tuberculosis to limit the increase in IRF-1 mRNA expression that typically follows transcriptional synergism may contribute to the pathogenicity of M. tuberculosis.

Hasan, U., C. Chaffois, et al. (2005). "Human TLR10 Is a Functional Receptor, Expressed by B Cells and Plasmacytoid Dendritic Cells, Which Activates Gene Transcription through MyD88." J. Immunol. **174**(5): 2942-2950.

http://www.jimmunol.org/cgi/content/abstract/174/5/2942

Human TLR10 is an orphan member of the TLR family. Genomic studies indicate that TLR10 is in a locus that also contains TLR1 and TLR6, two receptors known to function as coreceptors for TLR2. We have shown that TLR10 was not only able to homodimerize but also heterodimerized with TLRs 1 and 2. In addition, unlike TLR1 and TLR6, TLR10 was expressed in a highly restricted fashion as a highly N-glycosylated protein, which we detected in B cell lines, B cells from peripheral blood, and plasmacytoid dendritic cells from tonsil. We were also able to detect TLR10 in a CD1a+ DC subset derived from CD34+ progenitor cells which resemble Langerhans cells in the epidermis. Although we were unable to identify a specific ligand for TLR10, by using a recombinant CD4TLR10 molecule we also demonstrated that TLR10 directly associates with MyD88, the common Toll IL-1 receptor domain adapter. Additionally, we have characterized regions in the Toll IL-1 receptor domain of TLR10 that are essential in the activation of promoters from certain inflammatory cytokines. Even though TLR10 expression has not been detected in mice, we have identified a partial genomic sequence of the TLR10 gene that was present but nonfunctional and disrupted by a retroviral insertion in all mouse strains tested. However, a complete TLR10 sequence could be detected in the rat genome, indicating that a functional copy may be preserved in this species.

Bacci, A., C. Montagnoli, et al. (2002). "Dendritic Cells Pulsed with Fungal RNA Induce Protective Immunity to Candida albicans in Hematopoietic Transplantation." J. Immunol. **168**(6): 2904-2913.

http://www.jimmunol.org/cgi/content/abstract/168/6/2904

Immature myeloid dendritic cells (DC) phagocytose yeasts and hyphae of the fungus Candida albicans and induce different Th cell responses to the fungus. Ingestion of yeasts activates DC for production of IL-12 and Th1 priming, while ingestion of hyphae induces IL-4 production and Th2 priming. In vivo, generation of antifungal protective immunity is induced upon injection of DC ex vivo pulsed with Candida yeasts but not hyphae. In the present study we sought to determine the functional activity of DC transfected with yeast or hyphal RNA. It was found that DC, from either spleens or bone marrow, transfected with yeast, but not hyphal, RNA 1) express fungal mannoproteins on their surface; 2) undergo functional maturation, as revealed by the up-regulated expression of MHC class II Ags and costimulatory molecules; 3) produce IL-12 but no IL-4; 4) are capable of inducing Th1-dependent antifungal resistance when delivered s.c. in vivo in nontransplanted mice; and 5) provide protection against the fungus in allogeneic bone marrow-transplanted mice, by accelerating the functional recovery of Candida-specific IFN-{gamma}-producing CD4+ donor lymphocytes. These results indicate the efficacy of DC pulsed with Candida yeasts or yeast RNA as fungal vaccines and point to the potential use of RNA-transfected DC as anti-infective vaccines in conditions that negate the use of attenuated microorganisms or in the case of poor availability of protective Ags.

Du, C. and S. Sriram (2002). "Increased Severity of Experimental Allergic Encephalomyelitis in lyn-/- Mice in the Absence of Elevated Proinflammatory Cytokine Response in the Central Nervous System." J. Immunol. **168**(6): 3105-3112.

http://www.jimmunol.org/cgi/content/abstract/168/6/3105

lyn, a member of the src kinase family, is an important signaling molecule in B cells. lyn-/- mice display hyperactive B-1 cells and IgM hyperglobulinemia. The role of lyn on T cell function and development of Th1-mediated inflammatory disease is not known. Therefore, we examined the effect of disruption of the lyn gene on the development of experimental allergic encephalomyelitis (EAE), a well-established Th1-mediated autoimmune disease. Following immunization with myelin oligodendrocyte protein (MOG) p35-55, lyn-/- mice had higher clinical and pathological severity scores of EAE when compared with wild type (WT). The increase in the severity of EAE in lyn-/- mice was not associated with a commensurate increase in the production of proinflammatory cytokines in the CNS. lyn-/- mice with EAE showed elevation in serum anti-IgM MOG Ab levels over that seen in WT mice, along with a modest increase in the mRNA levels of complement C5 and its receptor, C5aR, in the spinal cord. Transfer of serum from MOG-immunized lyn-/- mice worsened EAE in WT mice, suggesting a pathogenic role for anti-MOG IgM Abs in EAE. These observations underscore the potential role of lyn in regulation of Th1-mediated disease and the role of autoantibodies and complement in the development of EAE.

Thornton, M. V., D. Kudo, et al. (2004). "Degradation of NF-{kappa}B in T Cells by Gangliosides Expressed on Renal Cell Carcinomas." J. Immunol. **172**(6): 3480-3490.

http://www.jimmunol.org/cgi/content/abstract/172/6/3480

T cells from cancer patients are often functionally impaired, which imposes a barrier to effective immunotherapy. Most pronounced are the alterations characterizing tumor-infiltrating T cells, which in renal cell carcinomas includes defective NF-{kappa}B activation and a heightened sensitivity to apoptosis. Coculture experiments revealed that renal tumor cell lines induced a time-dependent decrease in RelA(p65) and p50 protein levels within both Jurkat T cells and peripheral blood T lymphocytes that coincided with the onset of apoptosis. The degradation of RelA/p50 is

critical for SK-RC-45-induced apoptosis because overexpression of RelA in Jurkat cells protects against cell death. The loss of RelA/p50 coincided with a decrease in expression of the NF-{kappa}B regulated antiapoptotic protein Bcl-xL at both the protein and mRNA level. The disappearance of RelA/p50 protein was mediated by a caspase-dependent pathway because pretreatment of T lymphocytes with a pan caspase inhibitor before coculture with SK-RC-45 blocked RelA and p50 degradation. SK-RC-45 gangliosides appear to mediate this degradative pathway, as blocking ganglioside synthesis in SK-RC-45 cells with the glucosylceramide synthase inhibitor, PPPP, protected T cells from tumor cell-induced RelA degradation and apoptosis. The ability of the Bcl-2 transgene to protect Jurkat cells from RelA degradation, caspase activation, and apoptosis implicates the mitochondria in these SK-RC-45 ganglioside-mediated effects.

Boniface, K., F.-X. Bernard, et al. (2005). "IL-22 Inhibits Epidermal Differentiation and Induces Proinflammatory Gene Expression and Migration of Human Keratinocytes." J. Immunol. **174**(6): 3695-3702.

http://www.jimmunol.org/cgi/content/abstract/174/6/3695

IL-22 belongs to a family of cytokines structurally related to IL-10, including IL-19, IL-20, IL-24, and IL-26. In contrast to IL-10, IL-22 has proinflammatory activities. IL-22 signals through a class II cytokine receptor composed of an IL-22-binding chain, IL-22RA1, and the IL-10RB subunit, which is shared with the IL-10R. In the present study, we show that short-term cultured human epidermal keratinocytes express a functional IL-22R but no IL-10R. Accordingly, IL-22 but not IL-10 induces STAT3 activation in keratinocytes. Using a cDNA array screening approach, real-time RT-PCR, and Western blot analysis, we demonstrate that IL-22 up-regulates, in a dose-dependent manner, the expression of S100A7, S100A8, S100A9, a group of proinflammatory molecules belonging to the S100 family of calcium-binding proteins, as well as the matrix metalloproteinase 3, the platelet-derived growth factor A, and the CXCL5 chemokine. In addition, IL-22 induces keratinocyte migration in an in vitro injury model and down-regulates the expression of at least seven genes associated with keratinocyte differentiation. Finally, we show that IL-22 strongly induces hyperplasia of reconstituted human epidermis. Taken together, these results suggest that IL-22 plays an important role in skin inflammatory processes and wound healing.

Iizasa, H., H. Yoneyama, et al. (2005). "Exacerbation of Granuloma Formation in IL-1 Receptor Antagonist-Deficient Mice with Impaired Dendritic Cell Maturation Associated with Th2 Cytokine Production." J. Immunol. **174**(6): 3273-3280.

http://www.jimmunol.org/cgi/content/abstract/174/6/3273

Dendritic cell (DC) maturation at the site of inflammation and migration into draining lymph nodes is fundamental to initiate Ag-specific immune responses. Although several proinflammatory cytokines, including IL-1, are known to promote DC maturation in vitro, their contributions to DC activation and migration within peripheral inflamed tissue compartments are not yet fully understood. We show here that endogenous IL-1 receptor antagonist (IL-1ra) controls the activation state of liver-recruited DCs and their migration in a Propionibacterium acnes-induced murine granulomatous liver disease model. After P. acnes treatment, formation of portal tract-associated lymphoid tissue was conversely impaired in IL-1ra-deficient mice. IL-1ra-deficient mice developed hepatic granulomas within 3 days after P. acnes administration and showed a more pronounced granuloma formation than wild-type mice. Although sinusoidal granulomas contained numerous CD11c+ DCs at day 7, expressions of CCR7, IL-12p40 by these DCs were dramatically decreased in IL-1ra-deficient mice, suggesting aberrant DC maturation and sinusoid

portal migration in the absence of endogenous IL-1ra. This was accompanied with enhanced intrahepatic Th2 cytokine production and severe hepatocellular damage. Thus, hepatocyte-derived IL-1ra may control optimal activation and migration of inflammatory DCs within the liver and thereby determine the local immune responses in granulomatous liver disease.

Linard, B., S. Bezieau, et al. (2002). "A ras-Mutated Peptide Targeted by CTL Infiltrating a Human Melanoma Lesion." J. Immunol. **168**(9): 4802-4808.

http://www.jimmunol.org/cgi/content/abstract/168/9/4802

Ags derived from commonly mutated oncogenic proteins seem ideally suited as targets for tumor immunotherapy. Nonetheless, only a few mutated epitopes efficiently presented by human tumors have thus far been identified. We describe here an approach to identify such epitopes. This approach involves: 1) identifying tumors expressing a ras mutation and isolating the tumor-infiltrating lymphocytes (TIL); 2) transfecting COS cells to induce expression of unknown mutated peptides in the context of a patient's HLA class I molecules; and 3) screening epitope recognition by using TIL from the tumors expressing a ras mutation. By using this approach, there appeared to be a N-ras mutation (a glutamine-to-arginine exchange at residue 61 (Q61R)), detected in a melanoma lesion, which was recognized specifically by the autologous TIL in the HLA-A*0101 context. The ras peptide 55-64Q61R was the epitope of these TIL and was regularly presented by Q61R-mutated HLA-A*0101+ melanoma cell lines. This peptide and its wild-type homolog (55-64wt) bound to HLA-A*0101 with similar affinities. However, only the mutated peptide could induce specific CTL expansion from PBL. All the CTL clones specific to the mutated peptide, failed to recognize the wild-type sequence on both COS and melanoma cells. These data thus show that oncogenic protein mutations can create shared tumor-specific CTL epitopes, efficiently presented by tumor cells, and that screening for oncogene-transfected COS cell recognition by TIL (from tumors containing mutations) is a powerful approach for the identification of these epitopes.

Zwijnenburg, P. J. G., T. van der Poll, et al. (2003). "IL-1 Receptor Type 1 Gene-Deficient Mice Demonstrate an Impaired Host Defense Against Pneumococcal Meningitis." J. Immunol. **170**(9): 4724-4730.

http://www.jimmunol.org/cgi/content/abstract/170/9/4724

The fatality rate associated with Streptococcus pneumoniae meningitis remains high despite adequate antibiotic treatment. IL-1 is an important proinflammatory cytokine, which is up-regulated in brain tissue after the induction of meningitis. To determine the role of IL-1 in pneumococcal meningitis we induced meningitis by intranasal inoculation with 8 x 104 CFU of S. pneumoniae and 180 U of hyaluronidase in IL-1R type I gene-deficient (IL-1R-/-) mice and wild-type mice. Meningitis resulted in elevated IL-1{alpha} and IL-1{beta} mRNA and protein levels in the brain. The absence of an intact IL-1 signal was associated with a higher susceptibility to develop meningitis. Furthermore, the lack of IL-1 impaired bacterial clearance, as reflected by an increased number of CFU in cerebrospinal fluid of IL-1R-/- mice. The characteristic pleocytosis of meningitis was not significantly altered in IL-1R-/- mice, but meningitis was associated with lower brain levels of cytokines. The mortality was significantly higher and earlier in the course of the disease in IL-1R-/- mice. These results demonstrate that endogenous IL-1 is required for an adequate host defense in pneumococcal meningitis.

Lebbink, R. J., T. de Ruiter, et al. (2004). "The Mouse Homologue of the Leukocyte-Associated Ig-Like Receptor-1 Is an Inhibitory Receptor That Recruits Src Homology Region 2-Containing Protein Tyrosine Phosphatase (SHP)-2, but Not SHP-1." J. Immunol. **172**(9): 5535-5543.

http://www.jimmunol.org/cgi/content/abstract/172/9/5535

We report the molecular cloning and characterization of the first leukocyte-associated Ig-like receptor 1 (LAIR-1) homologue in mice that we have named mouse LAIR-1 (mLAIR-1). The mLAIR-1 gene maps to the proximal end of mouse chromosome 7 in a region syntenic with human chromosome 19q13.4 where the leukocyte receptor cluster is located. The protein shares 40% sequence identity with human LAIR-1, has a single Ig-like domain, and contains two immunoreceptor tyrosine-based inhibitory motif-like structures in its cytoplasmic tail. Mouse LAIR-1 is broadly expressed on various immune cells, and cross-linking of the molecule on stably transfected RBL-2H3 and YT.2C2 cells results in strong inhibition of their degranulation and cytotoxic activities, respectively. Upon pervanadate stimulation, the mLAIR-1 cytoplasmic tail becomes phosphorylated, thereby recruiting Src homology region 2-containing tyrosine phosphatase-2. Interestingly, unlike human LAIR-1, Src homology region 2-containing tyrosine phosphatase-1 is not recruited to the mLAIR-1 cytoplasmic tail. Screening human and mouse cell lines for mLAIR-1 and human LAIR-1 binding partners identified several lines expressing putative ligand(s) for both receptors.

Biassoni, R., M. Fogli, et al. (2005). "Molecular and Functional Characterization of NKG2D, NKp80, and NKG2C Triggering NK Cell Receptors in Rhesus and Cynomolgus Macaques: Monitoring of NK Cell Function during Simian HIV Infection." J. Immunol. **174**(9): 5695-5705.

http://www.jimmunol.org/cgi/content/abstract/174/9/5695

An involvement of innate immunity and of NK cells during the priming of adaptive immune responses has been recently suggested in normal and disease conditions such as HIV infection and acute myelogenous leukemia. The analysis of NK cell-triggering receptor expression has been so far restricted to only NKp46 and NKp30 in Macaca fascicularis. In this study, we extended the molecular and functional characterization to the various NK cell-triggering receptors using PBMC and to the in vitro-derived NK cell populations by cytofluorometry and by cytolytic activity assays. In addition, RT-PCR strategy, cDNA cloning/sequencing, and transient transfections were used to identify and characterize NKp80, NKG2D, CD94/NKG2C, and CD94/NKG2A in M. fascicularis and Macaca mulatta as well as in the signal transducing polypeptide DNAX-activating protein DAP-10. Both M. fascicularis and M. mulatta NK cells express NKp80, NKG2D, and NKG2C molecules, which displayed a high degree of sequence homology with their human counterpart. Analysis of NK cells in simian HIV-infected M. fascicularis revealed reduced surface expression of selected NK cell-triggering receptors associated with a decreased NK cell function only in some animals. Overall surface density of NK cell-triggering receptors on peripheral blood cells and their triggering function on NK cell populations derived in vitro was not decreased compared with uninfected animals. Thus, triggering NK cell receptor monitoring on macaque NK cells is possible and could provide a valuable tool for assessing NK cell function during experimental infections and for exploring possible differences in immune correlates of protection in humans compared with cynomolgus and rhesus macaques undergoing different vaccination strategies.

Gough, M., M. Crittenden, et al. (2005). "Gene Therapy to Manipulate Effector T Cell Trafficking to Tumors for Immunotherapy." J. Immunol. **174**(9): 5766-5773.

http://www.jimmunol.org/cgi/content/abstract/174/9/5766

Strategies that generate tumor Ag-specific effector cells do not necessarily cure established tumors. We hypothesized that the relative efficiency with which tumor-specific effector cells reach the tumor is critical for therapy. We demonstrate in this study that activated T cells respond to the chemokine CCL3, both in vitro and in vivo, and we further demonstrate that expression of CCL3 within tumors increases the effector T cell infiltrate in those tumors. Importantly, we show that adenoviral gene transfer to cause expression of CCL3 within B16ova tumors in vivo increases the efficacy of adoptive transfer of tumor-specific effector OT1 T cells. We additionally demonstrate that such therapies result in endogenous immune responses to tumor Ags that are capable of protecting animals against subsequent tumor challenge. Strategies that modify the "visibility" of tumors have the potential to significantly enhance the efficacy of both vaccine and adoptive transfer therapies currently in development.

Kawauchi, K., K. Ihjima, et al. (2005). "IL-2 Increases Human Telomerase Reverse Transcriptase Activity Transcriptionally and Posttranslationally through Phosphatidylinositol 3'-Kinase/Akt, Heat Shock Protein 90, and Mammalian Target of Rapamycin in Transformed NK Cells." J. Immunol. **174**(9): 5261-5269.

http://www.jimmunol.org/cgi/content/abstract/174/9/5261

Human telomerase activity is induced by Ag receptor ligation in T and B cells. However, it is unknown whether telomerase activity is increased in association with activation and proliferation of NK cells. We found that telomerase activity in a human NK cell line (NK-92), which requires IL-2 for proliferation, was increased within 24 h after stimulation with IL-2. Levels of human telomerase reverse transcriptase (hTERT) mRNA and protein correlated with telomerase activity. ERK1/2 and Akt kinase (Akt) were activated by IL-2 stimulation. LY294002, an inhibitor of PI3K, abolished expression of hTERT mRNA and protein expression and abolished hTERT activity, whereas PD98059, which inhibits MEK1/2 and thus ERK1/2, had no effect. In addition, radicicol, an inhibitor of heat shock protein 90 (Hsp90), and rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR), blocked IL-2-induced hTERT activity and nuclear translocation of hTERT but not hTERT mRNA expression. hTERT was coimmunoprecipitated with Akt, Hsp90, mTOR, and p70 S6 kinase (S6K), suggesting that these molecules form a physical complex. Immunoprecipitates of Akt, Hsp90, mTOR, and S6K from IL-2-stimulated NK-92 cells contained telomerase activity. Furthermore, the findings that Hsp90 and mTOR immunoprecipitates from primary samples contained telomerase activity are consistent with the results from NK-92 cells. These results indicate that IL-2 stimulation induces hTERT activation and that the mechanism of IL-2-induced hTERT activation involves transcriptional or posttranslational regulation through the pathway including PI3K/Akt, Hsp90, mTOR, and S6K in NK cells.

Ruckert, R., K. Brandt, et al. (2005). "Blocking IL-15 Prevents the Induction of Allergen-Specific T Cells and Allergic Inflammation In Vivo." J. Immunol. **174**(9): 5507-5515.

http://www.jimmunol.org/cgi/content/abstract/174/9/5507

IL-15 has been shown to accelerate and boost allergic sensitization in mice. Using a murine model of allergic sensitization to OVA, we present evidence that blocking endogenous IL-15 during the sensitization phase using a soluble IL-15R{alpha} (sIL-15R{alpha}) suppresses the induction of Ag-specific, Th2-differentiated T cells. This significantly reduces the production of OVA-specific IgE and IgG and prevents the induction of a pulmonary inflammation. Release of proinflammatory TNF-{alpha}, IL-1{beta}, IL-6, and IL-12 as well as that of Th2 cytokines IL-4, IL-

5, and IL-13 into the bronchi are significantly reduced, resulting in suppressed recruitment of eosinophils and lymphocytes after allergen challenge. It is of clinical relevance that the airway hyper-responsiveness, a major symptom of human asthma bronchiale, is significantly reduced by sIL-15R{alpha} treatment. Ex vivo analysis of the draining lymph nodes revealed reduced numbers of CD8, but not CD4, memory cells and the inability of T cells of sIL-15R{alpha}-treated mice to proliferate and to produce Th2 cytokines after in vitro OVA restimulation. This phenomenon is not mediated by enhanced numbers of CD4+/CD25+ T cells. These results show that IL-15 is important for the induction of allergen-specific, Th2-differentiated T cells and induction of allergic inflammation in vivo.

Waetzig, G. H., D. Seegert, et al. (2002). "p38 Mitogen-Activated Protein Kinase Is Activated and Linked to TNF-{alpha} Signaling in Inflammatory Bowel Disease." J. Immunol. **168**(10): 5342-5351.

http://www.jimmunol.org/cgi/content/abstract/168/10/5342

Inflammatory bowel diseases (IBD)--Crohn's disease and ulcerative colitis--are relapsing chronic inflammatory disorders which involve genetic, immunological, and environmental factors. The regulation of TNF-{alpha}, a key mediator in the inflammatory process in IBD, is interconnected with mitogen-activated protein kinase pathways. The aim of this study was to characterize the activity and expression of the four p38 subtypes (p38{alpha}-{delta}), c-Jun N-terminal kinases (JNKs), and the extracellular signal-regulated kinases (ERK)1/2 in the inflamed intestinal mucosa. Western blot analysis revealed that p38{alpha}, JNKs, and ERK1/2 were significantly activated in IBD, with p38{alpha} showing the most pronounced increase in kinase activity. Protein expression of p38 and JNK was only moderately altered in IBD patients compared with normal controls, whereas ERK1/2 protein was significantly down-regulated. Immunohistochemical analysis of inflamed mucosal biopsies localized the main expression of p38{alpha} to lamina propria macrophages and neutrophils. ELISA screening of the supernatants of Crohn's disease mucosal biopsy cultures showed that incubation with the p38 inhibitor SB 203580 significantly reduced secretion of TNF-{alpha}. In vivo inhibition of TNF-{alpha} by a single infusion of anti-TNF-{alpha} Ab (infliximab) resulted in a highly significant transient increase of p38{alpha} activity during the first 48 h after infusion. A significant infliximab-dependent p38{alpha} activation was also observed in THP-1 myelomonocytic cells. In human monocytes, infliximab enhanced TNF-{alpha} gene expression, which could be inhibited by SB 203580. In conclusion, p38{alpha} signaling is involved in the pathophysiology of IBD.

Bulanova, E., V. Budagian, et al. (2003). "Mast Cells Express Novel Functional IL-15 Receptor {alpha} Isoforms." J. Immunol. **170**(10): 5045-5055.

http://www.jimmunol.org/cgi/content/abstract/170/10/5045

Mast cells previously have been reported to be regulated by IL-15 and to express a distinct IL-15R, termed IL-15RX. To further examine IL-15 binding and signaling in mast cells, we have studied the nature of the IL-15R and some of its biological activities in these cells. In this study, we report the existence of three novel isoforms of the IL-15R{alpha} chain in murine bone marrow-derived mast cells as a result of an alternative exon-splicing mechanism within the IL-15R{alpha} gene. These correspond to new mRNA transcripts lacking exon 4; exons 3 and 4; or exons 3, 4, and 5 (IL-15R{alpha}{Delta}4, IL-15R{alpha}{Delta}3,4, IL-15R{alpha}{Delta}3,4,5). After transient transfection in COS-7 cells, all IL-15R{alpha} isoforms associate with the Golgi apparatus, the endoplasmic reticulum, the perinuclear space, and the cell membrane. Analysis of glycosylation pattern demonstrates the usage of a single N-glycosylation site, while no O-glycosylation is observed. Importantly, IL-15 binds with high affinity to, and promotes the survival of, murine BA/F3 cells stably transfected with the IL-15R{alpha} isoforms. Furthermore, we report

that signaling mediated by IL-15 binding to the newly identified IL-15R{alpha} isoforms involves the phosphorylation of STAT3, STAT5, STAT6, Janus kinase 2, and Syk kinase. Taken together, our data indicate that murine mast cells express novel, fully functional IL-15R{alpha} isoforms, which can explain the selective regulatory effects of IL-15 on these cells.

Corcoran, L., I. Ferrero, et al. (2003). "The Lymphoid Past of Mouse Plasmacytoid Cells and Thymic Dendritic Cells." J. Immunol. **170**(10): 4926-4932.

http://www.jimmunol.org/cgi/content/abstract/170/10/4926

There has been controversy over the possible lymphoid origin of certain dendritic cell (DC) subtypes. To resolve this issue, DC and plasmacytoid pre-DC isolated from normal mouse tissues were analyzed for transient (mRNA) and permanent (DNA rearrangement) markers of early stages of lymphoid development. About 27% of the DNA of CD8+ DC from thymus, and 22-35% of the DNA of plasmacytoid pre-DC from spleen and thymus, was found to contain IgH gene D-J rearrangements, compared with 40% for T cells. However, the DC DNA did not contain IgH gene V-D-J rearrangements nor T cell Ag receptor {beta} gene D-J rearrangements. The same DC lineage populations containing IgH D-J rearrangements expressed mRNA for CD3 chains, and for pre-T{alpha}. In contrast, little of the DNA of the conventional DC derived from spleen, lymph nodes, or skin, whether CD8+ or CD8-, contained IgH D-J rearrangements and splenic conventional DC expressed very little CD3{epsilon} or pre-T{alpha} mRNA. Therefore, many plasmacytoid pre-DC and thymic CD8+ DC have shared early steps of development with the lymphoid lineages, and differ in origin from conventional peripheral DC.

Podojil, J. R. and V. M. Sanders (2003). "Selective Regulation of Mature IgG1 Transcription by CD86 and {beta}2-Adrenergic Receptor Stimulation." J. Immunol. **170**(10): 5143-5151.

http://www.jimmunol.org/cgi/content/abstract/170/10/5143

Stimulation of CD86 and the {beta}2-adrenergic receptor ({beta}2AR) on a B cell, either alone or together, is known to increase the level of IgG1 protein produced by a CD40 ligand/IL-4-activated B cell. It is also known that the mechanism by which CD40 and IL-4R stimulation on a B cell increases the level of IgG1 protein is by increasing germline {gamma}1 transcription, IgG1 class switching, and mature IgG1 transcription, while the molecular mechanism responsible for mediating the CD86- and {beta}2AR-induced effect remains unknown. In the present study using real-time PCR we show that the level of mature IgG1 transcription increases in CD40 ligand/IL-4-activated B cells following stimulation of either CD86 and/or {beta}2AR, and that this increase reflects the increase in IgG1 protein. Furthermore, we show that the CD86- and/or {beta}2AR-induced increase in mature IgG1 transcript is due to an increase in the rate of mature IgG1 transcription, as determined by nuclear run-on analysis. This effect is additive when both receptors are stimulated and is lost when B cells from CD86- and {beta}2AR-deficient mice are used. In contrast, the level of germline {gamma}1 transcription, the stability of mature IgG1 transcript, the number of IgG1-positive B cells, and the number of IgG1-secreting B cells did not change. These results provide the first evidence that CD86 and/or {beta}2AR stimulation on a CD40 ligand/IL-4-activated B cell increases the level of IgG1 protein produced per cell by increasing the rate of mature IgG1 transcription.

Shimoda, K., K. Kamesaki, et al. (2002). "Cutting Edge: Tyk2 Is Required for the Induction and Nuclear Translocation of Daxx Which Regulates IFN-{alpha}-Induced Suppression of B Lymphocyte

Formation." J. Immunol. **169**(9): 4707-4711.

http://www.jimmunol.org/cgi/content/abstract/169/9/4707

IFN-{alpha} inhibits B lymphocyte development, and the nuclear protein Daxx has been reported to be essential for this biological activity. We show in this study that IFN-{alpha} inhibits the clonal proliferation of B lymphocyte progenitors in response to IL-7 in wild-type, but not in tyk2-deficient, mice. In addition, the IFN-{alpha}-induced up-regulation and nuclear translocation of Daxx are completely abrogated in the absence of tyk2. Therefore, tyk2 is directly involved in IFN-{alpha} signaling for the induction and translocation of Daxx, which may result in B lymphocyte growth arrest and/or apoptosis.

van der Stoep, N., E. Quinten, et al. (2002). "Transcriptional Regulation of the MHC Class II Trans-Activator (CIITA) Promoter III: Identification of a Novel Regulatory Region in the 5'-Untranslated Region and an Important Role for cAMP-Responsive Element Binding Protein 1 and Activating Transcription Factor-1 in CIITA-Promoter III Transcriptional Activation in B Lymphocytes." J. Immunol. **169**(9): 5061-5071.

http://www.jimmunol.org/cgi/content/abstract/169/9/5061

The class II trans-activator (CIITA), which acts as a master regulator for expression of MHC class II genes, is expressed constitutively in mature B cells. This constitutive expression of CIITA is driven by CIITA promoter III (CIITA-PIII). However, little is known about the factors that control the B cell-mediated trans-activation of CIITA-PIII. In this study using B cells we have identified several cAMP-responsive elements (CREs) in the proximal promoter and in the 5'-untranslated region (5'-UTR) that are involved in the activation of CIITA-PIII. We show that activating transcription factor (ATF)/CRE binding protein (CREB) factors bind to the CREs in vitro and in vivo. Notably, our results also reveal that the 5'-UTR of CIITA-PIII functions as an important regulatory region in B lymphocytes. Furthermore, transient cotransfections of a CIITA-PIII luciferase reporter construct with either CREB-1 or ATF-1 boost CIITA-PIII trans-activation in a dose-dependent manner, which was further enhanced by addition of general coactivator CREB-binding protein. Transient transfections using mutant CIITA-PIII luciferase reporter constructs that either lack the (5'-UTR) or abolish binding of CREB-1 and ATF-1 to the CRE located in activation response element-2, displayed severely reduced promoter activity in B cells. A similar successive deletion of the CREs resulted in a subsequent reduction of CREB-1-induced activity of CIITA-PIII in B cells. Together our results argue for an important role of ATF/CREB factors and the 5'-UTR of CIITA-PIII in the trans-activation of CIITA-PIII in B cells.

Wang, H.-W., H. P. McNeil, et al. (2002). "{delta} Tryptase Is Expressed in Multiple Human Tissues, and a Recombinant Form Has Proteolytic Activity." J. Immunol. **169**(9): 5145-5152.

http://www.jimmunol.org/cgi/content/abstract/169/9/5145

Tryptases are neutral serine proteases selectively expressed in mast cells and have been implicated in the development of a number of inflammatory diseases including asthma. It has recently been established that the number of genes encoding human mast cell tryptases is much larger than originally believed, but it is not clear how many of these genes are expressed. A recent report suggested that the transcript for at least one of these genes, originally named mMCP-7-like tryptase, is not expressed. To further address this question, we screened tissue-specific RNA samples by RT-PCR, using primers designed to match the putative exonic sequence of this gene. We successfully generated and cloned the correctly sized RT-PCR

product from mRNA isolated from the human mast cell-I cell line. Two distinct clones were identified whose nucleotide sequence matched the published sequence of the mMCP-7-like I and mMCP-7-like II genes. Transcripts were detected in a wide variety of human tissues including lung, heart, stomach, spleen, skin, and colon. A polyclonal antipeptide Ab that specifically recognizes the translated product of this transcript was used to demonstrate its expression in mast cells that reside in the colon, lung, and inflamed synovium. A recombinant form of this protein expressed in bacterial cells was able to cleave a synthetic trypsin-sensitive substrate, D-Ile-Phe-Lys pNA. These results suggest that the range of functional tryptases is larger than previously recognized. For simplicity, we suggest that the gene, transcripts, and corresponding protein product be named {delta} tryptase.

Stievano, L., V. Tosello, et al. (2003). "CD8+{alpha}{beta}+ T Cells That Lack Surface CD5 Antigen Expression Are a Major Lymphotactin (XCL1) Source in Peripheral Blood Lymphocytes." J. Immunol. **171**(9): 4528-4538.

http://www.jimmunol.org/cgi/content/abstract/171/9/4528

To better characterize the cellular source of lymphotactin (XCL1), we compared XCL1 expression in different lymphocyte subsets by real-time PCR. XCL1 was constitutively expressed in both PBMC and CD4+ cells, but its expression was almost 2 log higher in CD8+ cells. In vitro activation was associated with a substantial increase in XCL1 expression in both PBMC and CD8+ cells, but not in CD4+ lymphocytes. The preferential expression of XCL1 in CD8+ cells was confirmed by measuring XCL1 production in culture supernatants, and a good correlation was found between figures obtained by real-time PCR and XCL1 contents. XCL1 expression was mostly confined to a CD3+CD8+ subset not expressing CD5, where XCL1 expression equaled that shown by {gamma}{delta}+ T cells. Compared with the CD5+ counterpart, CD3+CD8+CD5- cells, which did not express CD5 following in vitro activation, showed preferential expression of the {alpha}{alpha} form of CD8 and a lower expression of molecules associated with a noncommitted/naive phenotype, such as CD62L. CD3+CD8+CD5- cells also expressed higher levels of the XCL1 receptor; in addition, although not differing from CD3+CD8+CD5+ cells in terms of the expression of most {alpha}- and {beta}-chemokines, they showed higher expression of CCL3/macrophage inflammatory protein-1{alpha}. These data show that TCR {alpha}{beta}-expressing lymphocytes that lack CD5 expression are a major XCL1 source, and that the contribution to its synthesis by different TCR {alpha}{beta}-expressing T cell subsets, namely CD4+ lymphocytes, is negligible. In addition, they point to the CD3+CD8+CD5- population as a particular T cell subset within the CD8+ compartment, whose functional properties deserve further attention.

Masuda, A., Y. Yoshikai, et al. (2004). "The Interaction between GATA Proteins and Activator Protein-1 Promotes the Transcription of IL-13 in Mast Cells." J. Immunol. **173**(9): 5564-5573.

http://www.jimmunol.org/cgi/content/abstract/173/9/5564

IL-13 is considered to be a key modulator in the pathogenesis of Th2-induced allergic inflammation, although little is known about the regulation of IL-13 transcription in mast cells. In T cells, involvement of GATA-3 in cell type-specific expression of the IL-13 gene has been reported. However, the mechanisms that induce rapid transactivation of the IL-13 gene in response to various types of stimulation have hitherto remained unknown. In this report, we describe our investigation of the promoter region necessary for IL-13 transcription; we have found that both AP-1 and GATA proteins are indispensable for IL-13 transcription in mouse mast cells. In our investigation, we focused on the functional interaction between GATA and AP-1 in the IL-13 promoter context. Transfection experiments have revealed that GATA-1 and GATA-2 proteins are

able to associate with AP-1 proteins. We have also shown that overexpression of GATA-1 induced excess AP-1 binding to the IL-13 promoter as well as a significant increase in IL-13 production in mast cells. The results of the present study have shown that direct interaction between AP-1 and GATA proteins plays an important role in IL-13 transcription in mast cells.

Maertzdorf, J., A. D. M. E. Osterhaus, et al. (2002). "IL-17 Expression in Human Herpetic Stromal Keratitis: Modulatory Effects on Chemokine Production by Corneal Fibroblasts." J. Immunol. **169**(10): 5897-5903.

http://www.jimmunol.org/cgi/content/abstract/169/10/5897

Herpetic stromal keratitis (HSK) is an immunopathologic disease triggered by infection of the cornea with HSV. Key events in HSK involve the interaction between cornea-infiltrating inflammatory cells and resident cells. This interaction, in which macrophages, producing IL-1 and TNF-{alpha}, and IFN-{gamma}-producing Th1 cells play a crucial role, results in the local secretion of immune-modulatory factors and a major influx of neutrophils causing corneal lesions and blindness. The Th1-derived cytokine IL-17 has been shown to play an important role in several inflammatory diseases characterized by a massive infiltration of neutrophils into inflamed tissue. Here we show that IL-17 is expressed in corneas from patients with HSK and that the IL-17R is constitutively expressed by human corneal fibroblasts (HCF). IL-17 exhibited a strong synergistic effect with TNF-{alpha} on the induction of IL-6 and IL-8 secretion by cultured HCF. Secreted IL-8 in these cultures had a strong chemotactic effect on neutrophils. IL-17 also enhanced TNF-{alpha}- and IFN-{gamma}-induced secretion of macrophage-inflammatory proteins 1{alpha} and 3{alpha}, while inhibiting the induced secretion of RANTES. Furthermore, considerable levels of IFN-{gamma}-inducible protein 10 and matrix metalloproteinase 1 were measured in stimulated HCF cultures, while the constitutive secretion of monocyte chemotactic protein 1 remained unaffected. The data presented suggest that IL-17 may play an important role in the induction and/or perpetuation of the immunopathologic processes in human HSK by modulating the secretion of proinflammatory and neutrophil chemotactic factors by corneal resident fibroblasts.

Tsuchiya, T., S. Fukuda, et al. (2003). "Role of {gamma}{delta}T Cells in the Inflammatory Response of Experimental Colitis Mice." J. Immunol. **171**(10): 5507-5513.

http://www.jimmunol.org/cgi/content/abstract/171/10/5507

We examined the severity of experimental colitis induced by dextran sulfate sodium (DSS) using immunologically manipulated mice. C57BL/6 mice showed more severe colitis than BALB/c mice, but mice of both strains recovered fully from the disease after the removal of DSS from their drinking water. The infiltrated cells at the lesions were mainly granulocytes in normal littermates. However, C.B-17 scid, IL-7R{alpha} deficient, and TCR-C{beta}{delta} double-deficient mice showed severe colitis and did not recover from the disease even after the removal of DSS. It was found that the infiltrated cells at the lesions in the lethal strains were monocytes. Although both TCR-C{delta}-/- and TCR-C{beta}-/- mice showed severe colitis phenotypes, infiltration in the former is monocyte-dominant while that in the latter is granulocyte-dominant. Thus the type of cells that infiltrate at the lesions of DSS-induced experimental colitis may be controlled by functional T cell subsets. Immunohistological and RT-PCR analyses of the inflamed colon revealed that the murine homologue of human GRO{alpha} released by some cells under the control of {gamma}{delta}T cells is a possible candidate determining the severity of DSS-induced experimental colitis.

Saeed, R. W., S. Varma, et al. (2004). "Ethanol Blocks Leukocyte Recruitment and Endothelial Cell Activation In Vivo and In Vitro." J. Immunol. **173**(10): 6376-6383.

http://www.jimmunol.org/cgi/content/abstract/173/10/6376

Immune system impairment and increased susceptibility to infection among alcohol abusers is a significant but not well-understood problem. We hypothesized that acute ethanol administration would inhibit leukocyte recruitment and endothelial cell activation during inflammation and infection. Using LPS and carrageenan air pouch models in mice, we found that physiological concentrations of ethanol (1-5 g/kg) significantly blocked leukocyte recruitment (50-90%). Because endothelial cell activation and immune cell-endothelial cell interactions are critical regulators of leukocyte recruitment, we analyzed the effect of acute ethanol exposure on endothelial cell activation in vivo using the localized Shwartzman reaction model. In this model, ethanol markedly suppressed leukocyte accumulation and endothelial cell adhesion molecule expression in a dose-dependent manner. Finally, we examined the direct effects of ethanol on endothelial cell activation and leukocyte-endothelial cell interactions in vitro. Ethanol, at concentrations within the range found in human blood after acute exposure and below the levels that induce cytotoxicity (0.1-0.5%), did not induce endothelial cell activation, but significantly inhibited TNF-mediated endothelial cell activation, as measured by adhesion molecule (E-selectin, ICAM-1, VCAM-1) expression and chemokine (IL-8, MCP-1, RANTES) production and leukocyte adhesion in vitro. Studies exploring the potential mechanism by which ethanol suppresses endothelial cell activation revealed that ethanol blocked NF-{kappa}B nuclear entry in an I{kappa}B{alpha}-dependent manner. These findings support the hypothesis that acute ethanol overexposure may increase the risk of infection and inhibit the host inflammatory response, in part, by blocking endothelial cell activation and subsequent immune cell-endothelial cell interactions required for efficient immune cell recruitment.

Bengtson, P., A. Lundblad, et al. (2002). "Polymorphonuclear Leukocytes from Individuals Carrying the G329A Mutation in the {alpha}1,3-Fucosyltransferase VII Gene (FUT7) Roll on E- and P-Selectins." J. Immunol. **169**(7): 3940-3946.

http://www.jimmunol.org/cgi/content/abstract/169/7/3940

We recently identified several individuals carrying a missense mutation (G329A; Arg110-Gln) in the FUT7 gene encoding fucosyltransferase VII. This enzyme is involved in the biosynthesis of the sialyl Lewis x (Lex) epitope on human leukocytes, which has been identified as an important component of leukocyte ligands for E- and P-selectin. No enzyme activity was measurable in expression studies in COS-7 cells using the mutated FUT7 construct. One of the identified individuals carried this mutation homozygously. Flow cytometry analysis of polymorphonuclear leukocytes (PMN) from this individual showed a nearly complete absence of staining with mAbs directed against sialyl Lex and a diminished staining with an E-selectin IgG chimera. However, staining with P-selectin IgG chimera and Abs directed against P-selectin glycoprotein ligand-1 was not affected by the mutation. PMN from the homozygously mutated individual was further analyzed in an in vitro flow chamber assay. The number of rolling PMN and the rolling velocities on both E- and P-selectin were in the range of PMN from nonmutated individuals. FUT4 and FUT7 mRNA was quantified in PMN isolated from individuals carrying the FUT7 mutation. It was found that PMN from both FUT7 homozygously and heterozygously mutated individuals exhibited an elevated expression of FUT4 mRNA compared with PMN from FUT7 nonmutated individuals. The elevated expression of fucosyltransferase IV was reflected as an increased expression of the Lex and CD65s Ags on PMN from these individuals. The significance of the mutation was supported by transfection of BJAB cells.

Masuda, A., Y. Yoshikai, et al. (2002). "Th2 Cytokine Production from Mast Cells Is Directly Induced by Lipopolysaccharide and Distinctly Regulated by c-Jun N-Terminal Kinase and p38 Pathways." J. Immunol. **169**(7): 3801-3810.

http://www.jimmunol.org/cgi/content/abstract/169/7/3801

Mast cells secrete multiple cytokines and play an important role in allergic inflammation. Although it is widely accepted that bacteria infection occasionally worsens allergic airway inflammation, the mechanism has not been defined. In this study, we show that LPS induced Th2-associated cytokine production such as IL-5, IL-10, and IL-13 from mast cells and also synergistically enhanced production of these cytokines induced by IgE cross-linking. LPS-mediated Th2-type cytokine production was abolished in mouse bone marrow-derived mast cells derived from C3H/HeJ mice, suggesting that Toll-like receptor 4 is essential for the cytokine production. Furthermore, we found that mitogen-activated protein kinases including extracellular signal-regulated kinase 1/2, c-Jun N-terminal kinase, and p38 kinase were activated by LPS stimulation in bone marrow-derived mast cells. Inhibition of extracellular signal-regulated kinase activation has little effect on LPS-mediated cytokine production. In contrast, inhibition of c-Jun N-terminal kinase activation significantly suppressed both IL-10 and IL-13 expression at both mRNA and protein levels. Interestingly, although inhibition of p38 did not down-regulate the mRNA induction, it moderately decreased all three cytokine productions by LPS. These results indicate that LPS-mediated production of IL-5, IL-10, and IL-13 was distinctly regulated by mitogen-activated protein kinases. Our findings may indicate a clue to understanding the mechanisms of how bacteria infection worsens the clinical features of asthma.

Bas, A., S. G. Hammarstrom, et al. (2003). "Extrathymic TCR Gene Rearrangement in Human Small Intestine: Identification of New Splice Forms of Recombination Activating Gene-1 mRNA with Selective Tissue Expression." J. Immunol. **171**(7): 3359-3371.

http://www.jimmunol.org/cgi/content/abstract/171/7/3359

Two new 5'-untranslated region (5'UTR) exons were identified in the human gene for the lymphocyte-specific endonuclease recombination activating gene-1 (RAG1) required for the somatic recombination yielding functional Ag receptors. These 5'UTR exons were used in three different splice forms by jejunal lymphocytes of the T cell lineage. RAG1 mRNA containing the previously described 5'UTR exon was not expressed in these cells. Conversely, one of the new 5'UTR exons was not expressed in thymus. The new RAG1 mRNA splice forms were all expressed in immature T cells (CD2+CD7+CD3-). This cell population also expressed high levels of mRNA for the pre-T {alpha}-chain. In situ hybridization demonstrated jejunal cells expressing the new splice forms of RAG1 mRNA, both intraepithelially and in lamina propria. Pre-T {alpha}-chain mRNA-expressing cells were detected at the same sites. These results strongly suggest ongoing TCR gene rearrangement in human small intestinal mucosa, yielding T cells specially adapted for this environment. This seems to be achieved by two parallel processes, extrathymic T cell development and peripheral Ag-driven TCR editing.

Kikuchi-Maki, A., S.-i. Yusa, et al. (2003). "KIR2DL4 Is an IL-2-Regulated NK Cell Receptor That Exhibits Limited Expression in Humans but Triggers Strong IFN-{gamma} Production." J. Immunol. **171**(7): 3415-3425.

http://www.jimmunol.org/cgi/content/abstract/171/7/3415

Killer cell Ig-like receptor (KIR)2DL4 (2DL4, CD158d) was previously described as the only KIR expressed by every human NK cell. It is also structurally atypical among KIRs because it possesses a basic transmembrane residue, which is characteristic of many activating receptors, but also contains a cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM). We expressed epitope-tagged 2DL4 in an NK-like cell line to study receptor function. Three distinct 2DL4 cDNA clones were analyzed: one encoding the "conventional" 2DL4 with the cytoplasmic ITIM (2DL4.1) and two encoding different cytoplasmic truncated forms lacking the ITIM (2DL4.2 and 2DL4*). Surprisingly, one truncated receptor (2DL4.2), which is the product of a prevalent human 2DL4 allele, was not expressed on the cell surface, indicating that some individuals may lack functional 2DL4 protein expression. Conversely, both 2DL4.1 and 2DL4* were expressed on the cell surface and up-regulated by IL-2. Analysis of primary NK cells with anti-2DL4 mAb confirmed the lack of surface expression in a donor with the 2DL4.2 genotype. Donors with the 2DL4.1 genotype occasionally expressed receptor only on CD56high NK cells, although their expression was up-regulated by IL-2. Interestingly, Ab engagement of epitope-tagged 2DL4 triggered rapid and robust IFN-{gamma} production, but weak redirected cytotoxicity in an NK-like cell line, which was the opposite pattern to that observed upon engagement of another NK cell activating receptor, NKp44. Importantly, both 2DL4.1 and 2DL4* exhibited similar activation potential, indicating that the ITIM does not influence 2DL4.1 activating function. The unique activation properties of 2DL4 suggest linkage to a distinct signaling pathway.

Meusel, T. R. and F. Imani (2003). "Viral Induction of Inflammatory Cytokines in Human Epithelial Cells Follows a p38 Mitogen-Activated Protein Kinase-Dependent but NF-{kappa}B-Independent Pathway." J. Immunol. **171**(7): 3768-3774.

http://www.jimmunol.org/cgi/content/abstract/171/7/3768

The initial step in an immune response toward a viral infection is the induction of inflammatory cytokines. This innate immune response is mediated by expression of a variety of cytokines exemplified by TNF-{alpha} and IL-1{beta}. A key signal for the recognition of intracellular viral infections is the presence of dsRNA. Viral infections and dsRNA treatment can activate several signaling pathways including the protein kinase R pathway, mitogen-activated protein kinase (MAPK) pathways, and NF-{kappa}B, which are important in the expression of inflammatory cytokines. We previously reported that activation of protein kinase R was required for dsRNA induction of TNF-{alpha}, but not for IL-1{beta}. In this study, we report that activation of the p38 MAPK pathway by respiratory viral infections is necessary for induction of inflammatory cytokines in human bronchial epithelial cells. Inhibition of p38 MAPK by two different pharmacological inhibitors showed that expression of both TNF-{alpha} and IL-1{beta} required activation of this signaling pathway. Interestingly, inhibition of NF-{kappa}B did not significantly reduce viral induction of either cytokine. Our data show that, during the initial infections of epithelial cells with respiratory viruses, activation of the p38 MAPK pathway is associated with induction of inflammation, and NF-{kappa}B activation may be less important than previously suggested.

Mason, J. M., M. D. Naidu, et al. (2004). "IL-4-Induced Gene-1 Is a Leukocyte L-Amino Acid Oxidase with an Unusual Acidic pH Preference and Lysosomal Localization." J. Immunol. **173**(7): 4561-4567.

http://www.jimmunol.org/cgi/content/abstract/173/7/4561

IL-4-induced gene-1 (Il4i1 or Fig1) initially isolated as a gene of unknown function from mouse B lymphocytes, is limited in expression to primarily immune tissues and genetically maps to a region of susceptibility to autoimmune disease. The predicted Il4i1 protein (IL4I1) sequence is most similar to apoptosis-inducing protein and Apoxin I, both L-amino acid oxidases (LAAO; Enzyme Commission 1.4.3.2). We demonstrate that IL4I1 has unique LAAO properties. IL4I1 has

preference for aromatic amino acid substrates, having highest specific activity with phenylalanine. In support of this selectivity, IL4I1 is inhibited by aromatic competitors (benzoic acid and para-aminobenzoic acid), but not by nonaromatic LAAO inhibitors. Il4i1 protein and enzyme activity is found in the insoluble fraction of transient transfections, implying an association with cell membrane and possibly intracellular organelles. Indeed, IL4I1 has the unique property of being most active at acidic pH (pH 4), suggesting it may reside preferentially in lysosomes. IL4I1 is N-linked glycosylated, a requirement for lysosomal localization. Confocal microscopy of cells expressing IL4I1 translationally fused to red fluorescent protein demonstrated that IL4I1 colocalized with GFP targeted to lysosomes and with acriflavine, a green fluorescent dye that is taken up into lysosomes. Thus, IL4I1 is a unique mammalian LAAO targeted to lysosomes, an important subcellular compartment involved in Ag processing. F1"> WIDTH=200 HEIGHT=88 SRC="/small/zim0190481590001.gif" ALT=" "> View larger version (16K): F1">[in this window] F1" onClick="startTarget('F1', 590, 394); this.href='F1'" onMouseOver="window.status='View figure in a separate window'; return true" TARGET="F1">[in a new window] FIGURE 1. IL4I1 is most similar to LAAO. Schematic of mouse IL4I1 is shown to scale with N-linked glycosylation sites (N-Gly) ({blacksquare}) and potential tyrosine (Y) phosphorylation sites ({circ}). Predicted FAD cofactor binding domains are indicated by white boxes in the schematic corresponding to the three black lines underneath. The signal peptide and short regions of similarity to many known FAD-binding proteins (MANY) and to specific proteins (phytoene desaturase (PDS), monoamine oxidase (MAO), and tryptophan 2-monooxygenase (TMO)) are shown by the next row of black lines underneath the schematic, corresponding to the gray-spotted boxes. Regions of similarity to bacterial (Bacillus cereus) and eukaryotic (fish (Scomber japonicus) and snake (Crotalus atrox, C. adamanteus)) LAAO are shown by the remaining black lines underneath the schematic of IL4I1.

Adriouch, S., C. Dox, et al. (2002). "Cutting Edge: A Natural P451L Mutation in the Cytoplasmic Domain Impairs the Function of the Mouse P2X7 Receptor." J. Immunol. **169**(8): 4108-4112.

http://www.jimmunol.org/cgi/content/abstract/169/8/4108

The P2X7 receptor (P2X7R) is an ATP-gated channel that mediates apoptosis of cells of the immune system. The capacity of P2X7R to form large pores depends on its large cytoplasmic tail, which harbors a putative TNFR-related death domain. Previous transfection studies indicated that mouse P2X7R forms pores much less efficiently than its counterparts from humans and rats. In this study, we demonstrate that an allelic mutation (P451L) in the predicted death domain of P2X7R confers a drastically reduced sensitivity to ATP-induced pore formation in cells from some commonly used strains of mice, i.e., C57BL/6 and DBA/2. In contrast, most other strains of mice, including strains derived from wild mice, carry P451 at this position as do rats and humans. The effects of the P451L mutation resemble those of the E496A mutation in human P2X7R. These P2X7R mutants may provide useful tools to decipher the molecular mechanisms leading to pore formation.

Ge, B., O. Li, et al. (2003). "NF-{kappa}B Regulates BCL3 Transcription in T Lymphocytes Through an Intronic Enhancer." J. Immunol. **171**(8): 4210-4218.

http://www.jimmunol.org/cgi/content/abstract/171/8/4210

Exposure to soluble protein Ags in vivo leads to abortive proliferation of responding T cells. In the absence of a danger signal, artificially provided by adjuvants, most responding cells die, and the remainder typically become anergic. The adjuvant-derived signals provided to T cells are poorly understood, but recent work has identified BCL3 as the gene, of those tested, with the greatest differential transcriptional response to adjuvant administration in vivo. As an initial step in analyzing transcriptional responses of BCL3 in T cells, we have identified candidate regulatory

regions within the locus through their evolutionary conservation and by analysis of DNase hypersensitivity. An evolutionarily conserved DNase hypersensitive site (HS3) within intron 2 was found to act as a transcriptional enhancer in response to stimuli that mimic TCR activation, namely, PHA and PMA. In luciferase reporter gene constructs transiently transfected into the Jurkat T cell line, the HS3 enhancer can cooperate not only with the BCL3 promoter, but also with an exogenous promoter from herpes simplex thymidine kinase. Deletional analysis revealed that a minimal sequence of [~]81 bp is required for full enhancer activity. At the 5' end of this minimal sequence is a {kappa}B site, as confirmed by EMSAs. Mutation of this site in the context of the full-length HS3 abolished enhancer activity. Cotransfection with NF-{kappa}B p65 expression constructs dramatically increased luciferase activity, even without stimulation. Conversely, cotransfection with the NF-{kappa}B inhibitor I{kappa}B{alpha} reduced activation. Together, these results demonstrate a critical role for NF-{kappa}B in BCL3 transcriptional up-regulation by TCR-mimetic signals.

Shoham, T., R. Rajapaksa, et al. (2003). "The Tetraspanin CD81 Regulates the Expression of CD19 During B Cell Development in a Postendoplasmic Reticulum Compartment." J. Immunol. **171**(8): 4062-4072.

http://www.jimmunol.org/cgi/content/abstract/171/8/4062

CD81 is a widely expressed tetraspanin that associates in B cells with CD19 in the CD19-CD21-CD81 signaling complex. CD81 is necessary for normal CD19 expression; cd81-/- B cells express lower levels of CD19, especially cd81-/- small pre-BII cells, which are almost devoid of surface CD19. The dependence of CD19 expression on CD81 is specific to this particular tetraspanin since cd9-/- B cells express normal levels of CD19. Furthermore, expression of human CD81 in mouse cd81-/- B cells restored surface CD19 to normal levels. Quantitative analysis of CD19 mRNA demonstrated normal levels, even in cd81-/- pre-BII cells. Analysis of CD19 at the protein level identified two CD19 glycoforms in both wild-type and cd81-/- B cells. The higher Mr glycoform is significantly reduced in cd81-/- B cells and is endoglycosidase H (endo-H) resistant. In contrast, the low Mr glycoform is comparably expressed in cd81-/- and in wild-type B cells and is endo-H sensitive. Because endo-H sensitivity is tightly correlated with endoplasmic reticulum localization, we suggest that the dependency of CD19 expression on CD81 occurs in a postendoplasmic reticulum compartment where CD81 is necessary for normal trafficking or for surface membrane stability of CD19.

Boyartchuk, V., M. Rojas, et al. (2004). "The Host Resistance Locus sst1 Controls Innate Immunity to Listeria monocytogenes Infection in Immunodeficient Mice." J. Immunol. **173**(8): 5112-5120.

http://www.jimmunol.org/cgi/content/abstract/173/8/5112

Epidemiological, clinical, and experimental approaches have convincingly demonstrated that host resistance to infection with intracellular pathogens is significantly influenced by genetic polymorphisms. Using a mouse model of infection with virulent Mycobacterium tuberculosis (MTB), we have previously identified the sst1 locus as a genetic determinant of host resistance to tuberculosis. In this study we demonstrate that susceptibility to another intracellular pathogen, Listeria monocytogenes, is also influenced by the sst1 locus. The contribution of sst1 to anti-listerial immunity is much greater in immunodeficient scid mice, indicating that this locus controls innate immunity and becomes particularly important when adaptive immunity is significantly depressed. Similar to our previous observations using infection with MTB, the resistant allele of sst1 prevents formation of necrotic infectious lesions in vivo. We have shown that macrophages obtained from sst1-resistant congenic mice possess superior ability to kill L. monocytogenes in vitro. The bactericidal effect of sst1 is dependent on IFN-{gamma} activation and reactive oxygen

radical production by activated macrophages after infection, but is independent of NO production. It is possible that there is a single gene that controls common IFN-dependent macrophage function, which is important in the pathogenesis of infections caused by both MTB and L. monocytogenes. However, host resistance to the two pathogens may be controlled by two different polymorphic genes encoded within the sst1 locus. The polymorphic gene(s) encoded within the sst1 locus that controls macrophage interactions with the two intracellular pathogens remains to be elucidated.

Tagawa, T., H. Nishimura, et al. (2004). "V{delta}1+ {gamma}{delta} T Cells Producing CC Chemokines May Bridge a Gap between Neutrophils and Macrophages in Innate Immunity during Escherichia coli Infection in Mice." J. Immunol. **173**(8): 5156-5164.

http://www.jimmunol.org/cgi/content/abstract/173/8/5156

An influx of neutrophils followed a short time later by an influx of macrophages to the infected site plays a key role in innate immunity against Escherichia coli infection. We found in this study that V{delta}1-/- mice exhibited impaired accumulation of peritoneal macrophages but not neutrophils and delayed bacterial clearance after i.p. inoculation with E. coli. Peritoneal {gamma}{delta} T cells from E. coli-infected wild-type mice produced CCL3/MIP-1{alpha} and CCL5/RANTES in response to {gamma}{delta} TCR triggering in vitro, whereas such production was not evident in {gamma}{delta} T cells from E. coli-infected V{delta}1-/- mice. Neutralization of CCL3/MIP-1{alpha} by a specific mAb in vivo significantly inhibited the accumulation of macrophages in the peritoneal cavity after E. coli infection, resulting in exacerbated bacterial growth in the peritoneal cavity. These results suggest that V{delta}1+ {gamma}{delta} T cells bridge a gap between neutrophils and macrophages in innate immunity during E. coli infection mediated by production of CC chemokines, enhancing macrophage trafficking to the site of infection.

Bengten, E., S. M. A. Quiniou, et al. (2002). "The IgH Locus of the Channel Catfish, Ictalurus punctatus, Contains Multiple Constant Region Gene Sequences: Different Genes Encode Heavy Chains of Membrane and Secreted IgD." J. Immunol. **169**(5): 2488-2497.

http://www.jimmunol.org/cgi/content/abstract/169/5/2488

The {delta}-chain of catfish IgD was initially characterized as a unique chimeric molecule containing a rearranged VDJ spliced to C{micro}1, seven C domain-encoding exons ({delta}1-{delta}7), and a transmembrane tail. The presence of cDNA forms showing splicing of {delta}7 to an exon encoding a secretory tail was interpreted to indicate that membrane ({delta}m) and secreted ({delta}s) forms were likely expressed from a single gene by alternative RNA processing. Subsequent cloning and sequence analyses have unexpectedly revealed the presence of three {delta} C region genes, each linked to a {micro} gene or pseudogene. The first (IGHD1) is located 1.6 kb 3' of the functional C{micro} (IGHM1). The second (IGHD3) is positioned immediately downstream of a pseudo C{micro} (IGHM3P), [~]725 kb 5' of IGHM1. These two {delta} genes are highly similar in sequence and each contains a tandem duplication of {delta}2-{delta}3-{delta}4. However, IGHD1 has a terminal exon encoding the transmembrane region, whereas IGHD3 has a single terminal exon encoding a secreted tail. The occurrence of IGHD3 immediately downstream of a {micro} pseudogene indicates that the putative {delta}s product may not be expressed as a chimeric {micro}{delta} molecule. Western blots and protein sequencing data indicate that an IGHD3-encoded protein is expressed in catfish serum. Thus, catfish {delta}m transcripts appear to originate from IGHD1, whereas {delta}s transcripts originate from IGHD3 rather than, as previously inferred, from a single expressed {delta} gene. The third {delta} (IGHD2) is associated with a pseudo C{micro} (IGHM2P); its presence is inferred by Southern blot analyses.

Estes, J. D., B. F. Keele, et al. (2002). "Follicular Dendritic Cell-Mediated Up-Regulation of CXCR4 Expression on CD4 T Cells and HIV Pathogenesis." J. Immunol. **169**(5): 2313-2322.

http://www.jimmunol.org/cgi/content/abstract/169/5/2313

Follicular dendritic cells (FDCs) represent a major reservoir of HIV, and active infection occurs surrounding these cells, suggesting that this microenvironment is highly conducive to virus transmission. Because CD4 T cells around FDCs in germinal centers express the HIV coreceptor, CXCR4, whereas CD4 lymphocytes in many other sites do not, it prompted the hypothesis that FDCs may increase CXCR4 expression on CD4 T cells, thereby facilitating infection. To test this, HIV receptor/coreceptor expression was determined on CD4 T cells cultured with or without FDCs, and its consequence to infection was assessed by measuring virus binding and entry. FDCs had little effect on CCR5 or CD4 expression but increased CXCR4 expression on CD4 T cells. FDC-mediated up-regulation of CXCR4 on CD4 T cells occurred by 24 h and was sustained for at least 96 h in vitro, and FDC-CD4 T cell contact was necessary. Importantly, increased CXCR4 expression directly correlated with increased binding and entry of HIV-1 X4 isolates. Furthermore, CD4+CD57+ germinal center T cells expressed high levels of CXCR4 and supported enhanced entry of X4 HIV compared with other CD4 T cells from the same tissue. Thus, in addition to serving as a reservoir of infectious virus, FDCs render surrounding germinal center T cells highly susceptible to infection with X4 isolates of HIV-1.

Gray Parkin, K., R. P. Stephan, et al. (2002). "Expression of CD28 by Bone Marrow Stromal Cells and Its Involvement in B Lymphopoiesis." J. Immunol. **169**(5): 2292-2302.

http://www.jimmunol.org/cgi/content/abstract/169/5/2292

Young mice lacking CD28 have normal numbers of peripheral B cells; however, abnormalities exist in the humoral immune response that may result from an intrinsic defect in the B cells. The goal of this study was to assess whether CD28 could be involved in the development of B cells. CD28 mRNA was detected preferentially in the fraction of bone marrow enriched for stromal cells. Flow cytometry and RT-PCR analysis demonstrated that CD28 was also expressed by primary-cultured stromal cells that supported B lymphopoiesis. Confocal microscopy revealed that in the presence of B-lineage cells, CD28 was localized at the contact interface between B cell precursors and stromal cells. In addition, CD80 was detected on 2-6% of freshly isolated pro- and pre-B cells, and IL-7 stimulation led to induction of CD86 on 15-20% of pro- and pre-B cells. We also observed that stromal cell-dependent production of B-lineage cells in vitro was greater on stromal cells that lacked CD28. Finally, the frequencies of B-lineage precursors in the marrow from young (4- to 8-wk-old) CD28-/- mice were similar to those in wild-type mice; however, older CD28-/- mice (15-19 mo old) exhibited a 30% decrease in pro-B cells and a 50% decrease in pre-B cells vs age-matched controls. Our results suggest that CD28 on bone marrow stromal cells participates in stromal-dependent regulation of B-lineage cells in the bone marrow. The localization of CD28 at the stromal cell:B cell precursor interface suggests that molecules important for T cell:B cell interactions in the periphery may also participate in stromal cell:B cell precursor interactions in the bone marrow.

Lee, S.-K., J. Kalinowski, et al. (2002). "1,25 (OH)2 Vitamin D3-Stimulated Osteoclast Formation in Spleen-Osteoblast Cocultures Is Mediated in Part by Enhanced IL-1{alpha} and Receptor Activator of NF-{kappa}B Ligand Production in Osteoblasts." J. Immunol. **169**(5): 2374-2380.

http://www.jimmunol.org/cgi/content/abstract/169/5/2374

We examined the ability of 1,25 (OH)2 vitamin D3 (Vit D) to stimulate osteoclast-like cell (OCL) formation in cocultures of spleen cells and primary calvarial osteoblasts from wild-type (WT) and IL-1R type 1-deficient (knockout; KO) mice. Vit D dose dependently increased OCL in cocultures containing WT osteoblasts. In contrast, there was a 90% reduction in OCL numbers in cocultures containing KO osteoblasts. In cocultures with either WT or KO osteoblasts, treatment with Vit D increased receptor activator of NF-{kappa}B ligand mRNA by 17-, 19-, or 3.5-fold, respectively. Vit D decreased osteoprotegerin mRNA to undetectable in all groups. Intracellular IL-1{alpha} protein increased after Vit D treatment in cocultures containing WT, but not KO osteoblasts. We also examined direct effects of Vit D, IL-1{alpha}, and their combination on gene expression in primary osteoblasts. In WT cells, Vit D and IL-1 stimulated receptor activator of NF-{kappa}B ligand mRNA expression by 3- and 4-fold, respectively, and their combination produced a 7-fold increase. Inhibition of osteoprotegerin mRNA in WT cells was partial with either agent alone and greatest with their combination. In KO cells, only Vit D stimulated a response. IL-1 alone increased IL-1{alpha} protein expression in WT osteoblasts. However, in combination with Vit D, there was a synergistic response (100-fold increase). In KO cultures, there were no effects of IL-1, Vit D, or their combination on IL-1{alpha} protein. These results demonstrate interactions between IL-1 and Vit D in primary osteoblasts that appear important in both regulation of IL-1{alpha} production and the ability of Vit D to support osteoclastogenesis.

Voisine, C., F.-X. Hubert, et al. (2002). "Two Phenotypically Distinct Subsets of Spleen Dendritic Cells in Rats Exhibit Different Cytokine Production and T Cell Stimulatory Activity." J. Immunol. **169**(5): 2284-2291.

http://www.jimmunol.org/cgi/content/abstract/169/5/2284

We recently reported that splenic dendritic cells (DC) in rats can be separated into CD4+ and CD4- subsets and that the CD4- subset exhibited a natural cytotoxic activity in vitro against tumor cells. Moreover, a recent report suggests that CD4- DC could have tolerogenic properties in vivo. In this study, we have analyzed the phenotype and in vitro T cell stimulatory activity of freshly isolated splenic DC subsets. Unlike the CD4- subset, CD4+ splenic DC expressed CD5, CD90, and signal regulatory protein {alpha} molecules. Both fresh CD4- and CD4+ DC displayed an immature phenotype, although CD4+ cells constitutively expressed moderate levels of CD80. The half-life of the CD4-, but not CD4+ DC in vitro was extremely short but cells could be rescued from death by CD40 ligand, IL-3, or GM-CSF. The CD4- DC produced large amounts of the proinflammatory cytokines IL-12 and TNF-{alpha} and induced Th1 responses in allogeneic CD4+ T cells, whereas the CD4+ DC produced low amounts of IL-12 and no TNF-{alpha}, but induced Th1 and Th2 responses. As compared with the CD4+ DC that strongly stimulated the proliferation of purified CD8+ T cells, the CD4- DC exhibited a poor CD8+ T cell stimulatory capacity that was substantially increased by CD40 stimulation. Therefore, as previously shown in mice and humans, we have identified the existence of a high IL-12-producing DC subset in the rat that induces Th1 responses. The fact that both the CD4+ and CD4- DC subsets produced low amounts of IFN-{alpha} upon viral infection suggests that they are not related to plasmacytoid DC.

Bayley, J.-P., J. G. I. van Rietschoten, et al. (2003). "Allele-Specific Expression of the IL-1{alpha} Gene in Human CD4+ T Cell Clones." J. Immunol. **171**(5): 2349-2353.

http://www.jimmunol.org/cgi/content/abstract/171/5/2349

A number of reports have described the monoallelic expression of murine cytokine genes. Here we describe the monoallelic expression of the human IL-1{alpha} gene in CD4+ T cells. Analysis of peripheral blood T cell clones derived from healthy individuals revealed that the IL-1{alpha} gene shows predominantly monoallelic expression. Monoallelic expression was observed in Th0, Th1, and Th2 cell clones. In addition, we demonstrate monoallelic expression in T cell clones from rheumatoid arthritis patients derived from synovial fluid of the knee joint, suggesting that the occurrence of this phenomenon is not different from that in clones derived from healthy individuals. The finding of monoallelic expression of a cytokine gene in human CD4+ T cell clones provides evidence for allele-specific silencing/activation as another layer of regulation of IL-1{alpha} gene expression.

Weinstock-Guttman, B., D. Badgett, et al. (2003). "Genomic Effects of IFN-{beta} in Multiple Sclerosis Patients." J. Immunol. **171**(5): 2694-2702.

http://www.jimmunol.org/cgi/content/abstract/171/5/2694

The purpose of this report was to characterize the dynamics of the gene expression cascades induced by an IFN-{beta}-1a treatment regimen in multiple sclerosis patients and to examine the molecular mechanisms potentially capable of causing heterogeneity in response to therapy. In this open-label pharmacodynamic study design, peripheral blood was obtained from eight relapsing-remitting multiple sclerosis patients just before and at 1, 2, 4, 8, 24, 48, 120, and 168 h after i.m. injection of 30 {micro}g of IFN-{beta}-1a. The total RNA was isolated from monocyte-depleted PBL and analyzed using cDNA microarrays containing probes for >4000 known genes. IFN-{beta}-1a treatment resulted in selective, time-dependent effects on multiple genes. The mRNAs for genes implicated in the anti-viral response, e.g., double-stranded RNA-dependent protein kinase, myxovirus resistance proteins 1 and 2, and guanylate binding proteins 1 and 2 were rapidly induced within 1-4 h of IFN-{beta} treatment. The mRNAs for several genes involved in IFN-{beta} signaling, such as IFN-{alpha}/{beta} receptor-2 and Stat1, were also increased. The mRNAs for lymphocyte activation markers, such as IFN-induced transmembrane protein 1 (9-27), IFN-induced transmembrane protein 2 (1-8D), {beta}2-microglobulin, and CD69, were also increased in a time-dependent manner. The findings demonstrate that IFN-{beta} treatment induces specific and time-dependent changes in multiple mRNAs in lymphocytes of multiple sclerosis patients that could provide a framework for rapid monitoring of the response to therapy.

Garcia-Martinez, L. F., M. W. Appleby, et al. (2004). "A Novel Mutation in CD83 Results in the Development of a Unique Population of CD4+ T Cells." J. Immunol. **173**(5): 2995-3001.

http://www.jimmunol.org/cgi/content/abstract/173/5/2995

Using a mouse mutagenesis screen, we have identified CD83 as being critical for the development of CD4+ T cells and for their function postactivation. CD11c+ dendritic cells develop and function normally in mice with a mutated CD83 gene but CD4+ T cell development is substantially reduced. Additionally, we now show that those CD4+ cells that develop in a CD83 mutant animal fail to respond normally following allogeneic stimulation. This is at least in part due to an altered cytokine expression pattern characterized by an increased production of IL-4 and IL-10 and diminished IL-2 production. Thus, in addition to its role in selection of CD4+ T cells, absence of CD83 results in the generation of cells with an altered activation and cytokine profile.

Matsumoto, Y., Y. Tsukada, et al. (2004). "C Protein-Induced Myocarditis and Subsequent Dilated

Cardiomyopathy: Rescue from Death and Prevention of Dilated Cardiomyopathy by Chemokine Receptor DNA Therapy." J. Immunol. **173**(5): 3535-3541.

http://www.jimmunol.org/cgi/content/abstract/173/5/3535

Severe experimental autoimmune myocarditis and subsequent dilated cardiomyopathy (DCM) were successfully produced in Lewis rats by immunization with recombinant cardiac C protein. Seventy-five percent of immunized rats died between days 15 and 49 postimmunization, and all of the survived rats showed typical DCM characterized by the presence of ventricular dilatation and extensive fibrosis. Immunopathological and chemokine analysis during the acute phase revealed that there were marked macrophage infiltration with myocyte necrosis and up-regulation of MCP-1 and IFN-{gamma}-inducible protein-10 (IP-10). Based on these findings, we prepared plasmid DNAs encoding the binding site of CCR2 and CXCR3, which are receptors for MCP-1 and IP-10, respectively. The culture supernatant of cells transfected with these DNAs inhibited the migration of T cells and macrophages induced by MCP-1 and IP-10. Remarkably, administration of the DNAs to C protein-immunized rats prevented the disease progression and rescued animals from death. The present study has demonstrated for the first time that gene therapy targeting the chemokine receptor could be a powerful tool for the control of experimental autoimmune myocarditis and DCM.

Sasso, E. H., M. Martinez, et al. (2004). "Frequent Joining of Bcl-2 to a JH6 Gene in Hepatitis C Virus-Associated t(14;18)." J. Immunol. **173**(5): 3549-3556.

http://www.jimmunol.org/cgi/content/abstract/173/5/3549

The t(14;18) chromosomal translocation, which joins the Bcl-2 proto-oncogene to an Ig JH gene, has increased prevalence in patients chronically infected with hepatitis C virus (HCV). We now establish a link between the molecular structure and clinical occurrence of HCV-associated t(14;18). A t(14;18) was detected by PCR in leukocytes from 22 of 46 HCV-infected patients (48%) and 11 of 54 healthy controls (20%) ($p = 0.0053$). Nucleotide sequence analysis of the Bcl-2/JH joins found a JH6 gene in 18 of 22 (82%) t(14;18) from HCV+ patients, and 3 of 8 (38%) from controls ($p = 0.031$). The t(14;18) rarely contained JH gene mutations, or an intervening region sequence suggestive of D gene rearrangement or templated nucleotide insertion. Analysis of published t(14;18) nucleotide sequences established that the JH6 prevalence in t(14;18) from normal/nonneoplastic controls (48%) was significantly lower than in t(14;18) from our HCV+ patients ($p = 0.004$) or from non-Hodgkin's lymphomas (66%, $p = 0.003$). We conclude that the increased prevalence of t(14;18) in HCV+ patients occurs with a strong bias for Bcl-2/JH6 joins. In this regard, HCV-associated t(14;18) more closely resemble t(14;18) in lymphomas than t(14;18) from normal subjects.

Ueta, M., T. Nochi, et al. (2004). "Intracellularly Expressed TLR2s and TLR4s Contribution to an Immunosilent Environment at the Ocular Mucosal Epithelium." J. Immunol. **173**(5): 3337-3347.

http://www.jimmunol.org/cgi/content/abstract/173/5/3337

Epithelial cells are key players in the first line of defense offered by the mucosal immune system against invading pathogens. In the present study we sought to determine whether human corneal epithelial cells expressing Toll-like receptors (TLRs) function as pattern-recognition receptors in the innate immune system and, if so, whether these TLRs act as a first line of defense in ocular mucosal immunity. Incubation of human primary corneal epithelial cells and the human corneal epithelial cell line (HCE-T) with peptidoglycan or LPS did not lead to activation, at the level of

DNA transcription, of NF-{kappa}B or the secretion of inflammation-associated molecules such as IL-6, IL-8, and human {beta}-defensin-2. However, when incubated with IL-1{alpha} to activate NF-{kappa}B, the production by these cells of such inflammatory mediators was enhanced. Human corneal epithelial cells were observed to express both TLR2- and TLR4-specific mRNA as well as their corresponding proteins intracellularly, but not at the cell surface. However, even when LPS was artificially introduced into the cytoplasm, it did not lead to the activation of epithelial cells. Taken together, our results demonstrate that the intracellular expression of TLR2 and TLR4 in human corneal epithelial cells fails to elicit innate immune responses and therefore, perhaps purposely, contributes to an immunosilent environment at the ocular mucosal epithelium.

Manne, J., M. J. Mastrangelo, et al. (2002). "TCR Rearrangement in Lymphocytes Infiltrating Melanoma Metastases After Administration of Autologous Dinitrophenyl-Modified Vaccine." J. Immunol. **169**(6): 3407-3412.

http://www.jimmunol.org/cgi/content/abstract/169/6/3407

Administration of a vaccine consisting of autologous melanoma cells modified with a hapten, dinitrophenyl (DNP), induces T cell infiltration of metastatic sites. We have reported an analysis of these infiltrating T cells, indicating that certain TCR-V{beta} gene segments are greatly overexpressed. In this study, we investigate the rearrangement of the TCR-V{beta} as well as the junctional diversity in T cells infiltrating melanoma metastases following treatment with DNP vaccine. In 19 of 26 control specimens, V-D-J length analysis showed the expected polyclonal patterns. In contrast, postvaccine tumors from 9 of 10 patients showed dominant peaks of V-D-J junction size in one or more V{beta} families. Dominant peaks were seen most frequently in six V{beta} families (V{beta}7, 12, 13, 14, 16, and 23) and were never seen in seven others. Further analysis of the oligoclonal V{beta} products showed dominant peaks in the J region as well. Of particular interest was the finding that V{beta} and J{beta} peaks were similar in inflamed metastases obtained at different times or from different sites from the same patient. Although 6 of 10 patients expressed HLA-A1, there was no common pattern of TCR rearrangements among them. Finally, the amplified PCR products from seven of these specimens were cloned and sequenced and the amino acid sequence of the complementarity-determining region 3 was deduced. In six of seven specimens, the same complementarity-determining region 3 sequence was repeated in at least two clones and in five of seven in at least three clones. Our study indicates that DNP vaccine induces the expansion of particular T cell clones that may be agents of its antitumor effects.

Mencacci, A., C. Montagnoli, et al. (2002). "CD80+Gr-1+ Myeloid Cells Inhibit Development of Antifungal Th1 Immunity in Mice with Candidiasis." J. Immunol. **169**(6): 3180-3190.

http://www.jimmunol.org/cgi/content/abstract/169/6/3180

To find out whether polymorphonuclear neutrophils (PMN), abundantly recruited in disseminated Candida albicans infection, could directly affect the activation of Th cells we addressed the issues as to whether murine PMN, like their human counterparts, express costimulatory molecules and the functional consequence of this expression in terms of antifungal immune resistance. To this purpose, we assessed 1) the expression of CD80 (B7-1) and CD86 (B7-2) molecules on peripheral, splenic, and inflammatory murine Gr-1+ PMN; 2) its modulation upon interaction with C. albicans in vitro, in vivo, and in human PMN; 3) the effect of Candida exposure on the ability of murine PMN to affect CD4+ Th1 cell proliferation and cytokine production; and 4) the mechanism responsible for this effect. Murine PMN constitutively expressed CD80 molecules on both the surface and intracellularly; however, in both murine and human PMN, CD80 expression was differentially modulated upon interaction with Candida yeasts or hyphae in vitro as well as in

infected mice. The expression of the CD86 molecule was neither constitutive nor inducible upon exposure to the fungus. In vitro, Gr-1+ PMN were found to inhibit the activation of IFN-{gamma}-producing CD4+ T cells and to induce apoptosis through a CD80/CD28-dependent mechanism. A population of CD80+Gr-1+ myeloid cells was found to be expanded in conventional as well as in bone marrow-transplanted mice with disseminated candidiasis, but its depletion increased the IFN-{gamma}-mediated antifungal resistance. These data indicate that alternatively activated PMN expressing CD80 may adversely affect Th1-dependent resistance in fungal infections.

Nakajima, P. B. and M. J. Bosma (2002). "Variable Diversity Joining Recombination: Nonhairpin Coding Ends in Thymocytes of SCID and Wild-Type Mice." J. Immunol. **169**(6): 3094-3104.

http://www.jimmunol.org/cgi/content/abstract/169/6/3094

Initiation of V(D)J recombination results in broken DNA molecules with blunt recombination signal ends and covalently sealed (hairpin) coding ends. In SCID mice, coding joint formation is severely impaired and hairpin coding ends accumulate as a result of a deficiency in the catalytic subunit of DNA-dependent protein kinase, an enzyme involved in the repair of DNA double-strand breaks. In this study, we report that not all SCID coding ends are hairpinned. We have detected open J{delta}1 and D{delta}2 coding ends at the TCR{delta} locus in SCID thymocytes. Approximately 25% of 5'D{delta}2 coding ends were found to be open. Large deletions and abnormally long P nucleotide additions typical of SCID D{delta}2-J{delta}1 coding joints were not observed. Most J{delta}1 and D{delta}2 coding ends exhibited 3' overhangs, but at least 20% had unique 5' overhangs not previously detected in vivo. We suggest that the SCID DNA-dependent protein kinase deficiency not only reduces the efficiency of hairpin opening, but also may affect the specificity of hairpin nicking, as well as the efficiency of joining open coding ends.

Bave, U., M. Magnusson, et al. (2003). "Fc{gamma}RIIa Is Expressed on Natural IFN-{alpha}-Producing Cells (Plasmacytoid Dendritic Cells) and Is Required for the IFN-{alpha} Production Induced by Apoptotic Cells Combined with Lupus IgG." J. Immunol. **171**(6): 3296-3302.

http://www.jimmunol.org/cgi/content/abstract/171/6/3296

An ongoing production of IFN-{alpha} may be of etiopathogenic significance in systemic lupus erythematosus (SLE). It may be due to the natural IFN-producing cells (NIPC), also termed plasmacytoid dendritic cells (PDC), activated by immune complexes that contain nucleic acids derived from apoptotic cells. We here examined the role of Fc{gamma}R in the IFN-{alpha} production in vitro by PBMC induced by the combination of apoptotic U937 cells and autoantibody-containing IgG from SLE patients (SLE-IgG). The Fc portion of the SLE-IgG was essential to induce IFN-{alpha} production, because Fab fragments or F(ab')2 were ineffective. Normal, especially heat-aggregated, IgG inhibited the IFN-{alpha} production, suggesting a role for Fc{gamma}R on PBMC. Using blocking anti-Fc{gamma}R Abs, the Fc{gamma}RIIa,c (CD32) but not Fc{gamma}RI or Fc{gamma}RIII were shown to be involved in the IFN-{alpha} induction by apoptotic cells combined with SLE-IgG, but not by HSV or CpG DNA. In contrast, the action of all of these inducers was inhibited by the anti-Fc{gamma}RIIa,b,c mAb AT10 or heat-aggregated IgG. Flow cytometric analysis revealed that [~]50% of the BDCA-2-positive PBMC, i.e., NIPC/PDC, expressed low but significant levels of Fc{gamma}RII, as did most of the actual IFN-{alpha} producers activated by HSV. RT-PCR applied to NIPC/PDC purified by FACS demonstrated expression of Fc{gamma}RIIa, but not of Fc{gamma}RIIb or Fc{gamma}RIIc. We conclude that Fc{gamma}RIIa on NIPC/PDC is involved in the activation of IFN-{alpha} production by interferogenic immune complexes, but may also mediate inhibitory signals. The Fc{gamma}RIIa could therefore have a key function in NIPC/PDC and be a potential therapeutic target in SLE.

Clancy, R. M., C. B. Backer, et al. (2003). "Cytokine Polymorphisms and Histologic Expression in Autopsy Studies: Contribution of TNF-{alpha} and TGF-{beta}1 to the Pathogenesis of Autoimmune-Associated Congenital Heart Block." J. Immunol. **171**(6): 3253-3261.

http://www.jimmunol.org/cgi/content/abstract/171/6/3253

Although Abs to SSA/Ro-SSB/La are necessary for the development of congenital heart block (CHB), the low frequency suggests that fetal factors are contributory. Because CHB involves a cascade from inflammation to scarring, polymorphisms of the TNF-{alpha} promoter region and codons 10 and 25 of the TGF-{beta} gene were evaluated in 88 children (40 CHB, 17 rash, 31 unaffected siblings) and 74 mothers from the Research Registry for Neonatal Lupus (NL). Cytokine expression was assessed in autopsy material from two fetuses with CHB. Significantly increased frequency of the -308A (high-producer) allele of TNF-{alpha} was observed in all NL groups compared with controls. In contrast, the TGF-{beta} polymorphism Leu10 (associated with increased fibrosis) was significantly higher in CHB children (genotypic frequency 60%, allelic frequency 78%) than unaffected offspring (genotypic frequency 29%, p = 0.016; allelic frequency 56%, p = 0.011) and controls, while there were no significant differences between controls and other NL groups. For the TGF-{beta} polymorphism, Arg25, there were no significant differences between NL groups and controls. In fetal CHB hearts, protein expression of TGF-{beta}, but not TNF-{alpha}, was demonstrated in septal regions, extracellularly in the fibrous matrix, and intracellularly in macrophage infiltrates. Age-matched fetal hearts from voluntary terminations expressed neither cytokine. TNF-{alpha} may be one of several factors that amplify susceptibility; however, the genetic studies, backed by the histological data, more convincingly link TGF-{beta} to the pathogenesis of CHB. This profibrosing cytokine and its secretion/activation circuitry may provide a novel direction for evaluating fetal factors in the development of a robust animal model of CHB as well as therapeutic strategies in humans.

Nomura, I., E. Goleva, et al. (2003). "Cytokine Milieu of Atopic Dermatitis, as Compared to Psoriasis, Skin Prevents Induction of Innate Immune Response Genes." J. Immunol. **171**(6): 3262-3269.

http://www.jimmunol.org/cgi/content/abstract/171/6/3262

Atopic dermatitis (AD) and psoriasis are the two most common chronic skin diseases. However patients with AD, but not psoriasis, suffer from frequent skin infections. To understand the molecular basis for this phenomenon, skin biopsies from AD and psoriasis patients were analyzed using GeneChip microarrays. The expression of innate immune response genes, human {beta} defensin (HBD)-2, IL-8, and inducible NO synthetase (iNOS) was found to be decreased in AD, as compared with psoriasis, skin (HBD-2, p = 0.00021; IL-8, p = 0.044; iNOS, p = 0.016). Decreased expression of the novel antimicrobial peptide, HBD-3, was demonstrated at the mRNA level by real-time PCR (p = 0.0002) and at the protein level by immunohistochemistry (p = 0.0005). By real-time PCR, our data confirmed that AD, as compared with psoriasis, is associated with elevated skin production of Th2 cytokines and low levels of proinflammatory cytokines such as TNF-{alpha}, IFN-{gamma}, and IL-1{beta}. Because HBD-2, IL-8, and iNOS are known to be inhibited by Th2 cytokines, we examined the effects of IL-4 and IL-13 on HBD-3 expression in keratinocyte culture in vitro. We found that IL-13 and IL-4 inhibited TNF-{alpha}- and IFN-{gamma}-induced HBD-3 production. These studies indicate that decreased expression of a constellation of antimicrobial genes occurs as the result of local up-regulation of Th2 cytokines and the lack of elevated amounts of TNF-{alpha} and IFN-{gamma} under inflammatory conditions in AD skin. These observations could explain the increased susceptibility of AD skin to microorganisms, and suggest a new fundamental rule that may explain the mechanism for frequent infection in other Th2 cytokine-mediated diseases.

Rutella, S., G. Bonanno, et al. (2003). "Identification of a Novel Subpopulation of Human Cord Blood CD34-CD133-CD7-CD45+Lineage- Cells Capable of Lymphoid/NK Cell Differentiation After In Vitro Exposure to IL-15." J. Immunol. **171**(6): 2977-2988.

http://www.jimmunol.org/cgi/content/abstract/171/6/2977

The hemopoietic stem cell (HSC) compartment encompasses cell subsets with heterogeneous proliferative and developmental potential. Numerous CD34- cell subsets that might reside at an earlier stage of differentiation than CD34+ HSCs have been described and characterized within human umbilical cord blood (UCB). We identified a novel subpopulation of CD34-CD133-CD7-CD45dimlineage (lin)- HSCs contained within human UCB that were endowed with low but measurable extended long-term culture-initiating cell activity. Exposure of CD34-CD133-CD7-CD45dimlin- HSCs to stem cell factor preserved cell viability and was associated with the following: 1) concordant expression of the stem cell-associated Ags CD34 and CD133, 2) generation of CFU-granulocyte-macrophage, burst-forming unit erythroid, and megakaryocytic aggregates, 3) significant extended long-term culture-initiating cell activity, and 4) up-regulation of mRNA signals for myeloperoxidase. At variance with CD34+lin- cells, CD34-CD133-CD7-CD45dimlin- HSCs maintained with IL-15, but not with IL-2 or IL-7, proliferated vigorously and differentiated into a homogeneous population of CD7+CD45brightCD25+CD44+ lymphoid progenitors with high expression of the T cell-associated transcription factor GATA-3. Although they harbored nonclonally rearranged TCR{gamma} genes, IL-15-primed CD34-CD133-CD7-CD45dimlin- HSCs failed to achieve full maturation, as manifested in their CD3-TCR{alpha}{beta}-{gamma}{delta}- phenotype. Conversely, culture on stromal cells supplemented with IL-15 was associated with the acquisition of phenotypic and functional features of NK cells. Collectively, CD34-CD133-CD7-CD45dimlin- HSCs from human UCB displayed an exquisite sensitivity to IL-15 and differentiated into lymphoid/NK cells. Whether the transplantation of CD34-lin- HSCs possessing T/NK cell differentiation potential may impact on immunological reconstitution and control of minimal residual disease after HSC transplantation for autoimmune or malignant diseases remains to be determined.

Wright, G. J., H. Cherwinski, et al. (2003). "Characterization of the CD200 Receptor Family in Mice and Humans and Their Interactions with CD200." J. Immunol. **171**(6): 3034-3046.

http://www.jimmunol.org/cgi/content/abstract/171/6/3034

CD200 (OX2) is a broadly distributed cell surface glycoprotein that interacts with a structurally related receptor (CD200R) expressed on rodent myeloid cells and is involved in regulation of macrophage function. We report the first characterization of human CD200R (hCD200R) and define its binding characteristics to hCD200. We also report the identification of a closely related gene to hCD200R, designated hCD200RLa, and four mouse CD200R-related genes (termed mCD200RLa-d). CD200, CD200R, and CD200R-related genes were closely linked in humans and mice, suggesting that these genes arose by gene duplication. The distributions of the receptor genes were determined by quantitative RT-PCR, and protein expression was confirmed by a set of novel mAbs. The distribution of mouse and human CD200R was similar, with strongest labeling of macrophages and neutrophils, but also other leukocytes, including monocytes, mast cells, and T lymphocytes. Two mCD200 receptor-like family members, designated mCD200RLa and mCD200RLb, were shown to pair with the activatory adaptor protein, DAP12, suggesting that these receptors would transmit strong activating signals in contrast to the apparent inhibitory signal delivered by triggering the CD200R. Despite substantial sequence homology with mCD200R, mCD200RLa and mCD200RLb did not bind mCD200, and presently have unknown ligands. The CD200 receptor gene family resembles the signal regulatory proteins and killer Ig-related receptors in having receptor family members with potential

activatory and inhibitory functions that may play important roles in immune regulation and balance. Because manipulation of the CD200-CD200R interaction affects the outcome of rodent disease models, targeting of this pathway may have therapeutic utility.

Laan, M., S. Bozinovski, et al. (2004). "Cigarette Smoke Inhibits Lipopolysaccharide-Induced Production of Inflammatory Cytokines by Suppressing the Activation of Activator Protein-1 in Bronchial Epithelial Cells." J. Immunol. **173**(6): 4164-4170.

http://www.jimmunol.org/cgi/content/abstract/173/6/4164

Chronic smoking is characterized by immunosuppressive changes in the airways, leading to chronic colonization with bacteria, which in turn may contribute to the chronic obstructive pulmonary disease. The mechanisms causing this immunosuppression, however, are poorly characterized. This study evaluated whether cigarette smoke can inhibit endotoxin (LPS)-induced inflammatory cytokine production in bronchial epithelial cells and, if so, what the mechanisms are behind this effect. Pretreatment with cigarette smoke extract (CSE) concentration dependently inhibited the LPS-induced GM-CSF and IL-8 protein release, which was accompanied by decreased expression of mRNA in human bronchial epithelial cells (Beas-2B). The increase of neutrophil chemotaxis induced by conditioned medium from LPS-treated Beas-2B cells was also suppressed by CSE. In addition, the activity of LPS-induced transcription factor AP-1, but not NF-{kappa}B, was down-regulated by CSE. Notably, at the concentrations used, CSE had no effect on number or viability of Beas-2B cells. These data indicate that cigarette smoke possesses immunosuppressive properties by down-regulating the bacterial pathogen-induced neutrophil-mobilizing cytokine production via suppression of AP-1 activation in the airways. Hence, this study suggests a novel mechanism by which cigarette smoke may contribute to chronic colonization and chronic obstructive pulmonary disease in smokers.

Langerak, A. W., B. Nadel, et al. (2004). "Unraveling the Consecutive Recombination Events in the Human IGK Locus." J. Immunol. **173**(6): 3878-3888.

http://www.jimmunol.org/cgi/content/abstract/173/6/3878

In addition to the classical V{kappa}-J{kappa}, V{kappa}-{kappa} deleting element (Kde), and intron-Kde gene rearrangements, atypical recombinations involving J{kappa} recombination signal sequence (RSS) or intronRSS elements can occur in the Ig{kappa} (IGK) locus, as observed in human B cell malignancies. In-depth analysis revealed that atypical J{kappa}RSS-intronRSS, V{kappa}-intronRSS, and J{kappa}RSS-Kde recombinations not only occur in B cell malignancies, but rather reflect physiological gene rearrangements present in normal human B cells as well. Excision circle analysis and recombination substrate assays can discriminate between single-step vs multistep rearrangements. Using this combined approach, we unraveled that the atypical V{kappa}-intronRSS and J{kappa}RSS-Kde pseudohybrid joints most probably result from ongoing recombination following an initial aberrant J{kappa}RSS-intronRSS signal joint formation. Based on our observations in normal and malignant human B cells, a model is presented to describe the sequential (classical and atypical) recombination events in the human IGK locus and their estimated relative frequencies (0.2-1.0 vs <0.03). The initial J{kappa}RSS-intronRSS signal joint formation (except for J{kappa}1RSS-intronRSS) might be a side event of an active V(D)J recombination mechanism, but the subsequent formation of V{kappa}-intronRSS and J{kappa}RSS-Kde pseudohybrid joints can represent an alternative pathway for IGK allele inactivation and allelic exclusion, in addition to classical C{kappa} deletions. Although usage of this alternative pathway is limited, it seems essential for inactivation of those IGK alleles that have undergone initial aberrant recombinations, which might otherwise hamper selection of functional Ig L chain proteins.

Mackay, G. A., Z. Liu, et al. (2004). "Protection Against Late-Onset AIDS in Macaques Prophylactically Immunized with a Live Simian HIV Vaccine Was Dependent on Persistence of the Vaccine Virus." J. Immunol. **173**(6): 4100-4107.

http://www.jimmunol.org/cgi/content/abstract/173/6/4100

This is a 5-year follow-up study on 12 macaques that were immunized orally with two live SHIV vaccines, six with V1 and six with V2. All 12 macaques became persistently infected after transient replication of the vaccine viruses; all were challenged vaginally 6 mo later with homologous pathogenic SHIVKU-1. Two of the V1 group developed full-blown AIDS without evidence of vaccine virus DNA in tissues. The data on the 10 vaccinated survivors showed that all 10 became infected with SHIVKU-1 and that DNA of both vaccine and SHIVKU-1 viruses were present 6 mo postchallenge, with minimal replication of SHIVKU-1. During the following 5 years, these animals remained persistently infected, but with only one of the two viruses. Six animals eliminated their vaccine virus after variable periods of time and four of these succumbed to reactivation of the challenge virus and AIDS. Five years after challenge, four latently infected animals, two with V2 and two with SHIVKU-1, were reinoculated with SHIVKU-1. This resulted in transient superinfection and the animals promptly returned to their prechallenge status. Immunosuppression of the four animals 1 year later with Abs to CD8+ lymphocytes resulted in transiently productive replication of their respective latent viruses, and upon recovery of CD8+ lymphocytes, they reverted to their latent virus status. The major finding was that of eight animals that eliminated the vaccine virus, six developed AIDS. The two others harboring SHIVKU-1 remain at risk for developing late-onset disease. The primary correlate against AIDS was persistence of the vaccine virus.