SESSION I - **Friday May 19th**

Introduction, updates and unresolved issues

Chairman Prof P Beverley **Rapporteur Prof G D'Angio**

J Pritchard: The LCH jigsaw - a simple clinician's viewpoint

Dr Pritchard began his talk with a detailed review of the clinical pictures of the disease. He started with the origin of histiocytes, dividing them between antigen presenting cells, which designation includes the Langerhans cell, and the antigen processing cells: monocytes and tissue macrophages.

He then categorised the histiocytoses

Class I -	LCH
Class **II**	Haemophagocytic lymphohistiocytosis
	Sinus histiocytoses
	Juvenile xanthogranuloma
	Reticulohistiocytosis
Class **III**	Malignant histiocytosis

Next was a review of nosology, simplified by the identification by Nezelof and colleagues of the Langerhans cell as the lesional cell. This led to the present terminology from what previously had been termed Histiocytosis X.

A brief exposition of some of the cytohistopathology, including EM patterns of Birbeck granules, followed before Dr Pritchard showed examples of LCH in its several clinical manifestations. He called attention to the spontaneous healing of many lesions. The central distribution of the more severe forms of skin LCH was stressed, and the fact that the GI tract - starting with the mouth and especially the gums - is affected more often than has been appreciated.

He showed some unusual manifestations of this protean disease, eg. thymic enlargement protruding above the suprasternal notch as a mass low in the neck.

One part of the LCH clinical complex that deserves more attention is the wasting and failure of normal development exhibited by many of the patients. Dr Pritchard acknowledged there is no known explanation, and remarked that there almost seemed to be a catabolic enzyme associated with LCH.

He also noted that the disease, although often widely distributed throughout the body, exhibited some tropism in that the eye, muscle, kidney and gonad were rarely, if ever, affected.

LCH is commonly divided between single organ disease and multi-organ LCH. The former applies when the bone or lymph nodes are affected, although the skin may also

play a part. The latter includes disease in many parenchymatous organs, notably the lung, liver and spleen with or without bone, skin, or lymph nodal involvement.

Diabetes insipidus (DI) can be present in any patient with LCH early or late in the course of the disease. He noted that typical LCH lesions are not found in the liver. Evidence of liver dysfunction can be present nonetheless, manifest as low serum albumin and prolonged PT and PTT times - findings not commonly seen in childhood cancer. Likewise, bone marrow function may be depressed out of proportion to scanty or absent involvement of that organ.

Management ranges from observation to aggressive therapy depending on whether organ dysfunction is present or is threatened. LCH often responds to any of a wide variety of anti-cancer agents, usually associated with prednisolone. In others, more aggressive multi-agent regimens are needed.

He sketched the elements of the LCH-1 clinical trial guided by Professor H Gadner wherein the efficacy of vinblastine is being compared with etoposide in high risk patients. Objectives of the study are

1. to establish response rates, and
2. to determine whether the frequency of DI can be reduced as early studies have suggested.

This led to a discussion of the late effects of the disease and its treatment, of which DI is a particularly important complication. He showed the lesions now demonstrable by MRI with thickening of the pituitary stalk and loss of the normal bright signal in the posterior pituitary seen on T-1 weighted images. Other delayed adversities include depressed scars over bony defects, loss of teeth, xanthalssmic changes in scarred skin lesions, chronic draining sinuses from underlying lymph nodal LCH; and perhaps, most ominous, interstitial fibrosis in the lungs. Late changes in the liver also are seen eventuating in cirrhosis and hepatic failure.

Also now seen by MR.I are the progressive symmetrical lesions in the brain, often starting in the cerebellum with appropriate neurologic signs, and spreading upward to the cerebrum with progressive functional losses.

Turning to the future, Dr Pritchard described the pilot work done at GDS in collaboration with Peter Severely and Tony Chu, using a mouse monoclonal antibody (MoAb) to CD1a. Scintigraphic evidence was shown that demonstrated "homing" of tagged MoAb to known lesions in a child with LCH. Phase II work is underway. There is a need for humanizing the MoAb since a reaction to the mouse product has already been seen in one patient.

The nature and etiology of LCH remains enigmatic. He showed a case of "typical" LCH that turned out to be an atypical mycobacterium infection. While not suggesting that LCH is related to the tubercle bacillus, there are clinical similarities. These suggest a possible infectious agent as the initiation of the LCH process in patients with subtle immunodeficiencies such as were present in his patient.

Much more work is needed to unravel the significance of the clonality studies of LCH now confirmed in several laboratories. Sequential studies of clonality have been done in only one patient; more are needed along with the clonality of different lesions - synchronous or metachronous - in patients with multisite disease.

Other fundamental questions remain. Is there an LCH "gene"? Familial disease is very rare, but there may be a clue from "malignant LCH". Is disseminated LCH a metastatic disease? Spontaneous resolution would be all the more remarkable in that case, since regression is evidently apoptotic.

He ended with a suggestion that a future Symposium be devoted to a discussion of the mechanisms that lead to the often incapacitating damage seen in patients with controlled LCH: pulmonary fibrosis and hepatic cirrhosis to name two.

B Favara: - An update on the pathology of CNS LCH

Dr Favara noted that the neuropathology of CNS LCH has been studied in 22 cases and there have been 9 additional cases since the Br J Cancer article was written[(1)]. He then presented an informative case.

A 5 year old boy was admitted in July 1994 with a perplexing neurological disorder that progressed over the ensuing months and ultimately caused his death. Key aspects of the past history include lung and thyroid disease.

Lung disease - from 6 months of age the patient had wheezing thought to be asthma. Chest x-ray films showed chronic interstitial pneumonitis with fibrosis at 3 years of age and microcystic changes on admission at 5 years of age. This process was never considered to be of great clinical significance.

Thyroid disease - two and one half years before the onset of neurological disease, he presented with a goiter that seemed to arise over a period of 4 weeks. Studies revealed primary hypothyroidism with a diffuse inhomogeneous sonographic pattern. An isotype scan showed focal hyperfunctional areas and decreased uptake in others. T4 was 6.1, TSH 47, thyroglobulin 1.681 and no thyroid antibodies were found. He was treated with synthetic hormone with prompt resolution of the goiter and return to a euthyroid state that persisted with maintenance therapy. The presence of a thyroglossal duct cyst was questioned on several occasions.

Neurological disturbances - in July 1994 he presented with a history of headaches, diplopia, ophthalmoplegia and gait disturbance that evolved over a 4 week period. More recently he had manifest anger, profanity and crying at night, all without apparent cause. He was evaluated at another institution where central nervous system (CNS) imaging studies showed round, multifocal white matter lesions of the cerebellum and brain stem (Type 1 & 2 lesions of Grois, et at[(1)]). Grey matter was spared and ventricles were normal.

Suspecting that the neurological disorder was part of a systemic process, an open lung biopsy was done in the hope of finding pathology that might explain both the lung

and CNS disease. This showed idiopathic chronic interstitial pneumonia with fibrosis. A diagnosis of histiocytosis could not be made.

Stereotactic biopsies of the brain-stem revealed only non-diagnostic glial reactive changes. Neurological disease progressed with the development of acute hydrocephalus that required hospitalization,ventriculo-peritoneal shunt and subsequently shunt modification. The lung disease remained stable and essentially subclinical in nature. The child deteriorated and died 22 days after admission.

Autopsy revealed classical honeycomb lung that occurs in LCH, an atrophic thyroid and characteristic multifocal tumoral and nodular CNS lesions. Histology revealed only minor collections of pulmonary histiocytes, primarily xanthomatous histiocytes around atrophic thyroid follicles and minor collections of histiocytes in the area of the renal sinus.

A review of this case and others provides new information that suggests the pathogenetic event leading to two basic CNS reactions.

Small "histiocytomas" that form the Grois, et al. type 2 pattern seen on MRI examinations (well-defined changes in white and grey matter). These arise as perhaps reactive perivascular accumulations of histiocytes with various phenotypes. Most lack LC granules and are S-100+. Personal case CD la, 010, Factor XIIIA, CD68 + & EM neg.

Glial proliferative (reactive) lesions provide the type I pattern (poorly defined changes of white matter). These lesions may disappear and recur.

Also seen are large "histiocytomas", which are extraparenchymal, and constitute the type 3 imaging pattern described by Grois et al., and mixed patterns; ie, type 1 accompanying types 2 & 3.

The time of onset of signs of CNS LCH may be the opportunity to search for a blood-born agent in LCH.

Work remains to be done on existing material: 010 antibody, Factor X1 1 la and clonality if feasible.

Dr Nesbit called attention to the results of his epidemiologic study in which "thyroid trouble" was described significantly more often (21.6X) in the proband than in controls. The frequency also was increased significantly more often in family members, but not to the same degree. Since this was a questionnaire survey, the term "thyroid trouble" was not characterized further in that instrument, so questions concerning the nature of the "trouble" cannot be answered at this time. Dr Favara said the thyroid difficulty in the case he described seemed to be unrelated to LCH. Dr D'Angio asked whether the brain lesions Dr Favara described were in any way similar to those seen in kuru, known to be caused by a slow virus. The clinical pictures sound similar at least superficially. Dr Favara replied that they were very different pathologically. The virus disease is largely a demyelinating process with spongiform changes in the parenchyma, not at all like the pathology of cerebral LCH.

1. Grois N, Tsunematsu Y, Barkovich J, Favara BE: Central nervous system disease in Langerhans cell histiocytosis. BR J Cancer 1994; 70:524-528.

Clonality - unresolved issue

The impact of Cheryl Willman's work showing that all forms of LCH are monoclonal proliferative disorders is a shift in thinking. Dr Favara stated that LCH should now be considered a neoplastic disease until proven otherwise. The basis for the broad spectrum of biological behaviour requires definition. It is possible that the neoplastic process is virus-induced. The need for animal systems grows. The role, if any, of superantigen pathophysiology in LCH needs proof.

The monoclonal antibody 010 - unresolved issue

In late 1993 it was reported that the monoclonal antibody 010 identifies the CD1a antigen in formalin-fixed, paraffin embedded tissue[(/)]. Subsequently reports have appeared assuming specificity of the 010 monoclonal antibody for Langerhans cells in the context of histiocytosis syndromes while failing to provide further evidence of its specificity. Belief is widespread that cells reactive with 010 antibody bear the CD1a antigen and thus, when these cells are histiocytes, this reactivity identifies them as either normal or pathological Langerhans cells. This belief remains unproven and unchallenged.

A recent report found lesional cells of most cases of Rosai-Dorfmann disease to be 010 positive[(2)]. Previously larger numbers of cases had CD1a positive lesional cells. Based on his own limited experience with the antibody, kindly provided by Dr L Boumsell, Dr Favara has found the following:

A. alveolar macrophages in non-specific disorders to be 010-positive and CD1a-negative and
B. macrophages in certain infectious lesions to be 010-positive and CD 1 a-negative.

Although very sensitive in labeling the CD1a antigen, 010 also stains cells, including histiocytes, that do not possess CD1a antigenicity as tested by conventional means and/or in the context of pathology. It may, therefore, be a non-specific detector and, when misused, may cause confusion in a field of medicine in need of no further bewilderment. Until additional data are provided concerning the specificity of this antibody, conventional methods for demonstrating the presence of CD1a antigen should continue to be used in the context of diagnostic histopathology and cytopathology.

References

1. Krenacs L, Tisalvicz L, Krenacs T, Boumsell L Immunohistochemical detection of CD1a antigen in formalin-fixed and paraffin embedded tissue sections with monoclonal antibody 010. J Pathol 1993;171:99-104.

2. Paulli M, Feller AC, Boveri E, Kindl S, Berti E, Rosso R, Merz H, Facchetti F, Gambini C, Bonetti F, Geerts ML, Moller P, Magrini U, Samloff M, Solcia E. Cathepsin D and E co-expression in sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease) and Langerhans cell histiocytosis: further evidences of a phenotypic overlap between these histiocytic disorders. Virchows Archiv 1994;424:601-606.

SESSION II - **Saturday May 20th**

Lineage, growth, and biology of Langerhans' cells

Chairman Prof S Ladisch **Rapporteur Prof A Abbas**

J. Austyn: Migration of dendritic cells

1. Dendritic cells (DCs) are present in different tissues, and may be classified according to their location.
2. In lymphoid tissues: interdigitating dendritic cells (IDCs), DCs in marginal zones of splenic follicles
3. In non-lymphoid tissues: Langerhans' cells (skin), IDCs in solid organs
4. In circulation: veiled cells in lymph, DCs in blood

The functions of DCs in lymphoid tissues are to present protein antigens to T cells and to provide costimulation for T cell activation. Resting T cells require antigen presentation by DCs and costimulation to respond, whereas previously activated T cells may respond to antigens presented by other antigen-presenting cells (APCs) and exhibit a reduced requirement for costimulation. In the thymus, DCs play a role in the selection of developing T cells, ensuring that self-reactive lymphocytes are eliminated. Freshly isolated Langerhans' cells (LCs) are functionally and phenotypically immature, and develop to maturity upon culture in GM-CSF. This process is presumably the *in vitro* counterpart of the maturation that occurs during the migration

	Antigen uptake and processing	**Costimutation**
Fresh LCs	+++	.
Cultured LCs	+	+++
Lymphoid DCs	+	+++

of LCs from the skin to lymphoid tissues, where they reside as IDCs. Thus, LCs are thought to bind and process antigen, and transport it to lymphoid tissues, where they acquire costimulatory function and activate T lymphocytes.

	Fresh LCs	**Cultured LCs**
Expression of Fc receptors, mannose receptors	High	Low
Acidic endosomal vesicles	Numerous	Few
Synthesis and surface expression of class **II** MHC molecules	High	Low

The development of DCs occurs along the following sequence: bone man-ow progenitor → blood → non-lymphoid tissues (antigen uptake) → lymph, blood → secondary lymphoid tissues (antigen presentation); no further recirculation. This process has been analysed in several experimental models. If a mouse skin explant is floated on culture medium, within 4 hours the LCs enlarge and express increased levels of class **II** molecules. The cells migrate into the dermis in I day, and in ~ 3 days they exit via lymphatic vessels. DCs leave from cardiac allografts within a few days and migrate to the T cell-rich zones of the spleen, where they activate alloreactive T cells. Administration of endotoxin (LPS) leads to a rapid loss of DCs from solid organs, and recruitment of immature (class II-low) DCs.

J. Banchereau: Properties of dendritic cells generated in vitro from human hemopoietic progenitors

DCs can be readily grown from human $CD34^+$ hemopoietic progenitors by cultured in GM-CSF + TNFα or IL 4. TNFα augments GM-CSF-induced growth, and the cells that are recovered are $CD1a^+$ and have dendritic morphology.

Stimulation of progenitors via CD40, using CD40 ligand-transfected L cells, also results in the outgrowth of DCs. This is apparently independent of GM-CSF and TNFα, because these cytokines are virtually undetectable in cultures, and outgrowth of DCs cannot be blocked by specific anti-cytokine antibodies.

Germinal centres in lymphoid organs also contain DCs. These cells are class II-positive, and upon culture they become $CD40^+$ $B7^+$ and acquire the capacity to

stimulate strong allogeneic mixed lymphocyte reactions. Germinal Center DCs may function to transport antigens to germinal centres.

Immature DCs in non-lymphoid tissues, e.g. the liver, may play a role in maintaining tolerance to tissue antigens.

P. Ricciardi-Castagnoli: Generation of skin dendritic cell lines: new tools to investigate functional properties of Langerhans' cells

A mouse retroviral vector containing an activated *myc* gene (V-myc MH2) has been used to generate immortalised lines of mouse DCs. Cultures are infected with the retrovirus, and colonies picked on the basis of high growth. A subpopulation of cells is MHC class ll-positive and expresses other DC markers. These cells can be cloned by limiting dilution to establish cloned lines.

By this method, lines have been established from spleen and foetal skin. A foetal skin dendritic cell (FSDC) line has been characterised in detail. Its growth can be inhibited by a v-myc-specific antisense oligonucleotide. Cells are class II^+, $B7\text{-}2^+$, express high levels of ICAM-1, Mac-1 and CD45, and have a typical dendritic morphology by electron microscopy. The growth of FSDC is augmented by GM-CSF, even in serum-free medium, and inhibited by IFN-y and IL-4. The cells efficiently take up fluorescein-conjugated proteins and polysaccharides, and retain these in endosomes for > 24 hrs. This uptake is enhanced by GM-CSF and IL-4, and is more rapid than pinocytosis of macromolecules by macrophages and B cell lines. The ability of FSDC to stimulate an allogeneic mixed lymphocyte reaction is increased by GM-CSF and IL-4. The FSDC line presents protein antigens to T cells more efficiently than do B cell lines, using T cell hybridomas or naive T cells from a T-cell receptor transgenic mouse as the responders. FSDC cells prime naive mice for hapten-specific contact sensitivity reactions. Finally, a viral protein added to FSDC cultures can enter the MHC class-I antigen presentation pathway, and is recognised by $CD8^+$ T cells. Antigen-pulsed DC are more potent at stimulating $CD8^+$ T cells than peritoneal macrophages.

SESSION III

Animal models - Disorders related to LCH

Chairman Prof B Favara **Rapporteur Dr R Arceci**

J Dick: Modelling human haemopoiesis in SCID mice

Understanding the biology of hematopoietic stem cells in inherently difficult to do in humans. Many monoclonal antibodies directed to cell surface antigens have been developed in order to identify putative subsets of hematopoietic cells. Although both of these approaches have proven to be useful if the analysis of hematopoietic progenitors, they both have significant limitations. In the former instance, markers do not allow for proof of differentiative potential. *In vitro* methods suffer partly from our lack of knowledge of the best growth conditions for hematopoietic stem cells. For example, colony-forming units (CFUs) are able to assess the number of relatively late committed progenitors. In addition, other *in vitro* culture conditions are able to detect the presence of a subset of cells with some self-renewal potential and are termed Long Term Culture Iniating Cells (LTCIC). However, these approaches do not appear to be effective assays for the more primitive hematopoietic stern cell population. In an attempt to circumvent these problems, the development of methods to grow human bone marrow in immunodeficient mice has received much attention as an effective "in *vivo"* system.

Initial experiments have been done with the SCID (severe combined immunodeficiency) mouse. These mice are given a sublethal dose of total body radiation of 400 cGy. This is about half the dose required for normal mice because SCID mice have a DNA repair defect making them more susceptible to the affects of radiation. Total mononuclear cells from human bone marrow or from cord blood are then injected intravenously into these mice. The simultaneous administration of various human specific growth factors will increase the subsequent development of various hematopoietic lineages. For example, the administration of kit ligand along with GM-CSF or the addition of PXY321 (a chimeric molecule of GM-CSF and IL-3) will enhance growth of the myeloid lineage, while the administration if erythropoietin will increase development along the erythroid lineage.

Although the SCID mouse model engrafted with human bone marrow has been a very useful system, these mice still retain a significant amount of natural killer (NK) cell function, which might contribute to the relatively low frequency of human cells engrafting over long periods of time. Another immunodeficient mouse has recently been used in such experiments. The NOD/SCID mouse, developed by Dr L Shultz at the Jackson Laboratories in Bar Harbor, Maine, has defects in T and B cell function, but also in NK and macrophage function. From several groups, it appears that the deficiency in macrophage function is key to allowing human xenografts to efficently grow in these mice.

Engraftment studies in such mice have allowed the definition of a cell population termed the SCID Repopulating Cell (SRC). This more primitive hematopoietic precursor cell is known to be within a population which expresses the CD34 antigen, but is negative for the expression of CD38 and DR class II MHC expression. When cells from the bone marrow are infected with a retrovirus containing a marker gene, such as adenosine deaminase, the types of progenitors can be monitored by the detection of the marker gene. These types of marker experiments have shown a high efficiency (70-80%) of CPU-Cs being transduced with the marker gene. About 10% of LTCICs (more primitive than CFU-Cs) are marked and essentially no SRCs are transduced. These results suggest that it will be very difficult to perform gene therapy in humans by using conventional retrovirus systems to transduce bone marrow stem cells.

Human leukemia cells usually grow very poorly in culture. In addition, under special culture conditions with the addition of cytokines, one can determine leukemia-CFUs. These have limited proliferation capacity. In addition, it has been known for many years that most leukemias show signs of differentiation, suggesting that the leukemia stern call is the self-renewing cell in human leukemia and may actually represent a relatively small number of the total leukemia cell burden. The NOD/SCID mouse model has proven to be an excellent system to expand human leukemia cells. In addition, this has provided the opportunity to determine some of the phenotypic characteristics of the leukemic stem cell. In this chimeric human/mouse system, this cell type is referred to as the SCID-leukemia initiating cell (SL-IC).

Using this system and acute myeloid leukemia specimens, these suppositions have been confirmed. Whereas leukemia-CFUs may be 1 out of every 200 leukemia cells, SL-IC are probably represented in the I in 200,000 or more range. Furthermore, when cells are immunostained for surface markers, separated with a flow cytometer into different phenotypic classes, and then injected into SCID mice, the SL-IC population has been shown to reside in the CD34+, DC38- subset. This work has important implications for the ability to purge bone marrow contaminated with leukemia cells. Most current purging procedures use surface markers which are expressed on more differentiated cells and very likely not the leukemia stem cell. Such approaches will be doomed to fail as they do not kill the most primitive cell capable of propagating the leukemic clone.

The use of immunodeficient mouse models engrafted with human bone marrow cells or other human tissues has tremendous potential in the study of animal models for human disease. These marine models may also provide the best means by which to propagate the aberrant cells in histiocytic disorders such as Langerhans cell histiocytosis or the flemophagocytic Lymphohistiocytosis.

B Favara: Disorders related to LCH

At the light microscopic level certain disorders may be difficult to distinguish from LCH. It is, however, critical to discriminate these conditions because the treatment and prognosis is very different. To this end, Dr Blaise Favara discussed two histologic "look-alikes" to LCH.

The first condition discussed was Juvenile xanthogranuloma (JXG). Lesions with the histologic features of JXG represent the most frequent referrals for evaluation of LCH. The characteristic histopathological features characterising JXG include the accumulation of histiocytes, including those with xanthomatous morphology as well as multinucleated giant histiocytes. Some of these giant histiocytes contain the characteristic Touton configuration of nuclei. Some histopathological features, once thought to be specific for JXG, are sometimes observed in lesions of late-stage LCH, in CNS LCH and in leukemia cutis of chronic juvenile myelogenous leukemia.

No constant immunophenotype for JXG lesions has been described although the expression of Factor XIIIa antigen is commonly observed in the skin lesions. These Factor XIIIa containing histiocytes are variably present in extra-cutaneous lesions, which appear to occur in less than 1% of extra-cutaneous lesions of JXG. In addition, although deep lesions have .been reported as being positive for the expression of CD1a, these histiocytes do not appear to contain Birbeck granules and are negative for the expression of the S100 antigen.

The etiology of JXG is unknown. Hyperdiploid, aneuploidy and a high proliferative index has been observed in a giant scalp lesion of .IXG which followed a banal course. Clonality studies have not been done in JXG. Dermatofibromas, which closely resemble JXG histologically, are proliferative lesions caused by a virus in monkeys.

In addition to forming isolated skin lesions, JXG has also been associated in some cases with hepatosplenomegaly and cytopenias. In one case, JXG involvement of the liver was associated with cholangiocentric lesions, and like LCH, caused an obstructive hepatopathy.

Another disorder, xanthoma disseminatum, is a primarily skin-based disease that shares JXG histology and can be associated with diabetes insipidus. The distinction between LCH and JXG is extremely important since JXG, even when it presents as a large invasive deep-seated mass, undergoes spontaneous regression over a period of months to years without any therapy.

The non-LCH dendritic cell histiocytosis syndromes often present with widespread histiocytosis with lesions that feature histiocytes having a phenotype of the ordinary Histiocyte (macrophage) or the interdigitating dendritic cell. Such histiocytes are often S-100 antigen positive, CD antigen variable and Factor XIIla negative. In addition, they do not contain Birbeck granules by electron microscopy. The clinical behaviour of interdigitating dendritic cell lesions is difficult to predict. Some of these lesions follow a banal course, while others may be overtly malignant, interdigitating dendritic cell sarcomas.

Many previously reported cases of malignant histiocytoses are now generally conceded to represent lymphoid malignancies. This conclusion has been based on the use of advanced molecular and immunocytological methods to prove the cell lineage of origin. Although such data have been used to argue that histiocytic malignancies may not occur at all, there is good evidence that this is not the case. Three cases were reported which demonstrated lesional cells with features of the interdigitating dendritic cell subset of histiocytes. Two of these histiocytic sarcoma cases had quite

interesting clinical prodromes. One patient had been receiving granulocyte-colony stimulating factor (G-CSF) prior to developing the tumour and showed predominant osseous lesions, some of which were very LCH-like. The second case initially presented with panniculitis and subsequently developed a hemophagocytic syndrome and died of rapidly progressive malignant neoplasia. This patient had involvement of the testes, skin and eventually CNS disease, manifested as acutely increased intracranial pressure attributed to metastatic tumour. The third case was a localised tumour which was completely surgically resected.

Thus, the importance of distinguishing these disorders form classical LCH is extremely important as the course of the disease, treatment and prognosis may vary quite considerably.

References:

1. deGraaf JH, Timens W, Tamminga RYJ, Molenaar WM. Deep Juvenile Xanthogranuloma: A lesions related to dermal indeterinate cells. Hum Pathol, 1992; 23: 950-910.
2. Rabkin MSK, Jeldsberg CR, Hammond ME et al. Clinical Ultrastructural Immunohistochemical and DNA Content Analysis of Lymphomas Having Features of Interdigitating Reticulum Cells. Cancer, 61: 1594-1601.

M Egeler: A murine model with malignant histiocytosis

Malignant histiocytosis (MH) is a syndrome caused by a systemic neoplastic proliferation of cells related to tissue macrophages (histiocytes) and their precursors. A model of MH has been established by infecting mice with the malignant histiocytosis sarcoma virus (MHSV), an acutely transforming retrovirus (Franz et al, Nature 315: 149, 1985). As the pathogenesis induced by MHSV resembles that of MH found in humans, the viral infection provides a suitable model of the human disease.

MHSV (Malignant Histiocytosis Sarcoma Virus) - susceptible BALB/c mice are infected with approximately 10^3 SFFU (Spleen Focus Forming Units). This virus originated in isolated murine sarcomas that were induced by passage of a cloned Friend helper virus in newborn mice (Franz et al. Nature 315: 149, 1985). In the final stage of the disease, at day 12 to 14 after infection, spleens showed that the anatomical organization was severely disturbed, such that lymphoid white pulp areas were virtually absent and necrotic areas were found scattered through the organ. Transformed cells were consistently positive for the expression of the viral ras-encoded p21 antigen as determined by immunostaining with the Y13-259 monoclonal antibody. Viral antigen containing cells were preferentially associated with subcapsular fibroblasts. Malignant macrophages occurred intermingled with these fibroblasts in the subcapsular areas as well as throughout the whole spleen.

Interestingly, malignant macrophages expressed a characteristic cell surface phenotype. The general macrophage markers F4/80 and Mac-1 (CD 11 b) were present at low level, as is found more often for specific mature tissue-fixed

macrophage populations. In contrast, the mature macrophage subset markers Mac-2, Mac-3, MOMA-2 and ER-HR3 were expressed at high levels by malignant cells. Especially the presence of the latter marker is of significance, since this is characteristic of a mature macrophage subset and is normally associated with macrophages located at sites of active hemopoiesis such as the splenic red pulp or bone marrow. In addition, immunophenotyping at different times during the course of the disease revealed differential expression of markers. For example, at about 10 days after infection, there is a progressive increase of splenic cells positive for macrophage markers. In the later stages of the disease, the affected cells are positive for macrophage markers as well as for dendritic cell markers and may represent cells at different stages of activation.

Five cell lines from MH mice were generated. Three were derived from the bone marrow (BM) and two from the spleen. Phenotyping of these five cell lines demonstrated a pattern of antigen expression similar to that observed in the *in vivo* model. The cell lines were positive for the expression of the immature macrophage marker ER-MP58, variably positive for the immature macrophage marker ER-MP2O, as well as positive for the dendritic leucocyte marker ER-BMDM I. These cell lines do not react with B-and/or T-cell markers and all cell lines are capable of phagocytosis.

In order to test the ability of the cell lines to generate MH in mice, $2x10^6$ cells of each cell line were intravenously injected in BALB/c mice. Exploration of the mice between days 14-16 showed significant differences in spleen weight, liver weight and percentage of malignant cells in the bone marrow. In one BM-generated cell line, the malignant cells were primarily found in the liver. A second BM-derived cell line showed malignant cells primarily in the spleen. Two cell lines (one BM-derived and one spleen-derived) showed malignant cells in the liver, spleen and BM. The last spleen-derived cell line showed malignant cells primarily in BM and spleen.

The transformed splenic macrophages found in the current mouse model of malignant histiocytosis represent a mature, tissue-fixed subset of macrophages. The phenotypically normal counterparts represent cells which function as central hemopoietic island macrophages. Re-injection of transformed cell lines derived from either the BM or spleen of MH mice demonstrated differential preference of infiltration in specific organs by the different cell lines. These studies suggest the presence of differential homing-receptors for macrophages or locally important growth factors. Such an animal model, which shares some of the features of MH, may prove to be a useful experimental system for further investigations to identify novel homing receptors and for testing possible new treatments. This model also suggests that examining cells from patients with MH for activating ras mutations may prove worthwhile.

SESSION IV - **Sunday May 9th**

Molecular genetics of Langerhans cells

Chairman Dr R Arceci **Rapporteur Dr J Pritchard**

C Willman: Clonality and Genetics in histiocytic disorders

Cheryl stated that a key feature of neoplasia is its clonal origin. Several techniques can be applied to investigate clonality. Most frequently used are techniques that are based on X-chromosomal inactivation, that can be defined by the state of methylation of some genes. Three X-linked polymorphic loci have been used to analyse clonality and were briefly discussed: the loci for phosphoglycerate kinase (PGK), the hypoxanthine phosphoribosyltransferase (HPRT) loci and the locus DXS255. Another X-linked gene that can be used for assessment of clonality is the human androgen-receptor gene (HUMARA). The assay that is based on investigating this gene and that was discussed in greater detail is the HUMARA assay. With this assay various histiocytic disorders were investigated. In LCH, the CD1a positive cells in acute multisystem disease, multifocal bone disease and unifocal bone disease all appear to be clonal. No evidence has been found for X-linkage, to date, in LCH. An important question is whether consecutive lesions in LCH have the same pattern of X-linkage in LCH. An important question is whether consecutive lesions in LCH have the same pattern of X-chromosome inactivation as the 'primary' lesion. This question has been assessed in some cases. In one patient with LCH a mastoid lesion was found to be clonal and in a lymph node lesion that appeared 4 years later, the same pattern of X-chromosome inactivation was found. Another case showed that in two lymph node lesions (one primary, one appearing 2.5 years later) in one patient the same clonality pattern was found. The question arises whether there is a case for dormancy in LCH. Cheryl further showed data of a case of pulmonary LCH that appeared to be clonal. Also clonality in 4 cases of Sinus Histiocytosis with Massive Lymphadenopathy (SHML, or Rosai-Dorfman disease) was investigated with the HUMARA assay. The histiocytic cells in all 4 cases were polyclonal.

Further investigations on an acquired somatic mutation playing a role in LCH were discussed. Based on PCR analysis, in 10 cases of LCH no p53 mutations were found.

Mary Haag: Comparative genomic hybridization to characterise Langerhans cell histiocytosis

LCH is a rare enigmatic disease of unknown aetiology and pathogenesis. The lesions feature proliferation of histiocytes with a phenotype of the Langerhans cell. The clinical spectrum of the disease is diverse and includes significant morbidity and mortality, however the pathological characteristics of the disease are uniform. Monoclonality of the proliferating histiocytes has been recently demonstrated. This finding suggests that somatic mutation may play a role in the pathogenesis of the disease. Flow cytometry has revealed all but the rarest cases of LCH to be diploid (Omvold et al, Am J Pathol 136:1301). However, standard cytogenetic techniques to evaluate specific chromosomal abnormalities in LCH have been largely unsuccessful.

The development of comparative genomic hybridisation (CGH) for molecular cytogenetic analysis has been successfully applied in genetic characterisation of malignancies that are difficult to study by standard metaphase analysis (Kallioiemi et al, 1994, Genes Chrom Cancer 10:231). The basis of CGH is detection of the relative amount of differentially labelled DNA from lesional cells compared to that of normal cell DNA. Coloured image analysis of the fluorescent hybridisation pattern on a normal metaphase template produces a profile of the relative amount of lesional DNA and normal DNA hybridised to each chromosome. In this way a single comparative hybridisation can provide an overview of DNA sequence copy number changes from lesional cells and map these changes on the normal chromosomes. This technique has been used to detect tumour DNA losses, deletions, gains and amplifications and assign these changes to a particular chromosome or region of a chromosome.

Consistant location of a genetic change to a specific position on a chromosome is an initial step to provide insight into identification of the molecular mechanism in the pathogenesis of the disease. Mary Haag used the technique of CGHH to characterise 14 cases of LCH in an attempt to measure numerical chromosome abnormalities such as gain or loss of specific chromosomes or chromosome regions. Specimens were analysed by flow cytometry and image analysis for ploidy. Frozen specimens were then selectively dissected to include at least 75% of lesional cells for DNA isolation for CGH analysis. GCH profiles of these 14 LCH specimens appeared normal, with no visible regions of DNA loss or gain. One very homogeneous LCH sample, which contained 26% hyper diploid cells by flow and image analysis showed normal genome profile by GCH. The discrepancy between the hyperdiploid flow and image analysis and GCH analysis of this specimen may result from a subpopulation selection during specimen processing or from the dilution of the hyperdiploid tumour DNA by diploid cells, which decreases sensitivity of GCH analysis. The lack of an abnormal genome copy number in this series of LCH by GCH does not preclude somatic mutation below the limit of detection of this technique. However, this study does reduce the likehood of a gross amplification or deletion in the aetiology of LCH

Stephan Ladisch: p53 in LCH lesions

Stephan showed some results of an immunogistochemical study to p53 expression in LCH. In 6 out of 10 cases p53 expression was found. Immunogistochemical staining for p53 only detects the presence of p53, and does not indicate abnormalities of this protein, *per se,* ie mutations of p53.

Tony Chu: Clonality in normal Langerhans cells

Tony presented data on clonality in normal Langerhans cells. Large sheets of epidermis were used, after trypsinisation the CD1a positive cells were purified to about 90% purity by Dynal bead/Detachabead separation using antibodies against HLA DR. Clonality of these cells was investigated using a PCR based X-inactivation assay at the HUMARA locus. CD1 a negative keratinocytes were used as a normal cell comparator population for each skin specimen. LCs in some specimens were found to be clonal, LCs in others were found to be oligoclonal. In some cases the results appeared to be difficult to interpret. Keratinocytes generally showed random X-inactivation but in one specimen showed skewing but to the opposite allele to that

shown by LC. In conclusion it seems that, due to the few number of precursors of LCs in bone marrow, LCs in large regions of epidermis could be clonal.

The question that this study poses is whether LCH results from (somatic) mutation in a LC or LC-precursor causing proliferation of LCs, or whether some LCs may become proliferative, not necessarily by DNA-mutations but possibly induced by the microenvironment, resulting in a 'reactive' process involving LCs that is clonal.

SESSION V- **Monday May 22nd**

Clinical Variations

JS Malpas: Langerhans cell histiocytosis in the adult

Langerhans cell histiocytosis is rare in adults with an estimated incidence from literature searches of 1 adult per 600,000 of the population. The review of adult Langerhans cell histiocytosis given by Prof Malpas came from three sources:

1. Published material since 1950 - this excludes abstracts, patients under the age of 15 and those that were not documented adequately with good histology. 32 patients met the criteria.

2. A retrospective series of 16 patients diagnosed at St Bartholomew's Hospital over the last 25 years.

3. Cases reported in specialist journals where histiological verification of the diagnosis was not always available. The patients presented in this review were obviously biased as patients with solitary bone disease were not necessary included and most of the published reports were on unusual physical signs and symptoms.

Review of the literature since 1950

32 patients were reported in general medical journals. 24 presented with skin rash, 9 with weight loss, 9 with breathlessness, 6 with fever and drenching sweats, 3 with thirst and polyuria, 2 with aural discharge, 1 with lymphadenopathy, 1 with arthralgia and I with ataxia. The skin was the most common primary site of involvement with lung coming second. Patients presenting with breathlessness always showed persistent reticular shadowing in the lung. Pneumothoraces always occurred against a background of reticular or honey combed patterning of the lung. Of these 32 adults reported in the literature, 20 had a definitive diagnosis of LCH on the basis of CD la staining of Birbeck granules on electonmicroscopy. 12 showed the histiological features of LCH and therefore had a diagnosis of LCH. In 7 patients the disease was at a single site, in 16 it was multi system and in 9 patients there was evidence of organ failure affecting only the lung or the liver. Treatment programmes for these patients were very varied. 50% received no specific therapy and this may have been related to the fact that these patients were very elderly. Of those treated, most had chemotherapy with vinblastine with or without steroids. Cyclosporin and

Busulphan were used in only one patient each. 21 of the 32 reported patients died. 4 out of the 7 patients with single site disease died but only 1 was attributed to the LCH. The other three died of pneumonia, congestive heart failure and myeloma. 11 of the 16 patients with multi system disease died and 4 were attributed to LCH. 6 of the 9 with multi system disease and organ failure died of LCH.

Retrospective study of patients attending St Bartholomew's Hospital

Over the last 25 years, 16 adult patients with Langerhans cells histiocytosis have been seen at St Bartholomew's Hospital. Male to female ratio was 1:1 with a mean age of 31 and a range of 15-49 years. 12 patients presented with pain, 7 with pain in the skull, 3 with pain in the limbs, 1 with pain in the eye and 1 with pain in the back. 4 patients presented with polyuria and polydypsia, 2 with skin rashes, 1 with a fever and sweating, 1 with gum hypbertrophy, 1 with lymphadenopathy, 1 with amnesia and 1 was found as an incidental finding. The commonest primary site of involvement was the bone with 12 out of the 16 patients presenting in this way. All patients had biopsies performed; 14 showed histology compatible with LCH, but only 5 had a definitive diagnosis of LCH on staining with CD1a. No deaths were seen in this series of patients although 2 patients did have multi system disease with organ failure. Chemotherapy was given to 4 patients, 2 had vinblastin with or without steroids and 2 had oral etoposide with or without steroids. Responses were generally very good. Cyclosporin gave very disappointing results and was unsuccessful on two occassions even when therapeutic levels were achieved in the blood for over a month. 5 out of the 16 patients, ie, 30% had an associated malignancy. 1 patients had had Ewing's sarcoma at the age of 6 with LCH diagnosed at the age of 19. 1 patient developed LCH at the age of 26, shortly after a slowly growing astrocytoma had been successfully treated with radiotherapy. A 34 year old woman had breast carcinoma prior to developing LCH and 1 patient aged 43 developed chronic myloid leukaemia 6 years after receiving etoposide treatment for LCH. A further female patient developed breast cancer at the age of 37 with a diagnosis of LCH made later that same year.

Erdheim - Chester Disease

This is a very rare histiocytic syndrome in which lipid filled granulomas occur in bones, kidney, heart and lungs. Professor Malpas wondered whether this was a separate entity or part of the LCH spectrum perhaps representing burnt out LCH. He also wondered whether it could be a histiocytic response to malignancy. On immunohistochemistry the histiocytic cells in LCH and Erdheim-Chester disease both show reactivity to S 100, CD68 and Class II MHC molecules. There is no evidence about the expression of CD1 or Birbeck granules in Erdheim-Chester disease.

Langerhans cell histiocytoses with malignancy

In the large review by Martin Egeler of 91 cases of the association of LCH with malignancy nearly 50% were associated with malignant lymphomas but it was demonstrated that LCH could proceed, be concurrent with or occur subsequent to the diagnosis of malignancy. 16 cases of LCH with solid tumours were described of which 12 were lung cancer. Nearly all of these were concurrent. In the series of 16

patients at St Bartholomew's Hospital 5 patients developed a preceeding, concurrent or subsequent neoplasm the commonest being breast cancer. In one case report and review of the literature, 22 patients with LCH had an association with Hodgkins disease. In 9 patients the Hodgkins disease preceded LCH. In 14 it was concurrent and in **I** case LCH preceded the Hodgkins disease. Professor Malpas reviewed 980 patients with Hodgkins disease treated in his department since 1968 and found no association with Langerhans cell histiocytosis. In another reported study of 659 patients, however, 2 cases of LCH were identified.

R Arceci: 2^1-Chorodeoxyadenosine (2CdA) for Refractory Histiocytosis Syndromes in Paediatrics - Preliminary Multicentre Experience

Nucleosides have recently been shown to play major roles in the treatment of indolent lymphoid malignancies. In particular, 2CdA has been demonstrated to be highly efficacious in the treatment of hairy cell leukaemia refractory to other treatment modalities. 2CdA was originally synthesised by investigators at the Scripps Institute as a structural analogue of the purine deoxyadenosine. It was designed to replicate the metabolic consequences of adenosine deaminase (ADA) deficiency as the insertion of a chloride group renders 2CdA relatively resistant to the action of ADA. This results in the accumulation of 2CdATP as well as increased dATP, both of which may contribute to increasing the level of DNA strand breaks as well as inhibiting DNA repair and synthesis. 2CdA thus is able to be cytotoxic to both resting and dividing cells, resulting in their death by apoptosis. Cells of the lymphoid and

myelomonocytic lineages are particularly sensitive to the cytotoxic effects of 2CdA. For the above reasons, a number of centres began non-randomised trials in patients with refractory histiocytosis syndromes.

Results on the treatment of 6 patients with Langerhans cell histiocytosis (LCH) and 2 patients with Hemophagocytic Lymphohistiocytosis (HLH) were presented. Patients ranged in age from 10 months to 23 years. All patients had severe, multisystem disease refractory to multiple prior therapies, including corticosteriods, vinblastine and etoposide. The dosage of 2CdA ranged from 0.1 to 0.5 mg/kg continuous intravenous infusion for 5-7 days with cycles being given approximately every 4 weeks.

The 2CdA treatment was well tolerated in most instances with essentially no nausea or vomiting. Hematologic toxicity, characterised by Grade 2 amaemia, Grade 2 to 4 neutropenia and Grade 2 to 4 lymphopenia, was common. Importantly, Grade 4 thrombocytopenia was observed following greater than 3 cycles of treatment, suggesting a cumulative toxicity. One patient developed transient elevation in liver enzymes, two patients with Grade 2 gastrointestinal toxicity (diarrhoea, nausea and vomiting), one patient with Central Venous Line bacterial sepsis and two patients with transient Grade 3 to 4 renal toxicity (decreased glomerular filtration rate and/or renal tubular acidosis).

One patient died with progressive disease soon after starting to receive 2CdA and was considered an early death. Two patients (one with LCH and one with HLH) died from progressive disease despite treatment with 2CdA. One patient had a complete remission and is approximately 6 months off therapy. Two patients obtained partial

remissions and went onto bone marrow transplantation; both are survivors. One patient obtained a partial remission and is still on treatment with methotrexate and thioguanine. One patient has obtained a partial remission and continues on 2CdA therapy.

These results demonstrate that 2CdA is reasonably well tolerated and has therapeutic activity in these heavily pretreated patients with refractory disease. Some dramatic responses were observed although time to response in most patients was more prolonged. Some patients were refractory to 2CdA treatment. The potential cumulative toxicity, particularly on platelet numbers and renal function, may limit the chronic use of 2CdA. The optimal dose and schedule in paediatrics are unknown and further studies are recommended.