Meeting Proceedings - Nikolas XI

"Acute and chronic cytokine networks leading to tissue damage"

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INTRODUCTION: Langerhans cell histiocytosis (LCH) is a poorly understood disease affecting children and adults that is characterized by the development of inflammatory infiltrates and tissue destruction. LCH may involve one or more organs, including the bones, skin, lungs, liver, central nervous system, lymph nodes, and bone marrow, among others. By definition, LCH lesions are comprised of a population of CD1a+ histiocytes that resemble normal antigen-presenting Langerhans cells. However, in contrast to normal Langerhans cells, LCH "lesional cells" proliferate actively, and demonstrate differences in their morphology, expression of cell surface markers and functional capacities. In addition to Langerhans cells, LCH lesions contain a mixed cellular infiltrate consisting of T lymphocytes, monocytes, macrophages, and eosinophils. Despite improvements in our ability to classify the histiocytoses, and to treat patients with this disease, the causes of LCH remain unknown.

To help clarify the pathogenesis underlying LCH, Paul and Elizabeth Kontoyannis established the Nikolas Symposium in 1987. The aim of the Symposium is to facilitate a dialogue between clinicians familiar with LCH and basic scientists with expertise in immunology, dendritic cell biology, or other relevant research areas. Ultimately, it is anticipated that ideas stemming from these Symposia will facilitate development of rational and more effective treatments for patients with LCH. This year's symposium, Nikolas XI, focused on the interplay between the cellular components comprising LCH lesions. Specifically, the chemokines and cytokines influencing the recruitment and activation of T lymphocytes, macrophages, dendritic cells and normal or "lesional" Langerhans cells were explored. Increased knowledge explaining the molecular mechanisms regulating mononuclear cell recruitment, activation, proliferation and cytokine secretion should allow the pharmacological targeting of dysregulated pathways which may prevent disease onset, or decrease disease severity, in patients affected by LCH.

Saturday May 5, 2001 *"Lessons from biology"*
Session chair: Dr. Maarten Egeler, Rapporteur: Dr.Fred Racke

Dr. Hugh Perry *"Overview of Nikolas X: Inflammatory responses in the CNS: lessons for LCH"* Dr. Perry began this year's symposium by reviewing Nikolas Symposium X which focused on LCH involvement in the central nervous system (CNS). Due largely to the work of Nicole Grois and colleagues, CNS LCH is now separated into several classifications, based upon the location and radiological appearance of lesions (reviewed in Grois et al, *Heme. Onc. Clinics of North Amer.* 12(2):287, 1998). These include: 1) hypothalamic/pituitary involvement; 2) extraparenchymal involvement (meninges, choroid plexus, and possibly pineal gland); 3) intraparenchymal involvement; and 4) "neurodegenerative" disease resembling "multisystem atrophy" (rare neurodegenerative disorder of unclear etiology). The neurological symptoms of affected patients can very widely, depending on the site and severity of brain involvement.

Unfortunately, little is known about the pathogenesis underlying LCH in the brain. In order understand the biology of inflammatory CNS disease, it is important to understand the local influences of the brain microenvironment on either resident or infiltrating macrophages and dendritic cells. Towards this end, Dr. Perry's research focuses on elucidating the molecular mechanisms regulating the movement and activation of macrophages and dendritic cells during normal development, and in response to infectious or inflammatory challenges. Using the rat as a model system, Dr. Perry and others have identified that several subsets of macrophages exist within the brain, including "microglial" cells (~ 10% of non-neuronal cells within the cerebral cortex), "perivascular" macrophages (located in the meninges and choroid plexus), and pituitary macrophages. These subsets of brain macrophages differ in their expression of surface antigens, degree of "activation", function, and rate of migration into and out of the brain.

Dr. Perry also discussed the interplay between systemic immunity and CNS inflammation. Inoculation of antigen (such as the tuberculin reagent BCG) directly into the brain parenchyma may be insufficient to initiate an inflammatory response. However, following peripheral sensitization, dendritic cells appear within the CNS. These dendritic cells, in contrast to the resident microglial cells and perivascular macrophages, require active production in the bone marrow. Communication between cervical lymph nodes and CNS parenchymal spaces were hypothesized to occur via periarterial channels and the cribriform plate, and might be important for "cross talk" occurring between the peripheral immune system and the CNS. Interestingly, children with CNS LCH often have lesions involving the base of the skull. Dr. Perry speculates that this periarterial channel might allow the entrance of LCH lesional cells into the CNS. The factors influencing the tropism of normal macrophages and/or LCH lesional cells to the pituitary gland, choroid plexus and meninges remain unknown.

Finally, Dr. Perry discussed areas for future investigation that are relevant to CNS LCH. He proposed that longitudinal studies using magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS) might provide a way to look at "biochemistry *in vivo*". These technologies could potentially assess the "neurodegenerative" process that occurs in patients with "multi-system atrophy"-like brain involvement. In addition, more detailed studies examining the cognitive impairment of affected patients, and improved *in vivo* and *in vitro* systems for studying brain inflammation are required.

Dr. Ronald Jaffe *"Pathology of the dendritic cell"*. In this presentation, Dr. Jaffe provided a comprehensive overview of the histopathology and immunopathology of the pediatric dendritic cell disorders. Within the immune system, histiocytes can be broadly divided into two categories, including dendritic cells and macrophages. The macrophage component is more active in the immediate "innate" immune response, and contains the machinery required for engulfment and destruction of foreign materials and dying cells. In contrast, dendritic cells are members of the "inductive" or "adaptive" response and play a much larger role in the processing of engulfed material and presentation of processed antigen to T cells during the initiation and maintenance of an inflammatory response. Close interaction between the two cell types, however, is critical.

The ontogeny of dendritic cells, deduced mostly from *in vitro* experiments, includes at least three sets of precursors, including a germinal center dendritic cell, a CD34+ myeloid stem cell and a CD34+ lymphoid stem cell. These give rise to a variety of dendritic cells, including germinal center dendritic cells, interstitial dendritic cells, Langerhans cells, and plasmacytoid (lymphoid) dendritic cells, among others. There is close inter-relationship between dendritic cells and monocyte/ macrophages of similar origin, which are interconvertible by cytokine action at almost all levels of development.

Monocytes/macrophages and dendritic cells are immunophenotypically diverse. There exist large panels of antibodies relevant to these cells for functional and flow-cytometric analysis, but a much more restricted group of antibodies available for the study of archival collections of fixed dendritic cell lesions. The markers useful in embedded tissues can help distinguish the origins, affiliations, and degree of maturation of the dendritic lesional cells. In this respect, the pattern of staining (such as intracytoplasmic versus surface distribution) is of importance. Markers of interest include CD1a, S100, HLA-DR, Fascin, IL-3 receptor, CD68, and Factor XIIIa, among others. Using this panel of markers, along with the clinical information, it is possible to distinguish Langerhans Cell Histiocytosis (LCH), non-Langerhans Dendritic Cell lesions of mature or immature phenotype, and the Juvenile Xanthogranuloma family of disorders, local and

systemic. It is also possible to discern monocyte/macrophage participation in some dendritic cell disorders, and LCH with a hemophagocytic component is now being recognized

Dr. Peter Beverley *"Introduction to cytokine networks: the playing field and the players"* Dr. Beverley next discussed the pivotal role that dendritic cells play in the establishment and maintenance of an immune response. The complex interplay that occurs between dendritic cells and activated T lymphocytes may contribute to the chronic inflammation that characterizes LCH lesions. Dendritic cells are critical effectors of the immune response and are characterized by the following properties: 1) a large surface area on which to present antigen; 2) many surface receptors for antigen capture; 3) a high rate of endocytosis to facilitate antigen processing; 4) increased surface expression of MHC and co-stimulatory molecules that facilitate lymphocyte responses; 5) production of critical immunomodulatory cytokines, such as IL-12; and 6) active migration which allows traffic from sites of inflammation to draining lymph nodes, where additional contacts with T and B lymphocytes occur. Dendritic cells originate in the bone marrow, and under the stimulation of certain cytokines and external signals (see below), differentiate down one of at least two known paths, including: 1) a myeloid pathway in which dendritic cells share properties with monocytes and tissue macrophages; and 2) a lymphoid pathway leading to production of the CD8α+ dendritic cells found in lymphoid tissues. Despite significant advances in our understanding of how to differentiate dendritic cells *in vitro*, little is known about how dendritic cells develop *in vivo*.

Dendritic cells are capable of sensing "danger" signals, such as the lipopolysaccharide (LPS) or carbohydrate moieties of foreign microbes, and can then initiate a subsequent immune response. Following antigen uptake, and activation via a number of cell surface receptors (including Fc receptors, c-type lectins, and the Interleukin-1-Toll-like receptor family of molecules), tissue resident dendritic cells migrate to secondary lymphoid tissues where they drive the differentiation of T lymphocyte responses. This process is facilitated by exposure to certain cytokines such as IL-1 and TNFα (which activate dendritic cells), upregulation of the chemokine receptor CCR7 (which allows dendritic cell "homing" to draining lymph nodes), and changes in the expression of surface adhesion molecules (which enable detachment from tissues such as the skin). The development and coordination of an immune response is complex. Just how the various signals are integrated by the immune system is unclear. For example, several cytokines or chemokines may bind to one receptor, each exerting its own effect on immune cell function. Alternatively, one cytokine may bind to several receptors, again leading to different outcomes depending upon the receptor that is activated. Last, different "danger" signals may lead to the secretion of different cytokines, expression of different cell surface antigens, and initiation of different effector cell functions.

Dr. Beverley notes that the complex interplay between T lymphocytes, dendritic cells and their microenvironment is required to initiate and sustain a chronic inflammatory response. Chronic inflammatory responses require a "trigger", the activation and prolonged survival of reactive T cells, recruitment of other immune cell types, and secondary secretion of numerous cytokines. Dr. Beverley speculates that similar processes may lead to LCH, particularly when compounded by the possibility of abnormal genetics and/or contributory environmental factors. Several questions relevant to the development of LCH remain unanswered. For example, what is the "trigger" that initiates the formation of LCH lesions? What determines the propensity of disease to affect specific organs? Which cells are absolutely required to initiate LCH lesions (T lymphocytes, dendritic cells or both)? What are the cytokines or chemokines that are involved? If we knew better how to explain the nature of chronic inflammation, perhaps we could better understand and treat LCH.

Dr. Pieter Leenen "*Microenvironment influences in DC and macrophage differentiation"* Dr. Leenen's discussion focused on two general topics including an overview of dendritic cell differentiation, and a discussion of the "microenvironmental" factors that influence the differentiation and function of dendritic cells and macrophages. First, as regards differentiation, dendritic cells may arise from several different compartments including myeloid and lymphoid progenitor cells, as well as more mature monocytic cells (see above, discussion by Dr. Ron Jaffe). Normal Langerhans cell differentiation may constitute a separate lineage, or may develop from myeloid or lymphoid progenitor cells. All these pathways have been shown to occur in experimental conditions. Numerous questions were posed indicating our fundamental lack of knowledge regarding the differentiation of normal Langerhans and dendritic cells. For example, what is the nature of lymphoid dendritic cell progenitors? What is the Langerhans cell precursor in the "steady" state. Do Langerhans cells and other dendritic cells develop from the same or from different precursor populations?

In addition to genetic factors, dendritic cell development is influenced by the microenvironment. Among the potential microenvironmental influences, three in particular were discussed. First, cell:cell interactions mediated via numerous cell surface proteins (such as integrin receptors, MHC molecules, CD40, and CD43) were presented as potentially important regulators of dendritic cell development. In this regard, the inhibitory effect of E-cadherin on Langerhans cell maturation was provided as an example. Second, extracellular matrix components (such as collagen and elastin fibers, and glycans), that bind to various receptors (including integrins, lectins , CD44 and other receptors) may impart important regulatory effects. For example, when the monocytic cell line THP-1 is exposed to the extracellular matrix components such as type I collagen, fibronectin or laminin, unique patterns of gene expression occur, demonstrating differential regulation of a variety of important proteins including chemokines, cytokines, cell surface proteins, and metabolic factors (de Fougerolles et al. *Immunity* 13:749, 2000). Finally soluble mediators appear to be critical effectors of dendritic cell and macrophage differentiation. Included among these are cytokines, chemokines, hormones and heat shock proteins. Certain soluble mediators appear to be absolutely critical for specific types of responses, such as the requirement for interferon-γ for the listericidal effects of macrophages. In summary, the pathways from which dendritic cells and macrophages arise are diverse, and clearly regulated by the microenvironment. Targeted manipulation of microenvironmental factors may provide new strategies for treating diseases due to abnormal macrophage or dendritic cell biology.

Dr. Barrett Rollins *"Chemokine networks in LCH"* Chemokines constitute a family of over 50 proteins sharing a characteristic "chemokine structure" or chemokine "fold" These molecules, and their cognate receptors, play critical roles in regulating the migration and activation of various immune cells, including T and B lymphocytes, monocytes and macrophages, NK cells and dendritic cells. Chemokines are classified into subfamilies based on their amino acid structure, with distinct subfamily assignments reflecting selective differences in receptor binding. Interestingly, despite fairly widespread expression, mice engineered to lack specific chemokine receptors generally tend to have unique immunological phenotypes. Much of Dr. Rollins' work has focused on MCP-1 (monocyte chemoattractant protein-1), a "CC" chemokine expressed on monocytes, memory T lymphocytes and Natural Killer cells. MCP-1 binds to the receptor "CCR2" and knock-out studies have revealed an essential role for MCP-1 in the recruitment of monocytes during delayed-type hypersensitivity responses, thioglycollate-induced peritonitis, atherosclerosis, and models of brain injury, including the multiple sclerosis-like disorder experimental allergic encephalitis (EAE). In addition to leukocyte recruitment, chemokines and chemokine receptors have been shown to play an important role during the development of

secondary lymphoid organs. This is well demonstrated by BCR1 (CXCR5) knock-out animals which lack inguinal lymph nodes, intestinal Peyer's patches and splenic B cell follicles.

Based on the importance of chemokines in immune regulation, an interest has developed in investigating the role of these molecules during the development of LCH lesions. In this regard, chemokines regulate the trafficking of normal Langerhans cells into and out of the skin. For example, normal dermal Langerhans cells express the chemokine receptor CCR6, whose ligand MIP-3α, is expressed in the skin. Following trauma or other challenges involving the skin, MIP-3α secretion increases, recruiting Langerhans cells to damaged sites. Subsequently, activated Langerhans cells decrease CCR6 expression and upregulate CCR7 expression, thereby facilitating their release from the skin and movement to draining lymph nodes. In his evaluation of LCH lesions, Dr. Rollins has demonstrated dysregulated expression of CCR6 and CCR7 on lesional cells. He speculates that the aberrant expression of these receptors may contribute to the abnormal targeting of lesional Langerhans cells to both the skin and the lymph nodes. LCH cells also express chemokines that lead to the recruitment of T helper 2 cells, which Dr. Rollins notes may contribute to the inflammation and eosinophilia that is characteristic of lesions.

Session chair: Dr. Robert Arceci, Rapporteur: Kim Nichols

Dr. Jon Laman *"CD40 and CD40L expression and interactions in LCH"*. Engagement of the cellular receptor CD40 (which is present on B lymphocytes, macrophages and dendritic cells) by the CD40 ligand (CD40L; present on activated T lymphocytes), leads to the induction of effector cell functions and secretion of cytokines. These features suggest that the interaction between CD40 and CD40L bearing cells can stimulate the functional properties of both cell types. To better understand the cellular interactions and dysregulated cytokine secretion that take place within LCH lesions, Drs. Laman, Egeler and colleagues immunohistochemically examined the expression of CD40 and CD40L using lesional material from 15 pediatric patients with LCH involving the bone and lymph nodes (Egeler et al, *Eur. J. Cancer* 36:2105, 2000). These studies revealed a high expression of CD40 and CD40L on the cells present within LCH lesions. In addition, CD40+ CD1a+ lesional cells co-localized with CD3+ CD40L+ T lymphocytes. Based on these findings, Dr. Laman proposes that CD40:CD40L-mediated intercellular interactions may drive inflammation within LCH lesions.

Dr. Laman next discussed the possibility that interfering with the CD40:CD40L interaction might benefit patients with LCH, or other immune disorders, by modulating inflammatory responses that are dependent on the activation of T lymphocytes and antigen-presenting cells. Dr. Laman described several animal models of multiple sclerosis that investigate the therapeutic efficacy of agents that block the CD40:CD40L interaction. For example, he discussed a mouse model of experimental allergic encephalitis (EAE) in which antigen-challenged mice develop CNS infiltrates containing activated T lymphocytes and CD40+ macrophages. If these mice are treated with an αCD40L-imunotoxin to selectively target CD4+ T cells, one can almost completely abrogate the onset of EAE. Related work in marmosets reveals that the use of either a monoclonal or chimeric αCD40 antibody ameliorates or provides full protection from CNS disease. Therefore, αCD40 immunotherapy holds promise for the treatment of human inflammatory diseases, such as LCH. Considerations in the development of such antibody-based treatments include the limited bioavailability of drug, development of secondary antibody responses to the drug itself, and the widespread expression of CD40 within the immune compartment that might lead to generalized immunosuppression.

Last, Dr. Laman discussed the role of bacterial peptidoglycan (PG) in promoting organ-specific chronic inflammation. PG is an important part of the cell wall of gram positive bacteria, and like lipopolysaccharide (LPS), engages members of the Toll-like family of receptors. If one looks either in normal animals (monkey spleens), in animal models of rheumatoid arthritis, or in human brains from patient with multiple sclerosis, one often sees PG+ macrophages, or B cells making αPG antibodies. These findings suggest that dendritic cells, which normally "sample" the gut, may be capable of taking up PG from the bacteria residing as intestinal flora. These dendritic cells could then traffic to the lymph nodes, activate other immune cells, and lead to inflammatory states. Whether PG+ cells contribute to the pathogenesis of LCH remains to be determined.

Dr. George Tsangaris *"Evaluation of gene expression"* In recent years, investigators have focused on evaluating the patterns of gene expression as a way to better understand normal development, differentiation, and effector function. In addition, gene expression analyses have provided insights into human diseases associated with perturbations in these normal cellular processes. In this discussion, Dr. Tsangaris reviewed the process of gene expression profiling using cDNA microarrays, and described the preliminary results of his experiments in which he evaluated the upregulation of gene expression in three separate tissues (normal skin without Langerhans cells, skin containing CD1a+ Langerhans lesional cells, and bone marrow containing CD1a+ lesional cells. After isolating the genes whose expression levels differed by more than 4-fold, Dr. Tsangaris identified 36 genes that were upregulated in both LCH skin and bone marrow when compared with normal skin. Among these genes, 10 were found to encode transcription factors, 4 encoded kinases, and 2 encoded proteases. Specific genes that were discussed included the Notch family member Jagged 2, which is implicated in cancer and hematopoiesis, and tapascin, an immunoglobulin superfamily member that is important for the association of the TAP transported with MHC class I molecules in the endoplasmic reticulum. Whether upregulation of these genes is significant in the pathogenesis of LCH is not clear.

Dr. Hy Levitsky *"Lessons from tumor vaccines: tissue consequences of utilizing cytokine based vaccines for therapy"*. In this session, Dr. Levitsky reviewed the concept of tumor vaccination, and introduced the possible use of such vaccines as a novel therapeutic strategy for LCH patients. Tumor vaccination involves the active immunization of patients with tumor-derived antigens in order to initiate a systemic and long-lived immune response to an already established malignancy. In one approach, tumor vaccines can be based on the use of tumor cells themselves. In the tumor cell-based model, irradiated tumor cells can be injected directly into recipients, or they can be genetically modified prior to injection. Infection of tumor cells using retroviral vectors expressing certain cytokines, including IL-4, IL-6 and/or GMCSF, have been used as a means to increase the subsequent systemic immune response. In contrast, alternative tumor vaccination strategies use defined antigens, such as tumor-derived proteins, peptides, DNA or tumor-associated viruses.

Mouse models of tumor vaccination have revealed several important observations about tumor rejection. First, although previous work suggested that cytotoxic T cells were required for tumor cell killing, subsequent experiments have shown that mice deficient in CD8+ T lymphocytes can still reject a certain amount of tumor through CD4-dependent, CD8-independent mechanisms. Second, CD4+ T lymphocytes draining tumor vaccine injection sites express increased levels of Th1 and Th2 cytokines, indicating that CD4+ T helper cells are likely to play an important role in the process of tumor rejection. Third, vaccination experiments using mice deficient for one or more cytokine genes (such as IL-4, IL-5, and/or IFN-γ) have revealed a critical role for IFN-γ, without which mice can not mount an anti-tumor response. Fourth, in addition to T lymphocytes,

macrophages and eosinophils are actively recruited to tumors following vaccination. These cells are likely to contribute to the rejection of established tumors, and as effector cells, are shown to be dependent upon cytokines produced by the infiltrating CD4+ T cells. Last, experiments examining the fate of T cells specific for tumor antigens in the tumor-bearing host demonstrate they become tolerant over time. Paradoxically, this process does not result from a direct "tumor-T cell interaction" but rather from T cells encountering tumor antigen captures by host antigen presenting cells (APCs). Since host APCs are also central to *priming* tumor-specific T cell responses, much attention is now being given to factors that regulate APC activation as the key determinant of the ultimate outcome of presentation of tumor antigen to T cells.

Sunday May 6, 2001 "Specific systems"
Session chair: Dr. Ron Jaffe, Rapporteur: Dr. Hugh Perry

Dr. Jack *Gauldie* *"Lung: adenovirus models of cytokine secretion leading to pulmonary fibrosis"*. Chronic LCH can lead to fibrosis, most notably when the disease involves the lungs and/or liver. In this session, Dr. Gauldie discusses the process of tissue fibrosis and the contributory role of various cytokines. Fibrosis represents the end result of repeated immunological challenge and repair, and underlies a number of human diseases including idiopathic pulmonary fibrosis, systemic sclerosis, and asthma, among others. Pathologically, these disorders are characterized by the presence of fibroblastic foci which consist of an admixture of fibroblasts (which deposit collagen) and myofibroblasts (cells with a phenotype intermediate between fibroblasts and smooth muscle cells and which are also major sources of extracellular matrix).

Using a mouse model in which an adenoviral vector is delivered into the lungs of recipient mice, Dr. Gauldie has explored the role of various cytokines in the development of pulmonary fibrosis. Following delivery of the cytokine-expressing vector, mice are sacrificed at different time points, and the lungs are histologically examined. One of the molecules discussed was TNFα, a macrophage-derived cytokine that is associated with the development of fibrosis in transgenic mouse models. Interestingly, in Dr. Gauldie's experimental system, the transient expression of TNF-α resulted in only minimal deposition of matrix, transient myofibrobastic changes, and minimal scarring. In contrast, expression of IL-1B, a macrophage-derived pro-inflammatory cytokine, produced systemic effects, increased release of IL-1B, TNF-α and IL-6 into bronchoalveolar lavage fluid, and led to the development of persistent pulmonary parenchymal inflammation with destruction of alveoli, deposition of matrix proteins, and development of fibroblastic foci. Last, the role of TGFB, an immune regulator that differentiates myofibroblasts *in vitro* and is a primary stimulator of matrix deposition, was examined. TGFB exists in a "latent" form, expressed by cells such as fibroblasts and myofibroblasts and an "active" form, generated by enzymatic cleavage by tissue macrophages. Expression of latent TGFB resulted in the development of a transient and minimal inflammatory infiltrate despite massive amounts of TGFB expression. In contrast, expression of activated TGFB led to the recruitment of many infiltrating mononuclear cells, and early scar formation. Despite the absence of tissue destruction early in the course of disease, there was very active tissue remodeling with progressive fibrosis and organ dysfunction. Additional studies using a SMAD3 knock-out mouse revealed abrogation of TGFB-induced fibrosis, reflecting the requirement for SMAD3 in TGFB-dependent signaling. Interestingly, in SMAD3 deficient mice, connective tissue growth factor (CTGF) was expressed, suggesting that this gene by itself may not induce fibrosis. From these and other studies, Dr. Gauldie concludes that active TGFB and IL-1B are among the most important cytokines contributing to the pulmonary fibrogenic process.

Dr. Mick Arthur *" Liver: stellate cell and macrophage cooperation leading to hepatic fibrosis"* As with pulmonary LCH, inflammation and scarring are common features resulting from LCH involving the liver. In this session, Dr. Arthur reviewed the pathogenesis of liver fibrosis, which is characterized by the activation of hepatic stellate cells (HSCs) that produce matrix proteins and regulate matrix degradation. The HSC, which is very granular and stores vitamin A, resides in the space of Disse between the hepatocyes and the hepatic sinusoids. These cells are maintained in a quiescent phenotype in part because of the microenvironment in which they reside. When HSCs are removed from this environment (for example by placement into tissue culture), they become "activated", and acquire a myofibroblast-like appearance that is characteristic of that seen in fibrotic liver. During liver injury or inflammation, HSCs may become activated by apoptotic hepatocytes, or via the release of PDGF, TGFB and other factors by activated Kupffer cells. Once activated, HSCs lose their vitamin A droplets, proliferate, become contractile, release chemoattractants (such as MCP-1, IL-10, and SCF), and both degrade liver matrix and lay down new collagen. During the acute phase of liver injury, there is increased expression of matrix metalloproteinases (MMPs) as well as their tissue inhibitors (TIMPs). Fortunately, there is increasing evidence that liver fibrosis can be reversed, as long as the inciting cause is corrected early. Examples that are given include the early treatment of hepatitis C, and withdrawal of the toxin CCL4 in an experimental animal model of liver injury. Resolution of fibrosis is associated with heightened apoptosis of HSCs, increased levels of collagenase activity, and rapid decreases in TIMP-1 and TIMP-2 expression.

Based on Dr. Arthur's discussion, strategies for the treatment of diseases associated with acute liver injury and fibrosis include 1) inhibition of HSC proliferation and activation; 2) inhibition of the paracrine and autocrine effects of cytokines and growth factors released during the process of liver inflammation; 3) regulation of interactions between various inflammatory cells; 4) promotion of HSC apoptosis,; and 5) promotion of matrix degradation. The use of such treatments could conceivably improve the outcomes for patients with liver LCH or with sclerosing cholangitis, a related disorder associated with HSC activation and myofibroblast proliferation resulting in fibrosis of the small and large bile ducts. In liver LCH, it is not yet known whether HSC activation is driven by lesional LCH cells, or by the cytokines that they release.

Dr. Stuart Lipton *"HIV infection and CNS damage and dementia"* Over the past years, it has become apparent that a subset of LCH patients develop CNS disease (see above, discussion by Hugh Perry). Although involvement of the CNS can take one of several forms, one of the most problematic involves a progressive "neurodegeneration" of unclear etiology. In this session, Dr. Lipton discusses the pathobiology of a syndrome associated with cognitive and motor dysfunction observed following infection with human HIV-1 known as HIV-associated dementia (HAD). Using HAD as a model of brain inflammation and injury, it is possible that we may learn more about the etiology of CNS LCH.

Among patients with AIDS, ~ 7-10% present with dementia as their AIDS-defining illness. Pathological evaluation of affected brains demonstrates microgliosis with multinucleated giant cells, reactive astrocytosis, edema of the myelin, and neuronal injury and loss. The major pathway by which HIV enters the brain is through infected peripheral blood monocytes. It is generally felt that HAD does not result from virus-mediated injury to neurons. Rather, the pathogenesis of HAD is believed to involve the activation of macrophages and microglial cells, and their subsequent release of toxic substances that lead to neuronal damage. In addition, activated macrophages and microglial secrete cytokines that inhibit the uptake of glutamate by neurons. The increased extracellular glutamate acts as an excitatory stimulus that produces

excessive activation of neurons via glutamate receptors, primarily of the M-methyl-D-aspartate subtype. Moreover, stimulation of glutamate receptors can lead to increased glutamate release from neurons, exacerbating their excitation. NMDA-mediated neuronal cell activation results in calcium influx into neurons, activation of p38 MAPK, mitochondrial release of Cytochrome c, generation of nitric oxide and reactive oxygen species, activation of caspases, and ultimately, neuronal cell death (Kaul et al, *Nature* 410:988, 2001).

Dr. Lipton proposes that if one could prevent the excitatory component resulting from excessive glutamate-mediated signaling, it may be possible to prevent or ameliorate HIV-associated CNS disease. Currently, Dr. Lipton and others are investigating new agents designed to block the NMDA receptor. These investigations have revealed that if one directly blocks this receptor, patients can become sleepy due to the high concentration of receptors on the reticular activating system. Instead, drugs that interfere with sites that modulate receptor activity may have improved efficacy and less toxicity. Among these agents is included Memantine, a drug that enters the NMDA channel and inhibits the delivery of current. Interestingly, as extracellular glutamate levels increase, blockade of the receptor increases, indicating that pre-activation of the receptor by glutamate is required for optimal activity. Memantine has been shown to have therapeutic efficacy in transgenic mouse models of HIV CNS injury, and is currently under investigation in clinical trials of human diseases including alzheimer's disease, as well as multi-infarct and HIV-associated dementia. In addition to blocking the NMDA receptor, additional therapeutic strategies focus on interfering with downstream signaling intermediates. Examples of such strategies include the prevention of caspase and/or p38 MAPK activation. Whether related therapies will prove useful for treating patients with CNS LCH remains to be determined.

Dr. Frederic Racke *"CD1d expression in reactive and neoplastic histiocytic proliferations: a potential target for immunotherapy"* CD1d is a member of the MHC-like β2 microglobulin family of proteins and is important in the presentation of lipid antigens. An invariant population of T lymphocytes also known as NKT cells recognizes this molecule. NKT cells are felt to be important in a variety of immunological functions including development of autoimmunity, maintenance of pregnancy, tumor surveillance and granulomatous inflammation. Although CD1d was previously known to be expressed on some human T lymphocytes, B cells, monocytes hepatocytes and intestinal epithelial cells, little was known to explain the functional relevance of interactions occurring between NKT cells and CD1d expressing cells.

To learn more about the implications of NKT cell:target cell interactions, Dr. Racke and colleagues performed a series of expression and functional studies (Yang et al, *J. Immunol.* 165:3756, 2000). These experiments revealed that CD1d is expressed on cortical thymocytes, thymic medullary dendritic cells, cultured monocyte-derived dendritic cells, and lymph node paracortical dendritic cells. When placed in contact with CD1d+ dendritic cells, NKT cells lyse dendritic target cells. This lysis is CD1d-dependent because it can be blocked by an αCD1d antibody. Following exposure to CD1d+ dendritic cells, NKT cells also secrete IL-4 and IFNγ, suggesting that once they are activated, NKT cells can stimulate the recruitment and differentiation of additional dendritic cells.

Dr. Racke next examined the expression of CD1d in various human diseases associated with abnormal dendritic cell populations. Of note, 6/6 LCH cases were CD1d+. Dr. Racke speculates that CD1d expression by lesional cells could contribute to the immune dysregulation that underlies LCH, and possibly other dendritic cell hyperplasias. Next, he plans to study whether NKT cells are present in LCH lesions, and to determine the functional effects resulting from the *in vitro* interaction of LCH lesional cells with normal or patient-derived NKT cells.

Monday May 7, 2001 " Toward a rational approach to curing patients with LCH: summation and discussion of new directions and potential therapeutic targets"
Session chair: Dr. Robert Arceci, Rapporteur: Dr. Maarten Egeler

This morning's session provided an interactive opportunity for speakers to consider the topics that were discussed over the previous days, and to focus on unresolved questions relevant to the pathobiology of LCH. Many speakers noted that previous studies of LCH focused predominately on descriptions of the secondary effects resulting from abnormal LCH cell activation. More emphasis needed to be placed on getting at the underlying causes leading to disease. Various strategies to best to approach answering this question were discussed; however, it was agreed that clarifying the transcriptional profile of normal and lesional dendritic cells was required.

Drs. Hy Levitsky "*Vaccines and adoptive immunotherapy in LCH"* In this session, Dr.Levitsky bought up several unresolved issues relevant to LCH. These included: 1) What is the nature of the interaction between LCH lesional cells and infiltrating normal T cells?; Specifically, what are the molecules mediating the interaction between lesional cells and T lymphocytes? How does the interaction between LCH lesional cells and T cells effect the function of T cells? Is LCH a consequence of abnormal interactions between lesional cells and T cells?; 2) How might we better study the interaction between LCH lesional cells and T lymphocytes? Based on established practices of tumor immunology, one could isolate T cells from the draining lymph nodes or peripheral blood of affected patients and study the functional profile of T cells when placed in contact with lesional cells (using Elispot assays, proliferation assays, and possibly intracellular cytokine staining); 3) What can be done to improve the antigen-presenting capacity of LCH lesional cells? If lesional cells can be stimulated to be better antigen presenting cells, could they be used to drive an antigen-specific T cell response in an adoptive immunotherapeutic approach to treat LCH? What are the antigens recognized by reactive T cells following such an approach? In this regard, there are several technical challenges. Will it be possible to get enough lesional cells for a vaccine? Could one use a fusion protocol to generate a better antigen presenting cell?; 4) Is there evidence that LCH is due to genetic defects in a mechanism controlling LC homeostasis or function?; and 5) Are there ways to exploit evaluations of peripheral blood T cells or humoral responses that would ameliorate our understanding of the etiology of LCH?

Drs. Mick Arthur and Jon Laman *"Inhibiting T cell-DC interactions"* Dr. Arthur began this session by discussing the tolerogenic state of the normal liver. As an organ that filters nutrients and waste products from the blood, the liver is continually challenged with antigens. Despite this challenge, the liver does not "react" or develop inflammatory responses under normal conditions. The cellular and molecular mechanisms controlling the tolerizing capacity of the liver are not well known. Dr. Arthur speculates that liver dendritic cells may contribute to this process. Dendritic cells can be isolated from normal liver mononuclear cells following growth in the presence of the cytokines GM-CSF and IL-4. These dendritic cells have low expression levels of MHC and co-stimulatory molecules, decreased allostimulatory capacity in mixed lymphocyte reaction cultures. As a consequence, they are not good stimulators of naive T cells, and require significant exposure to antigen to "drive" a secondary immune response. In contrast to normal liver dendritic cells, LCH lesional cells, although "developmentally arrested", are associated with robust inflammation. If one could elucidate more about the transcription and other factors that control normal liver DC differentiation and function, perhaps one could elucidate the pathways that have gone awry in liver LCH. Next, Dr. Laman discussed the interaction between T cells and dendritic cells and raised the question "what are the molecular perpetrators of disease"? An increased understanding of the receptors mediating the interaction between T cells and dendritic cells, their down stream signaling pathways, and the genes regulating function is required. In

addition, Dr. Laman notes that better *in vitro* and *in vivo* assays for evaluating the function of normal and lesional dendritic cells must be developed.

Dr. Pieter Leenen "*Differentiation approaches for treating LCH"* Dendritic cell homeostasis results from a balance between the proliferation of cells remaining in an undifferrentiated state and maturation to a state that is no longer capable of dividing. In LCH, lesional cells appear "immature" and may retain the capacity to divide. Work by Geissmann and colleagues demonstrates that lesional cells express little CD86 co-stimulatory protein and are poor allostimulators in mixed lymphocyte reactions (Geissman et al, *Blood* 97:1241, 2001). When cultured with CD40L, lesional cells increase surface MHC class II and CD86 expression, and demonstrate increased allostimulatory capacity. Unfortunately, the factors associated with the abnormal maturation of LCH lesional cells are not well clarified. Dr. Leenen notes that in LCH lesions, monocytes and macrophages are present. These cells secrete numerous cytokines including PGE2, IL-10, TGFB, somatostatin, and thrombospondin, which may have inhibitory effects on Langerhans cell maturation. Interestingly, Dr. Geissman's study reveals that in self-healing LCH, fewer monocytes are present, and lesional cells have a more mature phenotype. Dr. Leenen suggests that if one could interfere with these inhibitory cytokines, or with anti-apoptotic cytokine combinations (such as TNFα + TGFB), perhaps one could improve the treatment of LCH. These observations lead to several fundamental questions: 1) Will therapies that promote dendritic cell maturation be useful?; 2) Are adverse effects expected?; 3) How will we achieve these effects?; 4) Is there a correlation between Langerhans cell maturation and healing of lesions?; 5) Is there an inverse correlation between healing and macrophage content?; 6) Because TGFB is important as an inhibitor of Langerhans cell maturation, stimulator of cell cycle entry and regulator of fibrosis, is there a central role for TGFB in all LCH lesions? 7) What is the role of TGFB-producing regulatory T cells in the development of LCH lesions?

SUMMARY: From these discussions, it is clear that numerous cellular and chemical factors are involved in the initiation and maintenance of both normal and abnormal inflammatory responses. Unfortunately, we are only beginning to understand the nature of these factors, and currently, many remain unknown. In addition, the normal immune response is remarkably complex. It involves many factors, and leads to divergent outcomes depending upon the inciting stimulus, and the microenvironmental and genetic factors of the host, among other features. A better understanding of the interplay between cellular and chemical components of the immune system may enable the development of improved treatments for diseases associated with abnormal inflammatory responses, like LCH.

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