EIGHTH NIKOLAS SYMPOSIUM
May 9-12, 1997

Migration and *in vivo* Interactions of Immune Cells

Friday Evening May 9 Rapporteurs: A. Abbas and M. Aricò.

Dr. Valerie Broadbent and Prof. Ronald Jaffe presented **Histiocytosis for basic scientists**, simultaneously with **What is LCH? A debate** by **Prof. Blaise Favara and Dr. Robert Arceci.** In this last presentation Dr. Arceci came with a working definition, which was based on the molecular, cellular and clinical data currently available. LCH (as pathologically defined by the Histiocyte Society Criteria) is a neoplastic process involving the dendritic cell lineage with heterogeneous clinical expression modulated by 1) the degree of somatic mutations inherent in the clonagenic cell; 2) host predisposition; 3) environmental stimuli and 4) disease elaborated factors. The chronic changes that occur in liver, lung and the central nervous system are consistent with a para-neoplastic process stimulated either by the initial presence then disappearance of clonagenic LCH cells (short range effect) or by disease associated alterations possibly related to stimulatory cytokines (long range effect).

During the final discussion there were some open questions raised by the 2 sessions:

1. Is LCH a genetic, inherited disease?
   Common clinical sense claims that LCH is not familial; yet some families with recurrent LCH have been recognized. Additionally, identical twins are concordant in most cases but this was not the case at least in one family.
2. What is the reason for non-random distribution of involved sites?
   Some systems/organs (as hypothalamus/pituitary) are very often involved, while others (eye, genito-urinary tract) are virtually never involved.
3. Why are the histopathological features often non-uniform between different patients but also in different sites of the same patient?
4. Have the Langerhans cells there been before?
   In some involved sites LC are very rare or absent at histopathological evaluation.
5. Should we look in different times/stages of the disease or should we look for mechanisms of "long-distance" tissue damage?

Saturday Morning May 10 Rapporteurs: M. Aricò and D. Webb.

Prof. Ronald Jaffe reviewed **the ontogeny of dendritic cells** (DCs). He explained that there were at least three lines of origin for dendritic cells - lymphoid, myeloid and follicular - although the latter are probably of a different, non-leukocyte lineage. Myeloid DCs are derived from CD34+ marrow precursors and can be cultured under the influence of growth factors and cytokines with eventual differentiation to either macrophages or DCs. Two lines of DCs may be obtained from these precursors - Langerhans cells and those with circulating/dermal DC phenotype. Antigen expression is not fixed, but changes with different stages of cell development and functions as cells become activated, leave the dermis and migrate centrally to reach lymph nodes. Fascin, an actin bundling protein, has been used as a marker of circulating and dermal DCs, but is not expressed by LCH cells. Understanding tissue homing of LCH cells is fundamental and it is unknown why certain sites (kidney and gonads) are spared even in widespread multisystem Langerhans cell disease. The demonstration of clonality in CD1a+ lesional cells raises many questions - for example do these clonal cells arise in the marrow, or at the site of lesions? Mechanisms of tissue damage need to be elucidated - it is now clear that bone lytic lesions are due to activation of osteoclasts. Mechanisms of hepatomegaly remain unclear although macrophage activation with lymphocytes and Kupffer cell hyperplasia in the portal tracts has been suggested. Dermal dendritic cells and circulating dendritic cells are factor XIIIa+, CD1a-. The proportion of LCs within lesions is variable and LCs are often not the dominant form of histiocyte in involuting lesions. Studies are required to identify the balance within lesions between local proliferation of LCs and recruitment of new LCs to the area. The role of adhesion molecules in allowing cell migration is unclear - E Cadherin appears important for adhesion between LCs and skin keratinocytes, and becomes negative prior to LC migration.

Dr. Jonathan Austyn discussed **the role of chemokines in dendritic cell function.** Maturation and migration pathways of DCs control how, when and where many immune responses are initiated and DC - endothelial cell interactions are central to migration and homing capacities of these cells. Several steps are involved in trans-endothelial migration within the vasculature - "rolling' and transient attachment to the endothelium mediated particularly by selections followed by firm adhesion and arrest, mediated in large part by integrins, and finally diapedesis into the tissues. The production of chemokines by endothelium and other cells allows chemoattractant gradients to be established and ligation of chemokine receptors delivers intracellular signals that can, for example, activate integrins. Interest in the interaction of chemokines with DCs has been stimulated by the finding that DCs express chemokine receptors that may function as co-receptors for HIV. There is evidence that RANTES, MIP1*a*, MCP-3 and MIP-1ß can induce trans-endothelial migration of DCs. When DCs mature, down regulation of receptors occurs with loss of pinocytic and phagocytic abilities, but cell surface peptide - MHC Jaw: II complexes are up-regulated. The cells also acquire co-stimulatory molecules. There is no evidence at present that dendritic cells can become macrophages or vice versa. DCs secrete several chemokines (e.g. DC-CK1 that attracts naive T cells), and express chemokine receptors including a possible unique receptor (CCR6).

Dr. Barrett Rollins discussed **the possible role that monocyte chemoattractant protein-1 (MCP-I) might play in LCH.** MCP-1 is a chemokine that attracts monocytes, memory T lymphocytes, and NK cells. In addition, there are some data that MCP-1 attracts DCs in vitro and in vivo. In vitro analyses have shown that, like other chemokines, the N-terminal domain of MCP-1 is critical for chemoattractant activity, and that peptide inhibitors of MCP-1 may exert their effects by forming inactive heterodimers with MCP-1. Because of the target cells affected by MCP-1 and its patterns of expression, MCP-1 is believed to be involved in the pathogenesis of inflammatory diseases such as atherosclerosis, multiple sclerosis, asthma, rheumatoid arthritis, pulmonary fibrosis, and glomerulonephritis. However, a causal association is unproved so far. To examine MCP-1's function in vivo, transgenic mice have been constructed that overexpress MCP-1. In one model, high levels of MCP-1 are expressed in several organs, but no monocyte infiltration occurs. Instead this mouse is susceptible to infection by intracellular pathogens suggesting that high levels of MCP-1 partially inactivate monocytes. In contrast, a second transgenic mouse expressing low levels of MCP-1 only in pancreatic islets suffers a monocytic insulitis without diabetes. Finally, an MCP-1-deficient mouse has been developed using targeted gene disruption. This mouse shows defects in monocyte trafficking, and is currently being tested in disease models. Although there is controversy about MCP-1's ability to attract DCs in vitro, a transgenic mouse expressing MCP-1 in the skin had higher numbers of Langerhans cells suggesting that MCP-1 may directly or indirectly attract DCs. Thus expressing of MCP-1 may contribute to DC accumulation in LCH. In addition, however, secretion of MCP-1 by DCs themselves may contribute to the accumulation of macrophages in LCH lesions. Either way, MCP-1 inhibitors may be beneficial in LCH.

The identification of chemokines involved in leukocyte recruitment to inflammatory cells was introduced by **Dr. Jose-Carlos Gutierrez-Ramos.** Work on eotaxin and MCP-5 was described with their possible role in asthma. Experiments with neutralizing antibodies to the first mouse eosinophil chemokine, eotaxin, show reduced eosinophils present in BAL fluid. The anti MCP-5 antibody reduced lymphocyte numbers. Studies of cell accumulation (model using OVA challenge and assessment of cell number by BAL) show that numbers of eosinophils and lymphocytes increase over several weeks. Monocytes accumulate early, and then the numbers fall. M-RNA expression of eotaxin and MCP-1 parallel the accumulation of specific leukocyte types. MCP-1 levels are raised earlier than those of eotaxin. The presence of lymphocytes is necessary for the generation of lung eosinophilia. The majority of chemokine production is by resident lung cells - epithelial cells (Eotaxin), macrophages and smooth muscle cells (MCP-5). The presence of lymphocytes is essential for the expression of MCP-5, but not other chemokines. Their specific contribution to the accumulation of eosinophils depends on the stage of the inflammatory process. Studies examining the role of chemokines in fibrosis using a glomerulonephritis model were described: anti-MCP-1 and anti-RANTES reduced inflammation, but only anti-MCP-1 reduced collagen deposition. There may therefore be a dissociation between inflammation and fibrosis (separate processes).

Saturday Afternoon May 10 Rapporteurs: B.E. Favara and R.M. Egeler

Dr. Ann Ager presented **leucocyte migration and the regulation of immune responses.** The effectiveness of the immune system depends on bringing together rare, antigen-specific lymphocytes with minute quantities of antigen. One arm of the immune response requires a mechanism for transporting antigen to secondary lymphoid organs, and the other ensures that lymphocytes have an opportunity to react to that antigen. Lymph nodes have therefore evolved specialized blood vessels, high endothelial venules (HEV), to extract lymphocytes irrespective of their receptor specificity. Specific lymphocytes are physically trapped by antigen on specialized presenting cells, such as the Langerhans cells, which transport antigen into the node from the surrounding tissues. Within a few days differentiated progeny of antigen-stimulated lymphocytes (effector and memory cells) re-enter the bloodstream, but with a different objective, e.g. to destroy an infectious agent. These cells infiltrate non-lymphoid tissues at sites of tissue injury or inflammation. The role that vascular endothelial cells play in regulating the migration of distinct populations of leucocyte from the bloodstream into tissues was stressed. In particular, the roles of L-selectin, α_4 integrins, chemoattractants and zinc-dependent metalloproteinases in regulating the migration of lymphocytes into lymph nodes *via* HEVs. Furthermore the role of integrin-mediated interactions with comp nests of the extracellular matrix in regulating the subsequent positioning and migration of leukocytes through tissues was pointed out. Recent studies of epidermal LCs have revealed a crucial role for α_6 integrin mediated interactions with laminin in regulating the positioning of LC within the epidermis and the initial stages of migration into the dermis across the underlying basement membrane.

Macrophage heterogeneity was stressed by **Dr. Paul R. Crocker.** Macrophages are often defined functionally by their phagocytic activity and expression of a diverse array of lineage-restricted markers, many of which are also expressed by LCs and DCs. The latter are usually defined by a combination of criteria including morphology, high levels of MHC class II molecules, accessory molecules and the ability to stimulate the proliferation of naive T cells, a property not shared by macrophages. However, the lineage inter-relationships between these cell populations has been controversial for many years. All three cell types show enormous heterogeneity with respect to the criteria which are used to define them, thereby making definitive lineage assignments and inter-relationships next to impossible. In recent years, however, the development of methods to derive LC- and DC-like cells from progenitors in vitro has been a major step forward, but many uncertainties remain, especially with the possibility of interconversion between these different cell types at different stages of their life histories. A key aspect of the biology of these related cell populations relates to tissue localization and migration. The molecular basis for these processes is poorly understood but is particularly relevant to LCH, since these cells can be found in any different tissues which are not normally inhabited by LC. Studies on tissue macrophages in mouse and man, with particular reference to sialoadhesin, a cell-cell and cell-substrate adhesion molecule that is highly restricted to subsets of macrophages, both in the steady-state and under inflammatory conditions was presented. This molecule is one of the few macrophage-restricted markers which is not apparently expressed on LC or DC, and further studies on its expression by LCH cell are required to investigate possible correlations between its expression and tissue localization of LCH cells.

Migration of LCH cells was presented by **Dr. Brian Nickoloff.** While LCs are typically distributed in skin, lymph nodes, lung and thymus, the tumor cells of LCH are present in bone, skin, lymph nodes, spleen, liver, thymus, bone marrow, CNS, and the gastrointestinal tract. This altered distribution profile indicates that aberrant migration of LCH cells play a role in the pathogenesis of this neoplastic process. The molecular basis for non-random trafficking patterns has been extensively studied for B and T lymphocytes, with considerably less known for LCs and tumor cells of LCH. It is important to try and determine which factors regulate the ebb and flow of tumor cells at the aforementioned anatomical sites. At first an overview of the basic principals of cell migration of bone marrow derived cells such as lymphoid cells and dendritic cells using the skin as model organ system was presented. Next, the role of adhesion molecules was highlighted as key determinants of cell migration patterns. Specific adhesion molecules that distinguish LCs and tumor cells of LCH were described that may contribute to the abnormal migratory behavior of these different cells. Finally, a novel human skin-SLID mouse transplantation system that will be useful to further define the molecular and cellular pathways relevant to the pathophysiology of LCH was described.

Sunday Morning May 11 Rapporteurs: G. Elinder and JI. Henter

Dr. Cheryl Willman gave **an update on studies of the molecular assessment of clonality in the histiocytic disorders.** It was stressed that one of the most important features distinguishing a neoplastic cell is its clonal origin of derivation from a single cell. The recent development of molecular techniques that reveal random (polyclonal) versus nonrandom (monoclonal) pattern of X chromosome inactivation now allow for clonality to be assessed in any cell lineage in female tissues. Molecular assays for the assessment of clonality are predicated upon the random process of X chromosome inactivation in females (the "Lyon Hypothesis"). Analysis of clonality at the

HUMARA locus is superior because of its high rate of informativeness (the maternal and paternally-derived X chromosomes can be distinguished at this locus in greater than 90% of females) and consistent patterns of methylation. Although the etiology and pathogenesis of LCH have remained unknown, the prevailing opinion had been that LCH was a reactive immunologic disorder. Using HUMARA clonality assays and phosphorimager quantitation of HUMARA alleles, this group demonstrated a variable percentage of clonal cells in each of nine cases of LCH. In each case, the percentage of clonal cells determined by the HUMARA assay correlated closely with the percentage of CD1a+ cells in the lesion. These and other studies indicate that LCH is most likely a clonal neoplastic disorder with highly variable biologic behavior and clinical severity. These studies have thus laid the foundation for the search for genetic lesions that promote the clonal expansion of Langerhans-type dendritic cells or their bone marrow precursors. Isolated pulmonary histiocytosis is most common in young adults, typically presenting in the third or fourth decade with a striking female preponderance. To determine if isolated pulmonary histiocytosis was in fact a polyclonal disorder or a clonal histiocytic neoplasm, this group recently assessed clonality in five females using the HUMARA PCR assay. Clonality in the lesional histiocytes was identified in all 5 cases.

To determine if Sinus Histiocytosis with Massive Lymphadenopathy (SHML) was in fact a polyclonal disorder or a clonal histiocytic neoplasm, this group recently assessed clonality in eight females (6 females with definitive nodal SHML and 2 females with extranodal SHML) using the HUMARA PCR assay. In 7 of the 8 cases, the microdissected SHML histiocytes were polyclonal; one case was not analyzable due to an inability to resolve the HUMARA alleles by gel electrophoresis. These findings imply that SHML is a reactive histiocytic disorder and provide molecular genetic data validating the separation of this disorder from other clonal histiocytic disorders, such as LCH.

It is important to observe that clonality is not equivalent to "cancer" or "malignant", but with the definition of neoplasm as "an unregulated growth of a clone of cells", LCH, most probably, is a neoplastic disorder. The biological significance of clonality is still not quite clear. Is the clone the "initial" underlying "mutation" or is it a prolonged "immunologic" "non-neoplastic" response? The latter hypothesis is hard to believe. A final question maybe not appreciated by everybody, is whether all clinically heterogeneous presentations of LCH are genetically uniform. The morphology may be uniform without the etiology being so and it may be important to separate different phenotypes during the search for the etiology.

Dr. David Lo gave **an update on Rel B.** RelB gene codes for an important differentiation factor for mature lymphoid dendritic cells. "Knock out" of this gene in mice results in a paucity of DC in lymphoid tissues, but the mice show some normal dendritic cell precursor forms such as skin LCs. Skin was cultured to observe migration of Langerhans cells and interestingly, they were able to migrate from the relB deficient skin at a much greater rate than from control skin. The DCs were capable of stimulating T lymphocyte proliferation in vitro, although at slightly lower efficiency. However, the adhesion between T-cells and DCs was abolished. This could explain the lymphoid disorganization in mutant relB mice. RelB is also involved in the chemokine expression in non-lymphoid cells. Without relB there is an overproduction of inflammatory chemokines after LPS stimulation.

It appears that relB is involved both in the resolution of acute inflammation (through its modulation of chemokine expression in fibroblasts), and the initiation of cellular immunity (through its induction of dendritic cell differentiation), leading to the hypothesis that relB is an important gene regulating the transition from innate to adaptive immunity in vivo.

Dr. George Kollias presented **the role of the tumor necrosis factor (TNF)/lymphotoxin (LT)/receptor system in the development and function of lymphoid tissue.** TNF and its close homologue LTα share a similar spectrum of biological activities. Recently, it has been shown LTα -/- mice lack peripheral lymph nodes and an essential lymphoid organogenetic function for LTα

has been deduced. In addition, it was shown that TNF knockout mice are defective in the formation of splenic B cell follicles, FDC networks and germinal centers. It was shown that in both TNF and p55TNF-R knockout mice Peyer's patches are present and contain distinct B and T cell areas and interdigitating but not follicular dendritic cells. Moreover, it was observed that TNFα knockout mice show impaired development of the macrophages that populate the splenic marginal zone suggesting an important role for TNFα in the differentiation of such specialized macrophage subsets.

These data demonstrate that the structural lymphoid abnormalities observed in TNF and p55TNF-R knockout mice are not associated with defects in lymphoid organogenesis but rather that this ligand/receptor pair is required for the correct formation of follicular structures and FDC networks in all secondary lymphoid organs and of specialized marginal zones in the spleen. It was concluded that: 1. TNF overexpression leads to enhanced activation and migrations of LCs; and 2. the absence of TNF suppresses the activation and migration of LCs.

The Artemis Fellowship Award Recipient **Dr. Marion Schneider,** presented **patterns of immunodeficiency in patients with hemophagocytic lymphohistiocytosis.** T-cell responsiveness against bacterial and viral antigens as well as mitogens was variable impaired in patients at different stages of active HLH. Thus impaired antigen-presenting function was a prominent feature. A fraction of hyperactivated mostly $CD4^+T\text{-}H_o$-cells appeared to function as antigen-presenters by coexpressing mostly CD86 and rarely CD80 in addition to HLA-DR. Functionally, these $T\text{-}H_0$ cells constitutively secrete IFNg as well as 1L-10 and thus successfully escape the $T\text{-}H_1/T\text{-}H_2$ regulatory circuits. They strongly induce the MIP-la secretion in resting lymphocytes. MIP-1α is a potent monocyte activator and an inhibitor of hematopoietic progenitor cell differentiation, which might explain some of the characteristic clinical features in HLH. This cytokine is elevated in HLH, markedly in familiar-HLH but less pronounced in "secondary' HLH. Oxidative stress induced by a low or completely negative NADP(H) Oxidoreductase-1 activity status in lymphocytes may constitute another parameter to trigger a hyper-inflammatory but low affinity T-cell response in about half of ± 20 patients tested. These results, however, need more detailed evaluation. Phagocyte function, however, appears to be normal.

Dr. Jose-Carlos Gutierrez-Ramos presented in a few minutes **gene identification strategies.** The importance of good collection of patients was stressed. In addition, the phenotype has to be very well defined. Another important step (cDNA approaches) may be facilitated by collaboration with pharmaceutical companies.

Dr. Klaus-Michael Debatin gave **an update on apoptosis.** He presented an overview of present opinions regarding the mechanisms of apoptosis. It is important to be aware that cytotoxic drugs may activate the CD95 system which in turn activates the cascades which causes cell death. Although LCH cells may express CD95, this does not mean anything more than that the cells have attained a certain state. By using a single cell approach it appears as if the expression of CD95 does not in general change with prolonged T-cell activation. One general conclusion was made: it is not sufficient to study only one molecule and instead try to make general analyses.

Monday Morning May 12 Rapporteurs: R.J. Arceci and KM. Debatin

LCH has been known for many years to show a predilection for involvement of the pituitary gland and hypothalamic stalk resulting in diabetes insipidus as well as disturbances of growth and puberty. More recently the involvement of other CNS sites by active LCH or gliotic changes has been recognized. Essentially nothing is understood about the biology or pathophysiology of CNS involvement by LCH. **Prof. Hugh Perry** presented **CNS inflammation** by describing several model animal systems of CNS injury in order to examine the inflammatory responses. Several results of these studies should be mentioned: 1. In a rodent model for hippocampal injury, extensive neuronal cell death occurs, but it is not accompanied by a typical local inflammatory response. Similarly when the proinflammatory cytokine, 1L-1, is injected into brain parenchyma, no local inflammatory response is observed, although the meninges demonstrated a typical leukocyte reaction. These results suggest that the CNS has a mechanism by which to resist inflammatory reactions. However, this *resistance* is acquired, as there is a short postnatal period when typical inflammatory responses can occur, but subsequent resistance to leukocyte infiltration subsequently develops. 2. In an attempt to determine whether at least part of the CNS resistance to leukocyte inflammation was

due to deficiencies of chemoattractants for WBCs, the MIP-2 chemokine was microinjected into the brain and infiltration of neutrophils into brain parenchyma occurred, resulting in a disruption of the blood-brain barrier and extravasation of serum proteins. Such experiments strongly suggest that the CNS displays a resistance to developing inflammatory reactions, in part, due to failure to express chemoattractant substances such as chemokines. 3. In experiments aimed at examining the primary immune responses in the CNS, heat killed BCG was injected into brain parenchyma. Although the BCG bacilli were ingested by perivascular macrophages and microglia, no immune reaction was observed. However, when these animals were challenged subcutaneously with BCG bacilli, an immune response developed at both the local site of injection as well as in the brain parenchyma where the initial injection had been made. Initially, T cells enter the inflammatory site along with macrophages followed by plasma cells. Such experiments demonstrate that the cells of the brain parenchyma are poor initiators of primary immune responses.

While the mammalian CNS has many bone marrow derived mononuclear phagocytes, such as the microglia and perivascular macrophages, typical primary immune responses or inflammatory responses are not commonly observed, and although these studies do not explain the unusual involvement of the CNS by LCH, they serve as a possible beginning to expand on our understanding its pathobiology. The need for non-invasive imaging methods to assess ongoing inflammatory responses in the CNS and other sites of chronic changes such as lung and liver, will be important to develop.

Eosinophil accumulation, repair and fibrosis was presented by **Prof. Timothy J Williams.** Eosinophil accumulation in tissues is a characteristic feature of helminth infections. Eosinophils are equipped to migrate to sites of such infection, attach to helminths and release toxic granular contents. However, in addition to this teleological role, eosinophils are also prominent in particular diseases eg. of skin, lung, heart and gut, notably in asthma and allergic conditions. It has been argued that in these diseases, eosinophils are recruited and activated inappropriately, causing tissue damage. In allergic reactions eosinophil recruitment appears to be regulated by Th2 lymphocytes. Notable in such reactions is the formation of fibrous tissue, which may be at least partly triggered in an attempt to restore tissue structure following eosinophil-mediated damage. There is evidence that transforming growth factor is important in this respect. Granulocyte macrophage colony stimulating factor (GM-CSF) administered by gene-transfer into rat airways induces eosinophilia and fibrotic reactions associated with TGFI3I induction. Further, eosinophils are a source of TGFα and TGFβ1.

For these reasons there is particular interest in mechanisms of eosinophil recruitment and in the chemical signals, 'chemoattractants', initiating this process. Recently a potent eosinophil-selective CC-chemokine, eotaxin, which is believed to be important in eosinophil recruitment was discovered. Eotaxin was purified and the protein sequenced from activity detected in bronchoalveolar lavage fluid from challenged/sensitized guinea pigs. Murine and human homologous have now been cloned. Eotaxin is unique amongst CC-chemokines in that it only acts on one receptor, CCR3, which is present in high numbers on eosinophils. Other CC-chemokines, RANTES, MCP3 and MCP-4 also appear to exert their effects on eosinophils via this receptor as well as acting on other cell types via different receptors. It has been shown that an important Th2 cytokine, IL-5, interacts synergistically with eotaxin to induce eosinophil accumulation; a prominent effect of IL-5 being an acute release of bone marrow eosinophils, making more cells available for recruitment from the blood.

These observations provide the basis for the development of a new class of drugs designed to block eosinophil accumulation selectively. Receptor antagonists acting on CCR3 are of particular interest in this respect. Thus, there is now the opportunity to inhibit the pathogenesis associated with the recruitment of eosinophils without the many side effects of non-specific anti-inflammatory compounds such as glucocorticosteroids. If eosinophils are shown to make an important contribution to the pathogenesis of LCH, drugs developed in the future for asthma may have a place in the treatment of LCH.

Discussion of Progress and Future Directions.

The summation discussion focused on the following areas of investigation and future directions:

I. Clonality, Chromosomes and Genetic Mutations

The update on clonality in LCH continues to support the concept that both initial and recurrent lesions are clonal, although more information on lesions in different anatomic sites as well as at different times during the course of the disease is needed. And while comparative FISH of chromosome spreads has not yet revealed any chromosomal

abnormalities, newer molecular and enhanced FISH methodologies should be used to attempt to find consistent somatic chromosomal mutations. It was agreed that there was a need for the construction of cDNA libraries from lesions at different anatomic sites for sequence comparison by microarray or chip technology and/or SAGE analysis with normal dendritic and Langerhans cells was agreed to be of importance. Several interesting *best candidate* genes should also be examined in LCH samples. In addition, based on the analogy with other clonal hematopoietic disorders, the possibility of alterations in genes predisposing patients to DNA damage was discussed. Thus, it may be of future interest to examine patients with LCH for mutations in genes involved in toxic drug metabolism. The importance of familial cases was stressed and the need and plans for a worldwide registry were discussed.

II. Langerhans Cell Migration

While there has been an increase within the past several years concerning cytokine and growth factor expression in LCH, it has remained unclear how the expression of these signaling molecules impact on the proliferation, circulation and recruitment of lesional cells. The need to better understand these processes may in part come from expression studies of new Langerhans cell surface markers such as specific integrins as well as on the relative newly described class of chemoattractants called chemokines, and their receptors. The known specificity of chemokines on the local recruitment of subsets of leukocytes make these signaling molecules particularly interesting in terms of LCH. The potential use of inhibitors of chemokine receptor interactions for consideration in therapeutic trials should await the results of expression studies.

III. The Role of Antigen Presentation and LCH cells

The potential for the lesions of LCH to spontaneously regress has been acknowledged for many years. This clinical behavior has raised the question of whether LCH cells are capable of either expressing unique surface antigens and serving as typical antigen presenting cells. This might potentially result in the development of an immune-mediated, anti-LCH response. In addition, this hypothesis is also suggested from studies showing the clonal nature of LCH. Ironically, preliminary studies suggest that LCH cells do not appear to function as typical antigen presenting cells in spite of the expression of costimulatory receptors. Sufficient numbers of lesional LCH cells will need to be isolated and maintained in vitro for definitive conclusions to be made concerning their ability to stimulate primary T lymphocyte responses.

IV. Possible Animal Models

The results presented on human skin xenografts and the ability to induce psoriasis in such explants by lymphocyte adoptive transfer provides for an interesting parallel with LCH. At the current time, it is unclear whether sufficient LCH lesional cells could be obtained from blood or other sites to perform such experiments. However, it is possible that a human skin graft might act as a tropic site for LCH cells. It was also agreed that further work on using immunodeficient mouse models for transferring LCH cells should be further explored using newly identified cytokines to which dendritic cells are responsive. Further exploration of other potential animal models such as found in the Burmese dog should be encouraged. There is also a remaining need for the development of LCH cell lines.

With the continued increase in scientific interest and questions concerning LCH, and the current lack of appropriate animal models, the development of satisfactory LCH tissue and paired normal tissue resources continues to take on an urgent importance. Plans were discussed to develop such resources and link their use to approved clinical and biological protocols which have undergone standardized review.

R. Maarten Egeler, M.D., Ph.D., Proceedings Editor 1997