SESSION 1 - **Friday May 7th**

LANGERHANS CELL HISTIOCYTOSIS - Clinical Features and Pathology

Stephan Ladisch

Chairman
G D'Angio

Rapporteur
B Favara

The clinical description of Langerhans cell histiocytosis (LCH) was published some 100 years ago and yet we know very little of the basic pathogenesis of the disorder.

Two types of histiocytes, both derived from bone marrow stem cells, are recognised. 1) the professional antigen presenting dendritic cells, among which the Langerhans cell is the key one, and 2) antigen processing cells that are characterised by phagocytic activity when activated. Langerhans cells (LC) may also be important reservoirs for **HIV.**

The classification of histiocytosis syndromes, as delineated by the Histiocyte Society, is dependent upon recognition of the features that distinguished these two types of histiocytes, with LC granules and CD1a surface antigen being the most definitive markers of LC.

The nature of the LC granule is still somewhat elusive but evidence of its derivation from the cell surface by invagination is convincing, likely secondary to an antigenic stimulation of some sort. Although the disorder affects all ages most cases are among young children.

Clinical manifestations of LCH are heterogeneous and become even more so, with the definition of CNS (especially cerebella) involvement. The list of signs and symptoms of LCH is headed by bone lesions with large and flat bones most often involved. Otitis, hepatomegaly and diabetes insipidus are of somewhat lower frequency. The number and kind of signs and symptoms vary greatly from patient to patient.

Multi-angle imaging of a lytic lesion of the skull shows that a lytic lesion of bone is usually exophytic or endophytic rather than flat and confined to the contour of the bone of origin and probably migrating into the bone (? the effect of cytokines rather than originating in the bone)

Once the pathological diagnosis is made, the clinical diversity takes secondary importance as a means of tracking the disease. This is because systemic therapy, applied somewhat independent of the state/details of involvement by disease, has been highly successful (DAL HX83), even if use of the individual agents (VP16) may still be controversial.

The most significant controversies about LCH include the six which are presented as background for a proposed list (one person's opinion [S.L]) of future experimental studies:

1. The Langerhans cell granule - study of its composition and its function. Better isolation and concentration methods are needed to study this organelle.

2. Lesional pathogenesis - role of the granule. Lesions in which LC granules cannot be demonstrate are less effectively treated. Liver and CNS which are organs that are essentially never the primary site of disease and in which LC granules are not or rarely found, can be used as examples. A possible hypothesis is that the presence or absence of the granule determines the biology of the disease at that site.

3. Clonality of cellular accumulations. Although knowledge of the clonal nature of lesions helps guide treatment it does not, in itself, mean that LCH is a malignant neoplastic disorder. Further study is necessary.

4. VP 16; this agent is highly effective in many cases of LCH but fear of leukaemogenicity is of concern.

a) VP 16 is a relatively weak agent in some ways; with respect to potential secondary problems ie leukaemia VM26 being ten times as disruptive to DNA as VP16.

b) In contrast to its use (VP16) in other disorders, ie concomitant use of alkylating and anthracycline agents this is not the case in LCH regimens. Therefore a separate analysis is necessary in LCH.

c) The underlying disorder, as a substrate for malignancy, may be an important factor in VP16 leukaemogenesis.

d) The association of LCH with hemopoietic malignancies is trying to tell us something. Is it therapy dependent? or is there another explanation?

5. ***Treatment approaches*** **-** In general therapy is directed at three types of disease:

a) Multifocal, single system that has a 95-100% survival

b) Multisystem disease without organ dysfunction that has a 44-100% survival

c) Multisystem disease with organ dysfunction that has a 33-54% survival. It is this later group that challenges therapists most.

A recent abstract by Ken McClain reported that 50% of 165 case of LCH treated with various regimens had complete resolution of disease whereas the other 50% had recurrent disease and a few died. It is assumed that treatment was not uniform.

In contrast the DAL HX83 study of 106 cases, treated intensively early in the disease, resulted in 87% complete resolution and only 18% of cases had recurrent disease.

Group A = 90% CR
Group B = 91%
Group C = 66%

This demonstrated justification for optimism. A pressing question is; How should group C be treated?

6. **Permanent consequences.** Role of therapy in individual cases remains a problem to therapists. That is, distinction between side effects of therapy and consequences of unsuccessfully or inadequately treated disease must be clearly distinguished, because the former suggest "less therapy" and the latter suggest "more" or more effective therapy should be used.

Pathology of LCH

Ron Jaffe

More and more pathologists are being asked to make diagnoses on smaller and smaller amounts of tissue, fine needle aspiration biopsy being an example. Diagnosis of LCH is dependent upon the demonstration of LC granule and/or CDIa positivity but there may be as few as 2% or as many as 79% of histiocytes in the lesion that bear the granule. Diagnosis of lung disease requires the presence of large numbers of LCs in broncheoalveolar lavage since LCs are

present in broncheoalveolar lavage in a variety of lung disorders. Large numbers of LCs and radiological compatibility are diagnostic. In the liver, bile duct tropism is striking. Destruction of bile ducts follows (role of adhesion molecules?) Some cases of "primary" infantile sclerosing cholangitis may be LCH.

The biological spectrum of disease is wide. Congenital disease of the reticular dermis may be self-limited. Solitary lesions -v- multiple ones demands consideration of how thorough the search has been for lesions. The terms recurrent, relapsing and progressive were discussed as they are used to describe the biology of LCH. A case of a disease free interval of 14 years was mentioned. A rare adult large cell lymphoma with LC phenotype has been described.

True "Malignant" dendritic cell tumours should have many of the following features:

Tumour with infiltrative behaviour
Malignant clinical course
Cells survive in culture and in nude mouse
DNA aneuploid
Cytogenetic abnormalities

The case of Pierre Russo's in which there was splenic disease and hepatic venous extension was mentioned in that it resolved without treatment. Reports of LC in placental foetal vessels were cited as examples of LC lesions without clinical manifestations. The implications of regional nodal lesions associated with skin and bone lesions are provocative. There are rare examples of LC involvement of only the white pulp of the spleen. It usually involves red pulp only. What controls homing of these cells? Possible local controlling factors were discussed and the influence of intervening lymphocytes, macrophages, eosinophils, local cytokine production and cytokine receptors were identified as homing signals for LCs. Another important issue was whether there was evidence of local turnover of the LCs. In one study, Dr Jaffe found 2-20% of cells in 15 LCH lesions from various sites expressed the proliferating cell nuclear antigen which shows that these cells are in S phase. Mitoses are common in lymph node disease.

Is LCH one disease or multiple diseases?

Are the LCs present in LCH normal or abnormal?

Critical Questions. Abul Abbas

Question that will be asked in the sessions are:

1) Concerning the LCH lesion

a. What are the components and what are they doing?
b. Clonality of lesional cells
c. Is there compositional heterogeneity?

2) Role of cytokines

a. Local factors
b. Systemic effects

3) ***What of viruses?***

4) What are reasonable strategies for therapy?

Summing Up

The symbiotic relationship between the Nikolas Symposia, the Histiocyte Society and the Histiocytosis Association of America was described.

Some of the products and research thrusts of past Symposia were noted and a status report given:

a) Determination of the role of viruses - One extensive study found no evidence of virus using molecular probes to a series of candidate agents (Ken McClain). Based on these data and general considerations, noted on several occasions by Robin Weiss, there is little reason why virus should be suspected.

b) The nature of the LCH lesion - This was explored partially by Peter Issacson who was distracted by the LC. He found the cell to be positive when stained with antibody to placental alkaline phosphatase. He failed to completely characterise associated cells. More work is needed to determine what the other mononuclear cells are and what they are doing in the LCH lesion.

c) Clonality - As evidence of malignancy is now being studied in three laboratories (Willman, Chu and Kannourakis) more cases and more relevant controls need to be studied.

d) The role of cytokines in the disorder - This is being explored by Kannourakis and other investigators have been given grants to study this area. More information is being awaited.

e) Epidemiology - Mark Nesbit and Maarten Egeler have a study underway and a leukemia study in UK includes some information about LCH but more on incidence of disease in various locations and other aspects are needed.

f) An ultrastructural study of lesional cells - Data on features other than LC granules was needed and has been completed by Gary Mierau and Edward Wills. These data were presented.

g) Finding an animal model - The moth-eaten mouse and the Burmese mountain dog are being evaluated but to date no model has been found.

h) Behaviour of LCH in nude and SCID mice - Work is underway by Ken McClain.

i) Better definition of CNS disease - Collaboration between Grois, Tsunematsu and Favara has resulted in several cases being studied and the scope of pathology defined.

k) Evidence for the presence of autoantibody - Studies are underway to examine the interaction between serum and lesional tissue in the same patient. Nothing reported to date.

1) Chromosomal studies are badly needed. No reports of activity in this area although Willman is studying the possibility of incubation of frozen tissue from lesions with growth factors as a means of determining the karyotype of lesional cells.

m) Definition of prognostic markers - Clinical features that serve as prognostic markers are being identified in the LCH-1 study that is in progress.

[n) The need for tissue and tissue banking - This was again discussed. Chu and Favara will solicit information and the Histiocyte Society will address the matter at its upcoming meeting in the fall.

SESSION II - **Saturday May 8th**

LANGERHANS CELL HISTIOCYTOSIS
Pathogenesis 1

Chairman
B Favara

Rapporteur
P Beverley

Introduction

The session dealt with the biology of Langerhans cells and dendritic cells (DC), their relationship to other myeloid cells and development from precursor cells. Recent advances in methods for isolation and culture of DC have for the first time allowed relatively pure populations to be isolated and maintained in culture. This has allowed questions of the lineage relationships of DC, their regulation and function to be addressed.

The nature of human Langerhans cells - Tony Chu

Dr Chu reviewed the features of normal Langerhans cells (LC) and the cells of Langerhans cell histiocytosis lesions. Normal LC represent up to 2% of normal epidermal cells, varying in density in different sites, and are found in the suprabasal layers of the epidermis. Mitotic cells are seldom observed but many LC are in S phase. LC with characteristic Birbeck granules are found in lymph nodes and thymus in addition to DC. They are found in other epithelia and are increased in the lung in heavy smokers. There is good evidence that they originate in the bone marrow and they may be involved in skin immune surveillance. These observations raise several questions relevant to research on LCH.

1. Does the LCH cell correspond to a specific stage of LC maturation?

2. Are the trophic factors which act on normal LC relevant to the pathogenesis of LCH?

3. Are LCH cells functionally active?

4. What cytokines do LCH cells produce and do these play a role in the tissue damage and the systemic manifestations of LCH?

Table 1 compares the phenotype of normal epidermal LC and LCH cells.

Table 1.

MARKER	LC	LCH
Surface ATP ase	+	+
MHC class II	+	+
MHC class I	+ - low	+ - ?
FcIgG receptor	+	+
FcIgE receptor	+	?
C3bi	+	+
CD1a and c	+	+
CD4	+ activated	+
CD45	+	+
CD14	+	?
CDw29	+	?
I L2 R	+ activated	+
CD1lb and c	+	+
S100	+	+
Peanut agglutinin		+
I F Nγ receptor	-	+
PLAP	+1-	+

There is obviously considerable similarity between LC and LCH cells. Placental alkaline phosphatase may be a useful marker in LCH. The finding that PLAP expression is transiently expressed by LC stimulated in mixed epidermal cell-lymphocyte cultures suggests that it may be an early activation marker for LC. This suggests that LCH may represent an "activated" LC phenotype.

CD1a. Dr Chu went on to review the function of the CD molecule since its presence is pathognomonic of LC and LCH cells. The MCH I-like structure and association with 132 microglobulin suggest that it may be an antigen presenting molecule. CD1 antigens have been shown to stimulate γδ T cells and Brenner has also reported that CD 1b can present mycobacterial antigen to a 13 CD4-8- T cells. When CD1a is labelled with anti-CD1a conjugated to gold particles, endocytosis occurs and particles can subsequently be observed by EM in Birbeck granules.

In stable transfectants, expression of CD1a slows the growth rate and the expression of the antigen is also temperature dependent, with optimum expression at 34°C. The low expression of MHC I on

LC might be accounted for by competition with CD1a for β2 microglobulin. This might make LC a protected site for viruses. Retroviral particles have been observed in LC in lymphoma and LC may be a reservoir for HIV in AIDS.

LC ontogeny LC precursors are CD34+ bone marrow cells, IL3, GM-CSF and TNFα all increase the number of DC colonies derived from bone marrow. These bone marrow precursors give rise to blood LC precursors. In normal adults <1% CD1a+ cells are found in blood but in severe burn patients up to 5% can be detected. Cord blood may have 20% CD1a+ cells present.

LC migration LC are migratory cells and this can be studied using whole epidermal sheets lying on top of filters. LC migrate into the filter in response to IL3, GM-CSF, IL8, C5a and epidermal conditioned medium. UV irradiation of the epidermal sheet also induces migration.

Function of LC These cells are central to the elicitation of contact allergic dermatitis. They may mediate immune surveillance in the skin and could present antigen to B cells via their high affinity FcIgE receptor. Following antigenic challenge to the skin LC migrate to the regional lymph nodes where they are found in contact with CD4+ T cells in the paracortex.

LC from human skin remain viable for up to 14 days in culture. They do not divide but upregulate MHC II 3 fold. CD1a is lost. Purified LC are very potent allostimulators. This and other functions may be mediated partly by cytokine production. Table II summarises information on cytokine production of LC, LCH cells and keratinocytes.

Table II

CYTOKINES	NORMAL LC	LCH CELLS	KERATINOCYTV
IL1 α β	+	+	+
1 L 2	-	+	+
1 L 3	-	+	+
I L 4	-	+	+
1 L 6	-	-	+
I L 8	-	+	+
TNFα	+	+	+
IFNγ	-	+	+
GM-CSF	-	+	+
TGFα	-	-	+

These results derive from antibody staining and identifying mRNA using a semiquantitive PCR methodology.

Differentiation and function of dendritic cells

Federica Sallusto

Dr Sallusto first discussed the underlying rationale of her experiments. Although DC have been cultured previously, their function alters in culture (Table III).

Table III

PROPERTY	FRESHLY ISOLATED LC	CULTURED LC
MHC CLASS I	+	**INCREASED**
MHC CLASS II	+	**INCREASED**
ICAM-1	+	**INCREASED**
LFA3	+	**INCREASED**
B7/13B1	+	**INCREASED**
FcgRII	+	**DECREASED**
NSE	+	**DECREASED**
F U N C T I O N		
MLR stimulation	+	**INCREASED**
Presentation of native antigens	+	**D E C R E A S E D**
Presentation of peptide antigens	+	+

These data (Schuler and Steinman, J Exp Med 161: 526, 1985 and Raoani et al Exp Med 169:1169, 1989) show that cultured DC lose the ability to process native antigens. They set out, therefore, to study factors which might maintain DC function. A culture system was devised which used the adherent fraction or a light Percoll gradient fraction of peripheral blood mononuclear cells (PBMC). These were depleted of CD2+ and CD19+ cells and cultured in GM-CSF and IL4.

The cultured cells express MHCI and II, CD1a, b, e, FcgRII and B7 (weakly). They are able to stimulate allogeneic lymphocytes and can present Tetanus Toxoid (TT) to TT specific T cell clones. These cells are 100-500x more potent allo-stimulators than PBMC and are as efficient as TT specific B cells in presenting this antigen. Furthermore, in the presence of immune complexes, cultured DC become even more efficient than antigen specific B cells. These

"immature" DC can be maintained in culture and retain the ability to use the FcgRII receptor.

Maturation Signals. Either TNFα or CD40 ligand can induce phenotypic and functional changes, summarised in table IV below.

Table IV

MARKERS	CULTURED DC	CULTURED DC + TNFa
MHC class II	**+**	**INCREASED**
ICAM	**+**	**INCREASED**
LFA3	**+**	**INCREASED**
CD44	**+**	**INCREASED**
B7	**+**	**INCREASED**
FcgRII - CD32	**+**	**DECREASED**
MLR stimulation	**+**	**INCREASED**
TT presentation	**+**	**DECREASED**
Effect of anti-TT	**INCREASED**	**NO EFFECT**

These data show that antigen presenting-competent DC can be maintained *in vitro* and that they can be functionally altered by the cytokine environment. These in vitro cultures provide a means of studying normal and LCH dendritic cells.

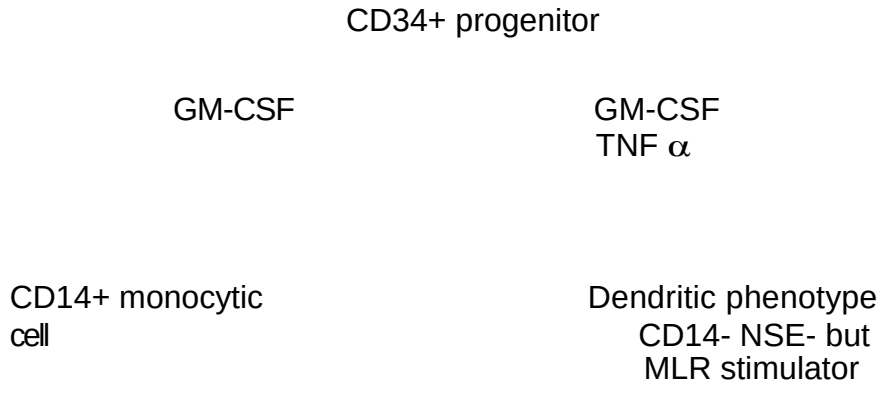
Development of dendritic cells and Langerhans cells from precursors

Ron Jaffe

Work with DC is complicated by the fact that there is no DC specific marker. Furthermore the functional and phenotypic properties of DC populations are highly dependent on methods of isolation and culture. Nevertheless a consensus view of DC is that they are motile cells with characteristic morphology and homing properties that are extremely effective allo-stimulators and express high levels of MHC II. Fresh DC isolated by negative selection from blood lack CD1a but CD1a+ cells can be obtained by cell sorting from blood. This raises the question of the relationship of these cell types.

The human haemopoietic progenitor cell for DC is probably a CD34+ MHC- cell. Culture in GM-CSF and TNFα gives rise to CD1a+ MHC+ cells.

Several authors have investigated culture conditions for growth of DC from cord or adult blood. Thus Santiago-Schwartz (1992) showed that cord blood non-adherent CD34+ cells could give rise to cells of varying phenotype depending on the combinations of cytokines added.

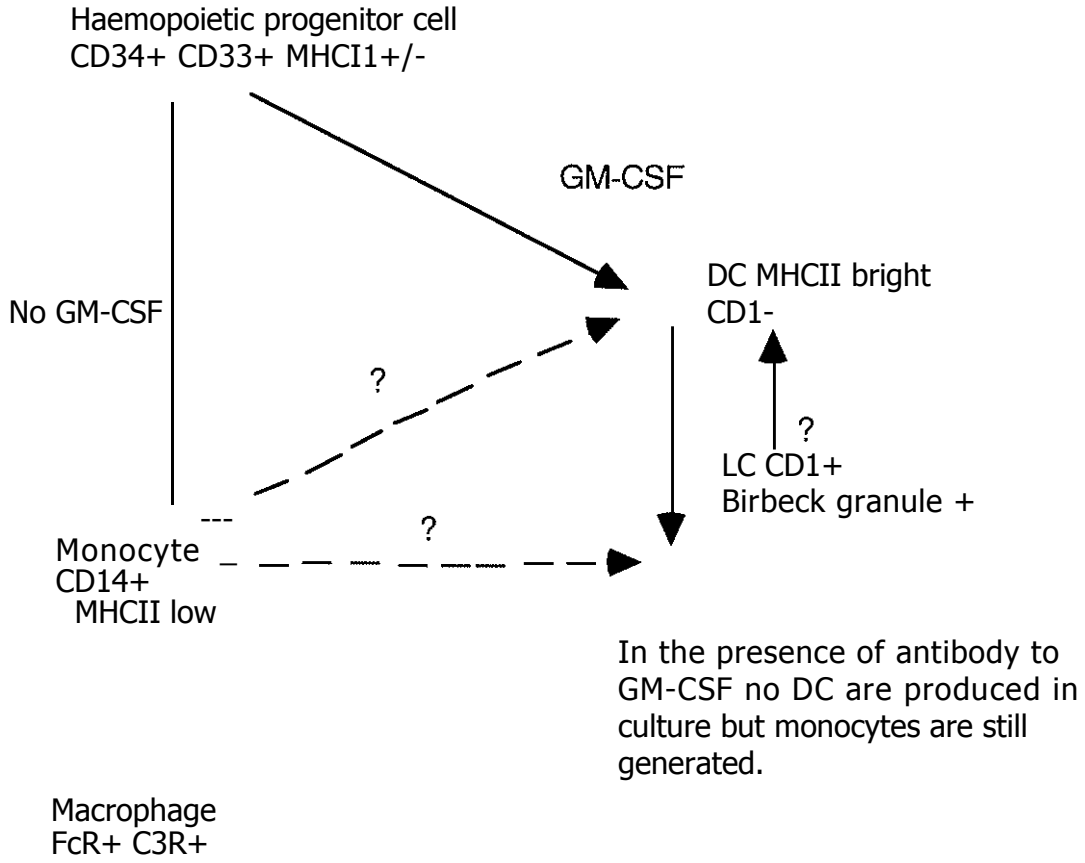


Similar results were obtained by Caux and Banchereau (1992) who showed that some GM-CSF/TNFα stimulated cells express MHCII, B7, CD40, CD11a, CD18 and CD54. About 20% of the CD1a+ cells had LC granules.

Reid (1992) showed that separated adult CD34+ cells could give rise to DC although the majority generated granulocytic cells.

In Dr Jaffe's own experiments, cord blood MNC are cultured with GM-CSF giving rise to clusters of cells with DC morphology. Motile cells migrate from the clusters. These are CD1a+ and CD68+ and appear to be a mixture of monocytic and dendritic cells. From these results and the literature reviewed above the following scheme for DC maturation is proposed (Fig I).

Fig I



SESSION III

Pathogenesis II

Chairman **Rapporteur**
Professor B Favara **Professor P Beverley**

Pre-LCH presenting in infancy Bob Arceci.

Dr Arceci described a histiocytosis patient who presented in infancy and had a strong family history.

JT was a full-term product of an uncomplicated pregnancy. On day 2 of life, a red plaque was noted on the tip of his nose. Over the course of the next two months of life, similar lesions appeared over the rest of his face, arms, hands, torso, buttocks, legs and feet. These lesions were erythematous, firm, raised papules or nodules which coalesced in many areas and in some regions were covered with long, dark hair. There were no other physical signs. He had a normal, peripheral white count and differential, mild microcytic anaemia and an elevated platelet count. Bone marrow examination showed only a slight increase in the AVE ratio. The remainder of the laboratory studies were normal. Radiographic findings were notable for "cupping" or "notching" of the distal ulna and proximal fibular metaphyses as well as the presence of permeative, destructive lesions in the phalanges. MRI of the head was normal. The family history was notable for identical lesions appearing during the first days of life in his father, a paternal aunt and an older male sibling. In all of these cases, the lesions regressed during the first month of life.

Skin biopsy demonstrated a pandermal granulomatous infiltrate comprised of aggregates of histiocytes and some giant cells. The nuclei were vesicular with irregular contours while the cytoplasm was faintly eosinophilic and vacuolated, although lipid could not be demonstrated. Intradermal micro-abscesses were present. Immunocytochemistry revealed most of the cells to be positive for HLA-DR, LeuM3 (CD14), KP-1 (CD68) and Leu3a (CD4). About 50% of the cells were positive for lysozyme. Scattered cells were positive for S-100 and some cells were positive for OKT6 (CD1a). Superficially disposed cells were positive for M241(CD1b), which recognises a subset of dendritic cells in the skin. Electron microscopy showed a dense, cellular infiltrate in the mid-and deep dermis comprised of monocytic cells. These cells had well developed, tapering processes. Lysosomes were present in some of these cells. Long, thin, granular-

like invaginations of the plasmalemma were present, suggesting to some pathologists, the formation of Birbeck granules, although no definitive Birbeck granules could be found.

In summary, the infiltrate had predominantly macrophage features. The staining of scattered cells for CD1a suggested some differentiation along the Langerhans cell series. The predominant lack of staining for S-100, including the CD1a positive cells, was felt to be consistent with an incomplete expression of the Langerhans cell phenotype, while the staining of superficially disposed cells for M241 and the absence of Birbeck granules by EM appeared to corroborate this conclusion. The apparent phenotypic evolution of cells from the deeper portion of the infiltrate, where the cells expressed mainly macrophage markers, toward the epidermis, where they assumed a phenotype more comparable to intraepidermal Langerhans cells, suggested to some pathologists that this might be a proliferative disorder of indeterminate cells.

Because of the disfiguring and progressive nature of the disorder in this patient, in contrast to the other affected members of the family, a trial of prednisone was given without significant response. Topical nitrogen mustard also produced no response. Vinblastine +1-prednisone resulted in no improvement. A trial of 6MP/MTX plus prednisone produced a mild improvement followed by a period of stable disease. However, while on this therapy, the disease again began to worsen. A trial of etoposide with prednisone resulted in a mild but transient improvement again followed by worsening disease. Cyclosporin A plus prednisone was then given, with some improvement and stabilisation of the disease. At age 3 years and while on Cyclosporin A, the disease again began to worsen and a -interferon was started. Quite significant improvement occurred during the first six weeks of therapy, particularly on the legs, torso, arms and hands. The radiographic abnormalities have completely resolved. The patient is currently in the second month of treatment with a-interferon.

What disease does this patient have? Does he have a familial form of an indeterminate cell proliferative disease or some other histiocytic precursor cell disease? Does he have a very early onset, familial form of multicentric reticulohistiocytosis? This case raises questions concerning the lineage relationship among the cells of the mononuclear, phagocytic system as well as the inherited nature of some forms of the histiocytic disorders.

Analysis of Clonality — Beverley Griffiths

Dr Griffiths discussed the analysis of clonality in LCH. Methods for analysis of clonality in most cells (other than lymphocytes, which express clonal receptors) depend on the observation that in female cells loci on one X chromosome are inactivated. Inactive DNA can be detected because it is methylated and resistant to certain restriction enzymes. Restriction fragment length polymorphisms (RFLPs) and variable number tandem repeats (VNTRs) provide polymorphic markers for specific X chromosome loci allowing paternal and maternal alleles to be distinguished. Differences in RFLP or VNTR size due to methylation make it possible to determine whether only one or both alleles are active in a population of cells. The more polymorphic the locus, the higher the proportion of cases in which the maternal and paternal alleles will differ and the analysis will be informative.

Three loci were examined in this study, PGK, informative in 30% of cases, M27β, in 90% and the androgen receptor in 90%. Two problems complicate clonality studies. The first is that X chromosome inactivation is not always random even in normal cells and the second, that the presence of normal cells in the population under study may obscure clonality of the abnormal cells.

Seven patients were studied and among these 2 were informative with PGK, 7 with M27β and the androgen receptor. In 6/7 normal tissue was available for comparison. Model experiments, in which clonal and non-clonal populations were mixed, showed that a clonal population could be detected at 50% when Southern blotting was used. Analysis by polymerase chain reaction was more sensitive to contaminants.

The results showed 2/2 cases with multisystem disease to be clonal, 1/3 with multifocal bone disease and 0/2 with unifocal bone disease. LCH appears therefore to be a heterogeneous disorder in which the more severe multifocal and multisystem forms may be clonal proliferations.

Critique of methods for clonal analysis — Jorge Yunis

Dr Yunis discussed the significance of studies of clonality. A firm conclusion is that clonality does not necessarily indicate a malignant or even a benign neoplasm, nor is X inactivation the sole method for analysis of clonality. Point mutations, chromosomal deletions and translocations may all be markers of clonal populations.

In myelodysplastic syndromes ras mutations may be detected without development of leukaemia and chromosomal abnormalities may also persist for long periods of time. An example of the latter is the 8:14 translocation involving the BCL2 gene, which is often present in lymphoma but has also been detected in benign hyperplasia without supervening lymphoma. An additional problem in analysis of haemopoietic cells is that they may show more skewing in X inactivation studies than most tissues, perhaps because small numbers of stem cells are dividing to give rise to clonal progeny at any one time.

SESSION IV **Sunday May 9th**

Pathogenesis III

Chairman M Nesbit **Rapporteur Tony Chu**

08.00-08.45	Viruses, implications	R Weiss
08.45-09.15	Cytokines in LCH	G Kannourakis
09.15-10.30	Comments	A Abbas/D Kamp

Viruses: implications

Robin Weiss

In a disease of unknown aetiology, there is always the possibility that either exogenous or endogenous viruses may be involved in its pathogenesis.

The evidence of a viral input into LCH is only tentative

1. Viral particles have been identified in LCH on electron microscopy.

2. Tissue from eosinophilic granuloma when disrupted, filtered and the resultant supernatant injected into animals, give rise to a similar disease in animals.

3 Initial studies reported at the 3rd Nikolas symposium by Ken McClain reported a high incidence of HTLVI in LCH. This has not been substantiated by a subsequent study.

Against a viral aetiology is the epidemiology of LCH- a rare and sporadic disease with no geographical or ethnic bias, which might be expected if a virus was involved.

There are however virally induced diseases in which geographical clustering is not involved ie. where the virus is ubiquitous and the disease is a rare outcome of infection. Examples of this include childhood Burkitt's lymphoma, nasopharyngeal carcinoma and epidermodysplasia verruciformis

Burkitt's lymphoma in Africa and Papua, New Guinea, although caused by EBV, geographically follows the distribution of malaria. In these patients, antigenic stimulation by malaria is an important cofactor in a population where EBV is ubiquitous.

In epidermodysplasia verruciformis (EV), a rare recessive genodermatosis, the incidence appears to be sporadic in the population. Individuals with EV are immunosuppressed, develop warts due to HPV5 and develop skin cancers in these warts when exposed to UVR. HPV5 is present in many members of the general population, but you need immunosuppression to develop such warts - ie. a relatively common and benign infection leads to skin cancer in patients with EV.

In HIV infection, the incidence of Kaposi sarcoma (KS) shows a massive bias to the gay patients (25%) compared with IV drug abuse patients (<5%) or those who acquired their HIV infection through transference of blood products (<2%). Probably a KS agent exists in addition to HIV, and KS appears as an opportunistic infection in immunosuppression.. It would seem that the KS agent is only transmissible sexually rather than by blood (or is it presence of sexually transmitted diseases that allows development of KS?)

In LCH there are as yet no clues as to which type of virus if any is involved. Which candidate viruses should be looked at:

a) ***Herpes virus*** - HHV6 and 7. HHV6 is found in AIDS patients and exanthamatum subitum. >50% of the patients carry the virus. It is lymphotrophic for T cells. Elaine Jaffe has found HHV6 in some cases of Rosae Dorfman disease. In LCH 16/30 patients were found to be carry HHV6 when archival material was subjected to PCR. It is unknown whether this represents the normal distribution in the population. Ken McClain has not been able to confirm these data.

b) ***Pesti/Toga virus ,*** such as Hepatitis C virus and

c) ***Arena virus*** - the cause of Lassa fever

d) ***Parvo virus***

e) ***Retroviruses*** - An unsupported report from Rudi Berger has demonstrated reverse transcriptase activity in an LCH cell line. Care must be exercised with interpretation of such findings since there may be laboratory sources of exogenous retrovirus that may contaminate cell cultures and endogenous retroviruses may be present. Most murine cell lines contain retrovirus and many laboratories use retroviral vectors. Zenotrophic viruses which have low activity in the mouse host may become active when infected into human cells. Many vertebrates have virus like genes and it is estimated that up to 0.1% human DNA is retroviral. It is possible that some human cells may produce non-infective retroviral proteins

Foamy viruses: These are common in non-human primates and have been isolated from nasopharyngeal carcinoma and from encephalapathy following renal dialysis.

Conclusion

To date there is no good evidence for a viral aetiology for LCH. Theoretically this is possibility, but there are no clues from the clinico pathological picture to suggest a specific type of virus that could be implicated. It is probably inappropriate to devote too much effort in looking for viral aetiology.

Cytokine overview Abul Abbas

There are four functional categories of cytokine.

I. Mediators of natural immunity

Proinflammatory -	TNF
	1L6
	IL 1
Chemokines-	1L8 - mainly granulocytes
	IL12 - the most potent inducer of IFNγ production by NK cells

2. Regulators of lymphocyte growth and differentiation

T cells especially CD4+ cells IL2
IL4
IL10 - inhibits accessory effect of macrophages to T cells. It is incorporated by EBV genome ie shuts off antiviral response.
TGFβ - produced by many different cell types and reduces lymphocyte proliferation.
IL13 - produced by T cells via CD28 rather than TCR

3. Activators of effector cells

IFNγ	macrophages
IL5	eosinophils
TNFβ	neutrophils

4. Haemopoetic growth factors

CSF's	
IL3	
IL7	B cell growth factor
IL11	megakaryocyte growth factor

Recent advances in cytokine biology

1. Identification of cytokine receptor families

2. Production of knockout mice 1L2, 1L4, IFNγ, IFNγ receptor, IL1O.

Issues still to be resolved

1. The biochemical mechanisms of actions of cytokines.
2. Physiological regulation of cytokines - different patterns of cytokine production in different infections and different diseases.

Role of Cytokines in disease

1 Allergic diseases - IL4 turns on IgE production by B cells. In atopic and non atopic asthma 1L5 appears to be important. 2 Autoimmunity in the effector phase - IL2 and IFNγ

3. Infection - TNF and IL1 in septic shock. In leprosy the phenotype of the disease is related to cytokine production. X linked SCID - defective mutation in IL2 receptor.
4. Lymphoproliferative disease. There is no hard evidence yet for a role of cytokines in these diseases

Clinical uses

Haematopoetic growth factors
IL1ra - use in septic shock
TNF receptor or binding protein in septic shock.

Cytokines in LCH

George Kannorakis

Lesions of LCH are composed of a mixture of histiocytes, lymphocytes, polymorphs, eosinophils, mast cells and plasma cells. Most of these cells are mature cells, cytologically.
If cells are extracted from lesions of eosinophilic granuloma, grown in vitro and the conditioned media used to stimulate bone marrow cells in colony forming assay, three distinct types of colonies are produced:

Erythroid,
Granulocyte/eosinophil and
Mixed erythroid and granulocyte colonies.

In studies in which EPO, GMCSF, IL3 and conditioned media were used to stimulate bone marrow cells, there was a synergistic effect of IL3 and GMCSF in producing non erythroid colonies and anti IL3 and anti GMCSF blocked the effect of conditioned media on the EPO effect on bone marrow cells. (Table VI)

Table VI

	Erythroid colonies	Non Erythroid colonies
EPO	**27+4**	**0**
EPO + GMCSF	**40+5**	**33+6**
EPO + IL3	**54+4**	**12+2**
EPO + IL3 + GMCSF	**57+6**	**70+8**
EPO + conditioned media	**51+3**	**35+5**
EPO + conditioned media + anti GMCSF	**40+4**	**19+2**
EPO + conditioned media + anti IL3	**23+2**	**22+4**
EPO + conditioned media + anti GMCSF + anti IL3	**25+3**	**2+1**

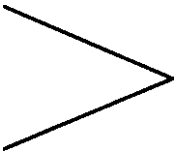
Methods used to identify cytokines in tissue include:

1. Reverse transcriptase PCR - can quantitate message - need non degraded RNA
2. In situ hybridisation using oligonucleotide probes
3. Immunocytochemistry - best to use intact tissue as separation techniques may lead to artefacts in cytokine repertoire ie activation or switch off of cytokine production.

Using in situ hybridisation, the following cytokines message have been detected in lesional tissue from LCH

I L1
I L3

IL4
GMCSF
TNFα
LIF



High levels

In addition LIF has been detected in high levels at the protein level by a bioassay system in six different conditioned media. Using in situ hybridisation, IL2, IL5, IL6 and IFNy were not found. A weak band for IL6 message was seen on RT-PCR.

LIF - Leukaemia inhibitory factor is a 38-67 Kd glycosylated protein. The LIF gene consists of 3 exons and 2 introns and mRNA is 4.2kb. Two forms are recognised

D - Diffusable form
M - Matrix associated form

Bioassays for LIF

1. Increase in IL3 colony formation of blast CFU's
2. Increase in megakaryocyte production
3. Suppression of clonogenicity of U937 and HL60 cells in the presence of GCSF and GMCSF.

LIF stimulates bone remodelling in vitro and may mediate hypercalcaemia in malignancy and HTLVI infection. It inhibits lipoprotein lipase in vitro and may act with TNF in the production of cachexia.
It has a stimulating effect on acute phase protein synthesis by hepatocytes.

In the nervous system LIF controls the choice of neurotransmitter phenotype made by sympathetic neurones in vitro.
There are many cellular sources for LIF

Long term bone marrow stromal cells
Thymine epithelial cells
Alloreactive T cells
Cultivated keratinocytes
SVK14 cells
Activated monocytes
PHA stimulated T cells.

Comments

Diane Komp

Dr Komp started the discussion by highlighting the need for age specific controls for the clonality, cytokine and the virology studies. The severe forms of LCH tend to be restricted to younger patients - could this be related to the way in which infants respond to biological insults with cell proliferation and possibly clonal expansion rather than polyclonal expansion. A possible model to examine this would be hamartomas in infants. Are these clonal?

Circulating cytokines change with the age of the infant from neonate to 1 year old and 2 year old. Infants have a greater variety of Vβ subsets of T cells especially in tissues. In the neonate, all subsets of Vβ T cells are present but by 8 days many have been deleted.

In a study of normal tissue, inflammatory dermatoses and LCH (table VI), the pattern of cytokine expression was similar in all tissue except that IL3 was only detected in LCH.

Table VI

Tissue	Site
CONTROLS	**Normal skin** **Inflammatory dermatoses** **Bone marrow trephines**
LCH	**Bone** **Lung** **Lymph node** **skin** **liver**

IL3 synergises with a number of other factors to induce proliferation, differentiation and chemoattraction. Abul Abbas made the comment that IL3 has not been demonstrated to have any direct effect on growth or migration but the addition of GM-CSF and IL3 to CDIa+ cells with lymphocytes leads to proliferation of CDIa+ cells.

Comments — Robin Weiss

Professor Weiss started by addressing the issue of the role of cytokines in LCH - are cytokines the cause or effect?

In cultures of cells from eosinophilic granuloma of the bone, cells will proliferate, but if penicillin is added to the cultures, proliferation is inhibited. One possible explanation is that bacteria present in the cultures stimulate cell proliferation. It is unlikely, however, in the long term, that bacterial infection will stimulate prolonged cytokine production. Cytokine production is generally, acute and short lived.

LIF expression in LCH could be responsible for the hepatomegaly seen in this disease. LIF is not produced by the LCH cells but possibly by circulating lymphocytes and resident Kupffer cells.

Professor Weiss then posed the question as to whether it was reasonable to consider the clinical use of IL3 and GM-CSF antagonists. Both these reagents are available and have been used in animal studies. If the LCH cell is the important cell in this disease, inhibition of cytokine to which these cells are dependant would be a rational approach to therapy.

The possible role of superantigen in LCH was then discussed. Superantigen is a potent stimulator of T cells and may stimulate the production of IL3. Only a very small percentage of T cells can be induced to produce IL3 - by using calcium ionophore and PMA this can be achieved but the cells virtually need to be killed to get them to produce IL3.

Experimental data that would be against the presence of superantigen on CD positive cells is that FACS purified CD1a cells when added to allogeneic T cells give only minor stimulation at day 6. If superantigen was present, a massive response would be expected.

SESSION V

CLINICAL ASPECTS OF LANGERHANS CELL HISTIOCYTOSIS

Chairman
M Nesbit

Rapporteur
D Komp

Interim report on the first International LCH-1 Study

Helmut Gadner

Since the most effective treatment approach for Langerhans cell histiocytosis(LCH) has yet to be established, in April 1991 the Histiocyte Society started the first International study (LCH-1). Only newly diagnosed, previously untreated patients up to the age of 18 years with multi-system disease and a definitive diagnosis of LCH are considered eligible to be enrolled in the study. The study requirements include:

i) the confirmation of diagnosis according to defined histopathological criteria (Histiocyte Society, Lancet 1987).
ii) a uniform patient stratification based on standardised diagnostic assessments of disease extent (Histiocyte Society, Med. Ped. Oncol. 1989) and
iii) Central data collection and recording.

The aims of this study were:

i) to prospectively test the role of chemotherapy started as soon as possible after confirmation of diagnosis and
ii) to compare two different treatment arms with respect to response, rates of failures and assessment of therapy related toxicity and/or late sequelae in a randomised way.

For this purpose, a clear definition of the disease state (active - AD or non active disease - NAD) and response criteria have been established. Regular evaluations are performed at fixed intervals (after 2, 4, and 8 courses of therapy) All patients with multi-system disease are randomly assigned to receive either etoposide (VP-16) (150mg/m^2 i.v. over 2 hours) given for 3 consecutive days every 3 weeks or vinblastine (6mg/m^2 i.v. bolus) weekly for a period of 24 weeks. Each drug is administered as a single agent,

only initially combined with one pulse of high dose methylprednisolone (HDMP) in an attempt to achieve a prompt improvement of general symptoms (ie fever, dyspnea, pain etc.) The chemotherapy protocol was designed to deliver treatment promptly after diagnosis, in order to rapidly decrease activity of the disease rather to wait for possible progression, to reduce mortality and to avoid recurrences, late sequelae and permanent consequences.

In Europe the clinical trial was opened on April 1st 1991, with the German/Austrian, Italian, Scandinavian and British groups participating. In the United States, Canada and Australia the study started on April 1st 1992. By September 1st 1993, 242 patients had been registered:

German/Austrian	142
Italy	42
UK/Ireland	33
Scandinavia	8
USA/Canada/Australia/South America	17

One hundred and ten of the registered patients had multisystem disease at diagnosis and 79 (38 males, 41 females) were randomised to one of the two treatment arms (90% with centrally confirmed definitive diagnosis). Forty three were assigned to vinblastine (arm A), and 36 to VP16 (arm B). The median age of randomised patients with multisystem disease was 18 months (range 6 days-14 years 6 months.) Remarkably, the interval from onset of symptoms to diagnosis had a median of only 3 months and therapy was started immediately after diagnosis (median 8 days). After a median observation time of 17 months (range 4-30 months) 54 out of 79 patients could be evaluated with respect to response after 2 courses (6 weeks of therapy). The assignation of these children to the three response categories was as follows:

10 progressive disease (AD "worse")
21 intermediate (AD stable or mixed response)
23 better (resolution/NAD or regression of AD)

There was a prevalence of involvement of more organs with liver and spleen involvement in the group of non responders ("worse"). However, considering the small patient numbers it has not yet been possible to determine a distinctive pattern of organ involvement at diagnosis which will predict the response to therapy.

In contrast, patients who did show a clear response after 2 courses/6 weeks clearly benefited from the protocol therapy. Out of 20 children, in whom full data was available following completion of the protocol therapy (24 weeks), 15 had a complete resolution of symptoms and signs (NAD), 3 were assigned to the response category better and 2 were still intermediate active. This observation supports the view treatment of LCH should continue as long as there is some evidence of response and no progression is visible.

On the other hand, 24 out of 79 patients included in the study were switched to the alternative therapy arm at different intervals, according to the recommendation of the study protocol in 1991. Remarkably, in 4 children who had switched after 12 weeks of treatment improvement was seen after the change of therapy. On the other hand, of 7 patients who were switched during the first 6 weeks (2 courses of therapy) because of non-responsiveness, 1 child showed an intermediate response, 2 patients suffered from progressive disease and 4 children died. Studying the outcome of all 10 patients categorised as worse after 2 courses of therapy it became evident that 6 of them died and only one survivor showed a clinical improvement, in the sense of a "better" response. Overall 10 children died because of non-responsiveness and disease progression during the observed period. None had achieved a "better" response after 2 courses of therapy (6 of them were non responders ("worse") and 4 intermediate complicated). The interval from diagnosis to death was median 8 months (range 3-22 months). All were less than 2 years old (median 13 months) and had liver and spleen involvement.

In summary the preliminary data shows that with the treatment applied in the LCH 1 study a regression of the disease within 6-12 weeks (2 to 4 courses) can be expected in approximately 50% of patients. The speed of initial response seems to be decisive in prognosis, as those patients who did not respond to treatment during the first 6 weeks of therapy fared worse than those that did. In the most severely ill children under 2 years with multi-system disease with multiple organs involved including liver and spleen even the switch to the alternative therapy arm (or to other salvage chemotherapy) after an initial non-response did not positively influence the outcome. This course seemed independent of which treatment arm these patients were receiving. However, if the switch occurred later, some patients clearly benefited from the change of therapy.

The analysis of LCH-1 data reveals that a small group of patients with a high risk of a poor outcome can be identified very early in their course, after only 6 weeks of treatment. This group includes a subset of patients suffering a form of disease with an 80% probability of early death, despite other chemotherapeutic approaches. For these patients the Histiocyte Society has established a Salvage Therapy Group to propose experimental therapeutic approaches, rather than continuing the apparently unsuccessful chemotherapeutic regimens used to date.

This treatment is described in detail in the LCH-1-S protocol of the Histiocyte Society and comprises the option of high dose immuno-suppressive therapy or allogeneic bone marrow transplantation. The protocol is considered a continuation of the systemic study of LCH by the Histiocyte Society and its members, and will be open only for those patients who have been actively treated on the LCH-1 protocol.

These achievement represent valuable progress in the efforts to find the optimal treatment for LCH and were only made possible through the fruitful International co-operation within the LCH-1 study group. However, a lot remains to be done to answer all questions of the on-going LCH-1 study, which requires the enrolment of many more patients.

Chemotherapy: Possible future directions

Maurice Slevin

In LCH, treatment of a patient that has had no active treatment before generally gives better results. If a patient had been heavily treated with chemotherapy before, the response is poorer.

The problems with treating LCH are enumerated below:

1) Few cells are cycling
2) LCH is not truly malignant
3) Spontaneous remissions are common
4) Mortality tends to be low
5) Responses to single agent and combination chemotherapy are similar

LCH bears some similarities with low grade non-Hodgkin's lymphoma and chronic leukaemias in that they are chemosensitive, difficult to cure, several remissions are possible and they are slow to develop resistance. As a broad principle, the

more the cell resembles the normal counterpart, the less likely is it that a cure will be achievable.

The broad principles for chemotherapy in LCH are:

1) Single agent
2) Drug without long term sequelae
3) Prolonged low dose administration
4) Oral if possible

Drugs used in LCH

VINBLASTINE

Weekly intravenously
Well tolerated in conventional doses
60-70% response rates

MERCAPTOPURINE

Active in ALL, AML, CML, NHL
Chronic oral dosing
Low toxicity

In a study of vinblastine and 6 mercaptopurine by Lahey in 1975, the following results were achieved (Table VII)

Table VII

AGENT	RESPONSE	COMPLETE REMISSION
Vinblastine	**60%**	**32%**
Vinblastine + prednisolone	**60 %**	**31 %**
6MP + Prednisolone	**44%**	**22%**

Non-responders to the primary line of therapy were unlikely to respond to the cross-over regime.

METHOTREXATE

Activity in a wide range of haematological and solid tumours
Chronic oral dosage may lead to cirrhosis
Long term oral weekly doses
Penetration into the CNS

VP16 or ETOPOSIDE

TABLE **VIII** gives the current data on VP16 in LCH.

Table VII

Patient numbers	Previous treatment	Response to VP16	Reference
18	18	83% (67%CR)	Cancer 1988
10	6	90%	MPO 1989
6	0	84%CR	MPO 1991
10	5	100 %	AJCO 1992
17	17	82%CR	MPO 1993

VP16 has been associated with the development of ACLL. In children treated for ALL with VP16, the risk of ANLL is about 6%. In small cell lung cancer treated with VP16 as a single agent, no patients have developed this second malignancy. In germ cell tumours treated with combination of bleomycin, VP16 and cis platinum, the risk of ANLL is about 4%.

The risk of ANLL is increased after a total dose of $2g/m^2$ of VP16.

VP16 has a phase specific cytotoxic action and responses are schedule dependant in both in vitro tests and animal studies.
In a study of small cell lung cancer with no previous treatment, VP16 was used as a single agent in two regimes:

A) A one day dose of $500mg/m^2$ as a 24 hour infusion
B) Five 2 hourly infusions of $100mg/m^2$.

Results of this study are given in Table IX

Table IX

Schedule	A	B
Number	20	19
Response	2 (10%)	16 (84%)
CR	0	1 *(5%)*
Overall response	10%	89%
Median duration	-	45 m
Median survival (giving conventional chemotherapy after failure of A or B	6.3m	10 m

If VP16 is given orally, there is enormous variability in bioavailability which is unpredictable. Intravenous infusion will achieve better and more consistent levels of the drug.

HYDROXYUREA

Wide range of activity in haematological and solid tumours
Oral long term treatment
Well tolerated
Good CNS penetration

FL UDARABINE

Fluorinated analogue of ARA A
Activity in CLL and low grade NHL
Mild toxicity

CYCLOSPORIN A

Cyclosporin has been tried both as a single agent and with prednisolone or vinblastine. In one trial of 18 patients, 2 had single system bone disease, one had single system CNS disease and 15 had multisystem disease. Age range was 1 month to 52 years. Patients were treated with Cyclosporin as a single agent at 6mg/kg twice daily. 6 patients died, 2 were stable but still active, 5 improved and 2 were too early to evaluate.

In a second study of 11 patients, 4 had limited disease with single system bone or bone and skin involvement and 7 had multisystem disease. The age range was 49 days to 14 years. The dose of Cyclosporin was 5 to 12 mg/kg twice daily with prednisolone or vinblastine. 6 patients died, three failed to respond, two improved.

Summary

It is unlikely that patient with LCH will be cured with current cytotoxic drugs.

The drugs to try are:

Etoposide - prolonged schedules
Methotrexate
Hydroxyurea
Fluarabine

Progress and problems in gene therapy

Malcolm Brenner

Only 2% of all diseases are genetic but many other diseases are influenced by gene abnormalities. If LCH is a clonal disease, gene therapy may be indicated. If only a minor population of patients represents a clonal disease, gene marking could identify this population who could be treated more aggressively with chemotherapy or bone marrow transplantation.

The applications for gene therapy are:

1) As a drug delivery system
2) To modify cell behaviour
3) To give new function to the cells
4) To replace defective gene function
5) For gene marking

The ideal features for gene therapy are:

1) High efficiency of insertion
2) Insertion into a specific cell type
3) Insertion into a specific site on the chromosome
4) Appropriate regulation
5) In vivo acting vector

Delivery systems that can be used are:

1) Physical methods including:
   Electroporation - this technique is mainly used in vitro in the laboratory rather than in vivo

   Liposomes

2) Viral vectors:
   Retroviruses which lead to permanent integration into the host cell

   Adenovirus as used in cystic fibrosis - these do not integrate and die with the cell

   Adeno-associated viruses - the wild type virus integrates into specific sites but in current vectors site specific binding sites are removed

To date, gene therapy has been of low efficiency and no regulation has been possible.

RETROVIRAL VECTORS

The retroviral vector used is usually a modified form of the mouse Malony leukaemia virus. The viral gene is removed, retaining the 5' and 3' LTR and the gene of interest is inserted with or without an internal promoter. The construct is produced in a packaging cell line so that once in the host cell, no free retrovirus is present. Exclusion of contaminating retrovirus is of major importance, as in monkey studies where high titre virus in packaging cell lines contaminated with retrovirus is given, thymomas develop in the monkeys containing wild type retrovirus.

GENE MARKING

Relapse of disease following autologous bone marrow grafting may be due to residual disease in the patient or in the bone marrow. Marrow can be purged of residual disease using pharmacological, immunological or physicochemical techniques but it is difficult to show that they are effective. Gene marking is one method of monitoring residual disease.

The advantages of gene marking are:

1) All progeny contain the marker
2) There is no dilutional effect, ie. the retrovirus is integrated into the cell genome and as the cell divides, the marker is passed onto all progeny.
3) No transfer to other cells

Normal cells can also be marked giving general information about the speed of recovery of the bone marrow and characteristics of engraftment.

Gene marker protocols

21 patients entered:
- 12 - AML
- 8 - Neuroblastomas

18 patients were evaluatable and of these there were 16 successful engraftments.

Gene marking did not damage or prevent engraftment of the cells. The gene marker was detectable for more than 2 years of follow up and was present in erythroid and myeloid cells and T and B cells.

Gene marking can be used to determine whether relapse was due to residual disease ion bone marrow grafts using a co-expressed marker for the malignant cells, eg. chromosomal translocation 8:21 which produces the ETO transcript to see if AML cells are marked or not. The use of marking can also be used to test the efficacy of purging of bone marrow.

CYTOKINE GENE TRANSFER

Cytokine gene transfer can theoretically be used in the treatment of tumours by increasing the immunogenicity of tumour cells. Failure of the body to attack tumour cells may be due to lack of tumour specific antigens or insufficient immune activation.

Benefits of immune activation in neuroblastoma:

1) Cells are derived from neurectodermal tissue which is not found in children
2) Spontaneous remissions are documented
3) Sensitive to immune system effectors

One protocol would be to modify the vector to include the 1L2 gene. In vitro studies have demonstrated that transfected neuroblastoma cells are surrounded by autologous lymphocytes and the cells are then destroyed. A similar effect can be achieved by adding exogenous IL2.

In patients, autologous transfected tumour cells were injected subcutaneously on day 1 and 8 and biopsies taken at day 8 and 22 respectively. Biopsies were subjected to immunohistochemical and PCR based analysis and a T cell infiltrate with no viable tumour cells was detected.

In LCH, no gene therapy is possible until the defect in LCH is found. However, there are 4 possibilities:

1) If a clonal disease - replace the marrow lesion
2) Modify LC using immunotherapy
3) Modify effector cells
4) Markers in bone marrow transplantation

Antibody Therapy - a Case Report

Kara Kelley

LCH cells express the CD1a glycoprotein that can be identified by a number of monoclonal antibodies. CD1a has a very restricted distribution in the human body being expressed only by Langerhans cells and cortical thymocytes. It thus represents the ideal target for antibody directed therapy. Antibodies against this molecule would potentially knock out LCH cells, Langerhans cells and cortical thymocytes but Langerhans cells and cortical thymocytes could repopulate from CD1a negative precursor cells.

We have access to the monoclonal antibody NA1/34 which was produced by Prof A McMichael at the John Radcliffe Hospital, Oxford. This has been produced in milligram amounts, purified and endotoxin treated and tested at the Imperial Cancer Research Fund.

KL was a 16 month old girl diagnosed at 13 months with multisystem, biopsy proven, LCH. The disease was progressive, despite high dose corticosteroids and etoposide. At the time of the study, the patient had gross hepatosplenomegaly with evidence of liver dysfunction - hypoalbuminaemia, prolonged prothrombin and partial thromboplastin time. The patient had a hypoceilular bone marrow and was transfusion dependant for red cells and platelets. Skeletal survey showed a single lytic lesion in the occiput.

NA1/34 was labelled with ^{111}In using diethylenetriamine pentaacetic acid, at 1mg antibody/37 MBq of isotope. 100µ.g of NA1/34 was first administered intradermally to test for type I hypersensitivity. As no reaction was observed over the next hour, 0.5mg of radiolabelled antibody was administered by slow intravenous infusion. Whole body images were obtained at 1, 4, 24 and 48 hours using the gamma camera. At 48 hours, the label was localised to the occiput - a site of known bone disease - spleen, liver and bone marrow.

The patient was then treated with increasing doses (6, 18 and 18mg) of unlabelled antibody by intravenous infusion over 30-60 mins on alternate days. Following this antibody treatment, the patient was subjectively better but the liver and spleen did not change in size and the patient remained transfusion dependant.

Localisation of the antibody as demonstrated on the HI In scans is encouraging as this does show that the antibody will localise *in vivo* to sites of known disease activity. The subjective response

seen following antibody therapy is difficult to interpret as LCH is known to have a fluctuating clinical course and it would be difficult to prove that the improvement seen was due to the antibody therapy. The results do, however, suggest that further studies in more patients are warranted to properly evaluate this modality of treatment.

A research project is presently underway in Tony Chu's laboratory to produce single chain antibodies for NA1/34. These will have the advantages of more rapid penetration of the agent through the tissues, lack of non-specific Fc binding to phagocytic mononuclear cells and reduced immunogenicity. Linkage of such reagents to radioisotopes or poisons could provide potential agents for salvage therapy in LCH.

FINAL SUMMING UP

Robin Weiss, Blaise Favara, Peter Beverley, Abul Abbas

A major problem with research into this disease was highlighted as difficulty in obtaining material from clinicians, particularly fresh tissue that could be used for clonality and karyotypic studies. It was agreed that protocols need to be written up for tissue collection and handling. These protocols should be presented to the Histiocyte Society to gain as much support as possible from clinicians dealing with patients.

The clonality studies have raised the issue that LCH may represent a malignancy, at least in part of the spectrum of disease recognised as LCH. Would this alter the way in which clinicians treat patients with LCH? Mark Nesbit said yes, although opinion was divided amongst other participants.
Further studies are needed to determine whether clonal LCH cells are reactive or truly malignant. Karyotypic studies were suggested as one way of achieving this.

Scientific studies that were given high priority were:

1) Clonality in different manifestations of the disease - single organ, polyostotic bone disease, multisystem disease. Could clonality be used as a prognostic index?

2) Cytogenetics - is the clonal LC malignant?

3) Biology of the LCH lesion - how does LCH tissue behave in culture

4) Other cells in the lesion - could cells such as the Vβ T cell be driving the disease?

5) Trigger for the disease - could LCH be the result of superantigen stimulation related to a persistent virus/bacterium?

Only with continued co-operation between the clinician and scientist with forums such as the Nikolas Symposia and the Histiocyte Society can we maintain the momentum we have already achieved in this disease.