Ninth Nikolas Symposium
May 8 - 11, 1998

Friday, May 8, 1998

Dr. Pritchard presented an introduction to the Nikolas Symposium and the main features of the precedent 8 sessions. **Drs. Egeler and Arceci,** presented a clinical overview of Langerhans cell histiocytosis (LCH). Although mortality is limited to 10 % late effects may be significant. Diabetes insipidus, the most frequent has a prevalence of 15 to 50 %. Fibrosis of liver and lung, hearing lost, growth retardation, dentition problems, and rare but severe CNS dysfunction may occur. The link between LCH and malignancies was discussed.

Pathological sessions:

Dr. Malone: "An Introduction to the Histopathology of Langerhans cell histiocytosis". Langerhans cells, normally present in the skin, thymus, nasopharynx, esophagus, lung and cervix, are not readily identifiable by H & E staining, but can be identified using immuno-histochemical staining for CD1a, (a cell surface molecule which is almost specific for Langerhans cells) and S-100 protein (an antigen which is not specific for Langerhans cells) structures, "Birbeck granules," are specific for Langerhans cells. Histologically, LCH is characterized by an infiltrate of large histiocytes, eosinophils, lymphocytes, and typically in bone, multinucleated giant cells. The natural history of LCH in many tissues is to progress to fibrosis, and "burnt out' LCH may consist of scar tissue only. Lesions are the same, in unifocal or multifocal disease and are not prognostic. The histopathological differential of LCH is wide.

Dr. R Jaffe: "New Findings in the Histopathology of LCH". He focussed on the relationship between the ontogeny of the dendritic cells. Even if LCH is the most frequent form, defined by clear criteria, other diagnoses must be considered. The use of different antigenic markers (CD1a, S100 protein, factor XIII, fascin, CD68, and Lag) is helpful.

Saturday May 9, 1998

Dr. Willman: "Biology and Etiology of LCH". Langerhans cells and related lesional cells are bone marrow — derived. In LCH there is no evidence to date for a viral etiology. Lesional LCs in all clinico-pathologic forms of LCH are clonally derived. Clonality has been shown in a few patients who have been studied sequentially and in biopsies for multiple sites. Lesional LCH cells have p53 protein, but no mutations have been detected to date. LCH is either a clonal neoplastic disorder of variable biologic severity and clinical course or a clonal but "non-neoplastic" aberrant immune response.

Dr. Chu: "What do we know about the biology and etiology of the "LCH-jigsaw". LCH is a clonal disease. Malignancies are clonal but not all clonal proliferations are malignant, so what does clonality mean in LCH? LCH is not restricted to sites of normal LCs. LCH cells show an activated phenotype, arrested at a particular stage of activation - an early stage of cell activation/maturation- but, importantly, it is a mature cell. What is the Birbeck granule and why is it there? Cytokines like IL-1, IL-3, IL-4, IL-8, GM-CSF, TNF-a, TNF-b and LIF have been found in increased amounts in lesions. LCs migrate towards GM-CSF, IL-3, TNF-a, IL-8 and IL-1. The LCs in LCH are functionally defective with decreased antigen presenting activity. Recent studies in Dr. Chu's laboratory have shown a lack of apoptosis in skin biopsies of LCH, whereas normal Langerhans cells undergo apoptosis following activation and migration from the epidermis.

Dr. Willman: "Biologic and Genetic Similarities between LCH and Pediatric Myelodysplastic Disorders (MDS): A Candidate Gene?" Is LCH a myeloid dendritic disorder related to MDS? A defect on chromosome 7 is common in MDS. There are interesting regions on 7q (7q22 and 7q32-34). Some familial syndromes with defective DNA repair on chromosome 7 and an association to MDS/AML:

- Fanconi anemia, is an autosomal recessive disorder with bone marrow failure and cancer susceptibility. MDS may occur and 20-40% develop AML by the age of 15. The leukemias frequently have -7/(7q-) or chromosome I abnormalities.
- Familial Pediatric MDS with -7 or inv (7)(q22.1q34).
- Severe congenital neutropenia may also develop AML with -7.

Research in LCH should perhaps focus on chromosome 7 abnormalities.

Dr. Betts: "Report of Chromosomal Abnormalities in LCH: t(7;12)(q11.2;p13) and evidence of genetic instability". Metaphases were studied in 9 specimens after mechanical disruption of the tissue and over- night culture. Most were single system isolated lesions and one patient had multi-system disease - one juvenile xanthogranuloma and three hemophagocytic lymphohistiocytosis. From the analysis of seven LCH lesions, six were found to be characterized by chromosomal instability. The instability took the form of simple chromatid or chromosomal breaks and in five cases non-clonal structural rearrangements. In one case there was a clonal t(7;12)(q11.2;p13) translocation thereby providing further evidence that LCH can be a neoplastic disorder. The only lesion, which did not show/ chromosomal aberrations, was from the sole patient with multi-system disease. Is the biology of multi-systemic disease different from other presentations of LCH? In no case was instability seen in non-infiltrated material from patients with LCH, providing evidence that the instability results from an acquired rather than constitutional event. Analysis of three sequential bone marrow samples from a patient with HLH may provide a model by which the cytogenetic abnormalities in LCH evolve. In the initial bone marrow aspirate no abnormalities were seen, a second aspirate, 3 months later, showed increased chromosomal instability and a third, 3 weeks, later contained a complex clone in the majority of metaphases. This patient and all the LCH patients are currently in complete remission, suggesting that the presence of cytogenetic abnormalities do not correlate with a poor prognosis. Discussion: It was pointed out that attempts to grow LCH cells in culture by several laboratories had failed and that these cells were almost never seen in mitosis culture. It is possible, therefore, that the cells examined were not LCH cells but other cells. Benign fibrous tumors have shown similar cytogenetic features to those seen in LCH but were generally less severe. It is likely, therefore, that the cells being examined in Dr. Betts' preparations were not LCH cells but were other cells. Benign fibrohistiocytoma has shown similar features to those seen in LCH but with fewer breakage points. Chromosomal instability is not a feature that indicates neoplasias; it is also seen in non-neoplastic diseases.

Dr. Aricò: "Update on LCH in families: a registry". LCH affects 4-5.4 per million of the population. There have been rare reports of familial disease including 5 cases of affected twins. Four were male twins and one was a female set. All developed LCH in infancy. In three sets the twins developed LCH at the same time at 4, 11 and 12 months. In two instances, one twin developed the disease 3-7 seven months before the other. There were six cases of familial (not twins) LCH reported between 1957 and 1993. Some of these cases may have been FHLH misdiagnosed as LCH. A registry of familial cases of LCH has been established. It contains 17 patients from nine families. There are four pairs of concordant twins and eight children with familial LCH includes a set of identical twins in which one twin developed LCH and the other did not. Most twins have similar involvement of body sites. In the non-twin familial cases there is less correlation with site of involvement and severity of disease. In one familial (non-twin) group both parents originated from the same village. In another familial group both parents were exposed to massive cesium 137 environmental pollution from industrial spillage. Dr. Aricò feels that the concordance in identical twins and concordance in age of onset in the twins suggests a genetic predisposition to LCH.

Dr. Merriman: "Gene Mapping for beginners". Type I diabetes mellitus (DM) is an autoimmune disease at least partially determined by the inheritance of susceptibility genes. Disease results from as yet unidentified environmental factor(s) impacting on a genetically susceptible individual. There are three general approaches to identifying disease susceptibility genes: (1) study of candidate genes, (2) study of positional candidate genes and (3) positional cloning. The candidate gene approach has lead to the identification of the MHC-HLA region on chromosome 6p and the insulin gene region on chromosome lip as susceptibility loci. The positional candidate approach has shown that CTLA-4 gene region on chromosome 2q harbors a DM susceptibility locus. Positional cloning approaches beginning with affected sibpair family linkage analysis have revealed that at least 13 loci influence disease development and illustrate the polygenic nature of type I DM. Gene mapping in complex disease is a complex process, but is essential as part of efforts aimed towards understanding of pathways leading to disease.

Dr. F. Cotter: Pediatric MDS Disorders and the role of Chromosome 7: Mapping Studies of 7q. Several pediatric syndromes were discussed which include a predisposition for the development of myelodysplasia (MDS) and acute myelogenous leukemia (AML) were discussed including Schwachman-Diamond Syndrome, Monosomy 7 and Fanconi Anemia. In each of these syndromes the MDS/AML which develops is frequently associated with abnormalities of chromosome 7. Ongoing work is being directed toward mapping regions on chromosome 7 which may show increased loss of heterozygosity, deletions, translocations and inversions associated with the MDS/AML observed in these syndromes. Several folate sensitive fragile sites were reported and may relate to increased cell turnover and apoptosis observed in MDS. These observations were further discussed in relation to the finding of a t(7;12) translocation reported in a recent case of LCH.

Dr. Mecucci: "Mapping of the chromosome 12p region: TEL/ETV6 and other genes. The discovery of a fusion protein between TEL/ETV6 and platelet derived growth factor receptor (PDGFBR) gene as a consequence of the typical t(5;12)(q33;p13) of chronic myeloid disorders has favored extensive studies for the molecular characterization of 12p chromosome changes in hematological malignancies. Cytogenetically a number of structural rearrangements of 12p have been described, included deletions, reciprocal and unbalanced translocations, inversions, and duplications. TEL is a versatile gene undergoing fusion with different chromosome partners. A t(12;21) involving ETV6 is one of the most frequent change in pediatric ALL. Breakpoints not involving ETV6 are clustering in well-defined regions, mainly telomeric to ETV6. In one patient t(7;12)(q12;p13) translocation have been observed in association with a monocytical malignancy (AML-M4) suggesting a role for TEL gene in the pathogenesis of histiocytosis.

"Etiology and biology of LCH: What can we do?" moderated by **Dr. Willman.** A genetic predisposition could play a role in the biology of LCH. Cytogenetic studies looking for increased chromosome fragility, chromosome breakages and other abnormalities are warranted. Future directions and priorities:

1) Collection of lesional material, blood and bone marrow samples (tissue repositorty) have been recognized as an important priority and pre-requisition for successful biological studies. Blood samples of the family members of patients with histiocytosis will be also required. Collaboration with parent associations from different countries could be very helpful.
2) Study all LCH lesions with sophisticated new technologies for cytogenetic and molecular genetic analysis. Such high-resolution techniques as automated spectral karyotyping (SKY), comparative genomic hybridization (CGH), high quality extended metaphases, and PCR for detection of direct gene mutation/fusion have been suggested for further application in studying LCH lesions.

3) Develop and identify family clusters. One registry (Dr. Aricò) could be a source for genetic studies and establishment of cell lines.
4) Develop suitable animal models (NOD/SCID, RAG/FCg, Knock-In/Out strategies). Clues may be given by Re1B-/- Mice (reduction of kB binding activity, multifocal, mixed inflammatory infiltrate, and reduced thymic Dendritic cells), or V-ReIER [conditional v-REL ER fusion} (Transformation of a common progenitor for neutrophils and dendritic cells).

Dr. Gilliland: 7q11.2, 12p13 and the role of TEUETV6 in myeloid disorders". LCH is a clonal neoplastic disorder as defined by X-inactivation based clonality assay. These data convincingly demonstrate that LCH is the consequence of an acquired somatic mutation that confers a proliferative advantage. However, the molecular genetic basis for LCH is unknown. An abnormal clone has recently been reported in a case of LCH that contains a balanced reciprocal translocation between human chromosomes 7q11.2 and 12p13 (see Dr. Betts). These regions of chromosome 7 and 12 are frequently involved in translocations and deletions in hematopoietic malignancy. The *TEL* gene is localized to 12p13 and is known to be involved in more than 20 different translocations in hematolotic malignancy. We first cloned the *TEL* gene in a patient with chronic myelomonocytic leukemia and t(5;12)(q33;p13) as a fusion gene with the platelet derived growth factor receptor gene *(PDGFJ3R).* It has subsequently been demonstrated that *TEL* gene rearrangements occur in a broad spectrum of hematologic malignancies. Examples include the *TEL/ABL* fusion associated with CMML, ALL and AML phenotypes; *theTEL/JAK2* fusion associated with CMML, B-ALL and T-ALL; and the *TEL/AML1* fusion associated with pediatric B-ALL, which is the most common gene rearrangement in any childhood malignancy. *TEL* is thus a reasonable candidate for involvement in LCH based both on genomic localization and association with a broad spectrum of hematopoietic malignancy. In addition, we have recently cloned a new gene associated with CMML that is localized to 7q11.2. The Huntingtin interacting protein *1 (HIP1)* gene is fused to the *PDGFfiR* gene as a consequence of t(5;7)(a33;q11.2) in a patient with CMML. *HIP1* was cloned by virtue of its interaction with Huntingtin, the gene that, when mutant, causes the neurodegenerative disorder Huntington's disease. It has been postulated that the HIP1:Huntingtin interaction may prevent apoptosis of neuronal cells. It is therefore plausible that the HIP1/PDGFBR, in addition to causing proliferation of cells by activation of the PDGFBR, may also contribute to the malignant phenotype by interference with normal apoptotic cell death. HIP1 and TEL are therefore both reasonable candidate genes for involvement in LCH by virtue of genomic localization, function, and association with other hematologic malignancy.

Sunday, May 10, 1998

Transcription factors regulating dendritic cell development was presented by **Dr. Shortman.**

The dendritic cells (DC) of mouse spleen are of two types, $CD8^{+,}$ CD1 I b^{-} DC of apparent lymphoid origin and $CD8^{-,}$ $CD11b^{+}$ DC of probable myeloid origin. Lymph nodes have a third group of $CD8^{-}$ CD1 lb^{-} DC, and these may include mature Langerhans cells (LC); however, the proportion of lymph node DC which are of LC origin is not clear. Flt3 ligand is the limiting cytokine in vivo for development of all classes of DC, but GM-CSF is also required for development of the myeloid-related DC. The Ikaros family of transcription factors (Ikaros, Helios and Deadelos) is required for the development of lymphoid cells, but not for most myeloid cells. Although LC are present in the epidermis of mutant mice where all Ikaros family transcription factors are suppressed, no DC of any type are found in any of the lymphoid organs; this deficiency is intrinsic to the stem cells and not just a consequence of lack of lymphoid cells.

The transcription factor ReIB is also involved in DC development; RelB mutant mice have CD8+ lymphoid-related DC but lack $CD8^{-}$ myeloid-related DC in lymphoid organs, although they do

have epidermal LC. Thus, both RelB and Ikaros appear to be required for the full development and/or migration to lymphoid organs of myeloid-related DC, but absence of these factors still allows the formation of the immature epidermal LC.

Dr. F. Cotter: Mouse Models and the Use of NOD-SCID Mice for Modeling of LCH and Other Disorders. Immunodeficient mice have been useful for the propagation of human solid tumor and leukemia xenografts as well as the maturation of human hematopoietic progenitors. The nude mouse model was initially used for such studies, but it has become clear that mice with increased levels of immune dysfunction provide for improved growth of xenografts. This has been shown using the SCID (severe combined) model. By cross breeding SCID mice with those of the non-obese diabetic mutation (NOD), the so-called NOD/SCID immunodeficient and has less natural killer cell activity than just SCID mice. The cross-breeding of mutants involving genes responsible for T cell receptor and immunoglobin gene rearrangement, such as the "rag" gene products, with mutants involving the gamma constant chain which is important for the signal transduction of various lymphocyte activating cytokines, results in an even more profoundly immunodeficient mouse. This rag minus/gamma constant chain minus animals provide a model system for improved efficiency of engraftment of human tissues and tumor types; these immunodeficient animals may also provide an excellent model for propagation of LCH lesions. The possibility that such animal models in conjunction with newly discovered dendritic cell stimulating cytokines, such as fit-3, offer reasonable experimental approaches to developing a small animal model for human LCH.

Dr. Moore presented **canine histiocytic disorders.** There are several well defined histiocytic diseases in dogs. Despite the large variation of clinical and pathological features of these diseases, in most instances the infiltrating histiocytes possess surface antigens characteristic of dendritic antigen presenting cells (APC) such as CD1, CD1 1 c and MHC class II. Cutaneous histiocytoma is a common, benign, cutaneous neoplasm which occurs in all breeds. Histiocytomas usually occur as solitary lesions which undergo spontaneous regression within several weeks co-incident with the invasion of CD8+ T cells. Histiocytomas have the phenotype of epidermal LCs; histiocytes are epidermotropic and frequently invade the epidermis. They express CD1a, CD lb, CD1c, MHC class II, and CD1 1 c, but not Thy-1 or CD4. Cutaneous and Systemic Histiocytosis (CH and SH) are histiocytic diseases that occur in all breeds, but are especially prevalent in the Bernese Mountain Dog, in which a familial relationship is apparent. They are considered reactive forms of LCH, either skin limited (CH) or systemic (SH). A wide range of clinical behavior is observed, with SH exhibiting more aggressive disease. Immuno-phenotypically CH and SH are identical; histiocytes in both diseases express markers expected of LCs (CD I a, CD lb, CD lc, MI-IC II, and CIA lc). However, the dermal and subcutaneous angio-centric nature of the lesions, and the expression of Thy-1 (a marker of dermal dendritic APC) and CD4 (a marker of LC activation) suggest that histiocytes in these diseases consist of activated dermal Langerhans cells. The clinical behavior, which ranges from spontaneous regression to persistent, aggressive disease, and the consistent response to immuno-suppressive therapy with agents capable of profoundly inhibiting T cell activation (cyclosporin A and Leflunomide) suggest that SH and CH occur as a consequence of disordered regulation of the interaction of dendritic APC and T cells. Malignant histiocytosis (MH), which is probably the disseminated form of Histiocytic sarcoma, is an aggressive, histiocytic neoplasm with rapid clinical progression in spite of treatment . MH occurs in many breeds but is especially prevalent in the Bernese Mountain Dog (familial association), Rottweiler, Golden Retriever, and Flat-coated Retriever. Primary lesions of MH occur in spleen, lymph node, lung and bone marrow. Secondary sites are widespread, but include liver (with splenic primary) and hilar lymph node (with lung primary). Clinical signs include anorexia, weight loss, and lethargy. Other signs depend on the organs

involved and are a consequence of destructive masses. Pulmonary signs have been seen, and CNS involvement has also been documented leading to seizures, incoordination and paralysis. Histologically the lesions of histiocytic sarcoma and MH are composed of large, pleomorphic, mononuclear cells and multi-nucleated giant cells which show marked cytologic atypia. Histiocytes express CD1, CD 1 1 c and MHC **II,** and may derive from lymphoid dendritic APC. Diffuse Thy-1 expression is unusual, and CD4 expression has not been observed even when skin is the primary site involved.

Monday May 11, 1998

Dr. Mason presented data on the use of **monoclonal antibodies to detect gene products in the diagnosis of haematological malignancies.** In malignant cells previously quiescent genes may be activated be genetic changes such as chromosomal translocations (e.g. the *BCL-2* gene is juxtaposed to Ig heavy chain gene in follicular lymphoma cells carrying the t(14;l 8) anomaly and its product is then expressed. Other examples of "re-activated" genes in lymphoma include *BCL1, BCL-6* and *c-MYC.* Chromosomal translocations and other aberrations can also create fusion genes encoding hybrid protein. It is difficult to raise antibodies specific for these chimeric proteins but antibodies to their constituent members may show diagnostically informative changes in expression patterns and / or subcellular localization. For example, the (2;5) translocation found in anaplastic large cell lymphoma creates a chimeric gene encoding a fusion protein in which a ubiquitously expressed protein, nucleophosmin (NPM, is joined to the intracellular portion of the ALK receptor tyrosine kinase. Since ALK is absent from normal lymphoid cells its immunocytochemical detection is essentially diagnostic for the (2;5) translocation. Tyrosine kinases are activated by genetic changes in many myeloid neoplasms (e.g. ABL in chronic myeloid leukemia) and LCH cells might contain similar lesions. Immunohistochemical techniques for detecting tyrosine kinase activity in tissue sections were shown. This approach can be supplemented with biochemical methods (e.g. *in vitro* kinase assays) when fresh tissue is available, The investigation of LCH samples in this way could prove informative in understanding the molecular pathogenesis of the disease.

Dr. Lench presented a familial syndrome characterized by neurosensory hearing loss, joint contractures and a type of histiocytosis with some pathologic similarity to Rosai-Dorfmann Syndrome **(Genetic Mapping of a Novel Form of Autosomal Recessive Histiocytosis).** This family, originally from Pakistan, was consanguineous and had multiple affected and unaffected members thus allowing the use of autozygosity genetic mapping methods. This approach revealed a homozygous region of approximately 1 cM at chromosome position I 1q25 in affected individuals. Studies are now being directed toward the identification of specific mutated genes in this region. While the histiocytosis associated with this syndrome is clearly not LCH, this work shows the first mapping of a genetic locus involved in the molecular pathogenesis of a form of histiocytosis.

<u>Several clinical ad hoc presentations were given.</u>

Dr. Pritchard presented preliminary data on the long-term follow-up of 28 patients with multisystem LCH who had been off therapy for 5 or more years. Data collected thus far indicate a high incidence of adverse neuroendocrinologic sequelae characterized by abnormal pituitary stalks, diabetes insipidus, growth hormone deficiency and pan-hypopituitarism. **In** addition, there was a history of abnormal eating patterns, obesity, temperature instability and aggressive "rage attacks" characterizing some patients. Also presented were several cases of basilar invagination of the odontoid process, the physiologic consequences of which are currently unclear although caution was urged during certain activities as well as during intubation of these patients for surgical procedures.

Dr. Henter presented several cases of LCH examined the PET scanning using 18F-fluoro-deoxy-glucose as a tracer. This technique provides the potential to simultaneously examine both structure and function of the brain. Several abnormalities were observed but the meaning of such observations is unclear. The potential of such imaging methods to more precisely define CNS pathology in patients with LCH is promising.

Dr. Chu presented an update on the use of the murine anti-cdl a monoclonal antibody, NA1/34 for imaging LCH disease activity. The NA1/34 monoclonal antibody, tagged with indium 111, is given intravenously and has been shown to localize to areas of LCH activity, particularly to bone lesions. Such an approach holds promise for both the detection and potential treatment of LCH, even in the central nervous system.

Dr. Donadieu reviewed four cases of LCH treated with trans-retinoic acid (ATRA). No significant responses were observed, all of these patients had end stage LCH and were highly refractory. Because of the lack of effective treatments for LCH involvement of the CNS, an adult patient with progressive signs of CNS involvement was treated with ATRA at 45 mg/m^2/day with stable signs and symptoms. Since stabilization of CNS disease in LCH has been observed without treatment, this case points out the need for careful, prospective, randomized trials when evaluating new treatments.

A **Summary Discussion** was given by **Dr. Arceci.** This discussion summarized the salient observations presented at the meeting and potential new experimental studies likely to prove important. The areas of discussion are outlined below:

1) Clinical and Pathological Issues

Two major themes emerged. The first was the heterogeneity of LCH. LCH has been known for many years to present in a multitude of ways, often mimicking other more common disorders. In addition, the clinical course and response to therapy may vary widely depending upon the age of the patient and the extent of the disease. More recently, the long-term sequelae of liver and lung fibrosis as well as CNS responses often characterized by gliosis, have been described in LCH. In addition, while general views on the pathology of LCH remain unchanged, it was acknowledged that there is often a significant amount of heterogeneity in lesions in terms of proliferation, apoptosis and cellular components. It is also accepted that dendritic cell disorders may include several other syndromes in addition to LCH. These include juvenile xanthogranuloma (A), solitary histiocytomas as well as rare conditions such as indeterminate cell disease. The observation that some patients may have LCH and JX contemporaneously suggests a certain degree of plasticity in the dendritic cell lineage or the existence of an altered gene which is important to the development, maturation or physiology of different types of dendritic cells. These observations point out the need to further understanding of dendritic cell biology and subsets.

2) Genetics

The clonality of the LCH cells in both solitary lesions as well as in patients with more extensive disease is well established. It is important to determine the clonality of normal Langerhans cells as well as myeloid progenitors in patients with LCH. While this information shows that LCH is a neoplastic process of clonal origin, it has not translated yet to improved management of patients. Nevertheless, it points out the possibility that there is likely to be a genetic basis for LCH.

The survey of familial cases of LCH is important. The cases of near simultaneous development of LCH in identical twins suggests possible transfer of disease by prenatal sharing of blood between twins. The one case of identical twins with only one twin being affected (even after four years) remains enigmatic. Although familial cases of LCH are rare, they suggest a genetic predisposition to the development of LCH in these families. A common environmental factor or a combination of both cannot be eliminated. They study of additional families with multiple affected members will prove critical for defining the vertical transmission of LCH and for the

mapping of genetic loci. The development of a means to collect and store blood and/or bone marrow or DNA from buccal swab preparations as well as the need to generate EBV-transformed cell lines for selected families will be critical.

Because of the clonality of LCH and the familial cases, investigators have started to search for potential candidate genes. The recent observation of a t(7;12) translocation being observed in a lesion from a patient with LCH raises important possibilities. Important members of the EVT family of genes (particularly EVT6 or TEL) have been shown to play critical roles in the development of subtypes of malignant myeloid disorders such as chronic myelomonocytic leukemia and myelodysplastic syndromes as well as in acute lymphoblastic leukemia. While only one example of the t(7;12) has been observed, it will be important to examine this case as well as other LCH cases for molecular alterations at the t(7;12) translocation. This conclusion is based partly on the analogy with common pre-B ALL of childhood in which cytogenetic rearrangements involving the TEL gene are relatively rare, but molecular analyses indicate upwards of 30% of cases may involve this gene. Thus, any cytogenetic observation of an abnormal clonal karyotype made in LCH should clearly be extended at the molecular level.

The observation that there may be increased chromosomal breakage in patients with LCH suggests a defect in either DNA repair or replication pathways. Confirmation of this observation will be critical, but the observation does suggest an additional class of potential candidate genes to be examined in LCH.

3) Animal Models?

Several important "knockout" or "null" mouse mutants generated though homologous recombination have provided important information concerning dendritic lineage and biology. However, no murine model for LCH has been identified from such mutant animals. Nevertheless, as more "knockout" mice are created, it will be important to examine their phenotypes for similarities to LCH.

Another approach to developing an animal model for LCH has been through xenografting human LCH lesions into immunodeficient mice. Thus far, there has been no consistent or convincing success. Strategies include the use of a variety of dendritic cell stimulatory cytokines such as GM-CSF or TNF-alpha as well as using progressively more immunodeficient strains of mice. Data presented at the symposium showed the generation of mice deficient in the "Rag" gene involved in T and B cell receptor rearrangements as well as deficient in the gamma-C gene involved in receptor signaling for multiple cytokines critical for lymphocyte functioning. These profoundly immunodeficient mice lack T and B cell function and a Natural Killer cell function and may thus serve as excellent targets for the growth of human LCH xenografts. The recent identification of additional dendritic growth factors such as flt3 may provide the necessary stimulus to effectively grow LCH lesional cells in immunodeficient mice.

Three canine dendritic cell disorders were presented as possible candidates for an LCH model. They included a 1) Cutaneous histiocytoma syndrome, 2) a malignant form of histiocytosis and 3) a cutaneous and systemic histiocytosis believed to be a immunoreactive disease. Of the three disorders, consensus was that the cutaneous histiocytoma syndrome shared many characteristics with LCH, including the variability of clinical course, ability to migrate to and involve lymph nodes as well as the occasional development of systemic disease. The disorder occurs at a relatively young age and can spontaneously remit. Of further interest is the high incidence of this disorder in Bernese Mountain dogs as well as the vertical transmission of the disease to offspring of an affected parent. Future work in this model will be to prove that the dendritic cells of the lesions are clonal. In addition, chromosomal mapping studies should be forthcoming and might reveal an important gene relevant to humans with LCH.

4) Diagnostics

Discussion of the expression of novel protein products including those resulting from mutations or translocations of specific genes holds much promise in further defining subclasses of human

dendritic disorders. If novel gene products resulting from somatic mutations are identified in LCH, then the hope of using such approaches should provide powerful diagnostic and prognostic tools.

Concluding Comments:

Defining the heterogeneity of dendritic orders remains an important goal. Investigations into the biology of dendritic cells should also provide possible explanations for the diverse biological manifestations and long-term sequelae of LCH as well as its association with various forms of cancer, particularly leukemia. The meaning of clonality and the possibility of finding abnormal genes involved in the etiology and pathogenesis of LCH should lead to more specific and rationale modes of therapy. Clearly, having an animal model would be very valuable for such studies. However, the growing power of genetic and molecular mapping methodologies may also provide a direct means by which important chromosomal loci can be defined and the relevant genes identified in humans.