SESSION I

INTRODUCTION

Chairman & Rapporteur Dr Jon Pritchard

Clinical review of LC II in Children vs Adults -
Drs Valerie Broadbent and Tony Chu.

Dr Broadbent described the clinical spectrum of LCH in children. Bones, then skin, are commonly affected, then lung, lymph nodes, pituitary gland, liver, spleen, bone marrow, gut including mouth, retro-orbital tissues, brain, thyroid and pancreas. Muscle, kidney adrenal, gonad and eye involvement have not yet been reported. Growth retardation (not necessarily due to GH deficiency) was a feature in some cases, and occasionally the presenting feature. Complete healing sometimes takes many months or years but "late effects" were seen after active disease had "burnt itself out". Diabetes insipidus was relatively common and lung and liver fibrosis also occurred.

Treatment depends on the extent of disease and symptomatology. Children may not need treatment, local treatment (intralesional steroids, topical mustine or (rarely) radiotherapy) or systemic treatment, with or without local treatment. Established systemic therapies include corticosteroids and chemotherapy particularly etoposide/vinblastine; experimental systemic treatments include cyclosporin A, interferon-a, indomethacin (for bone pain) and bone marrow transplantation. Generally speaking, systemic treatment is now monotherapy rather than combination therapy;

a) because the current view that LCH is a "reactive" condition, not a malignancy and

b) because the response rate to combination chemotherapy does not seem to be better than the response rate to monotherapy.

In 1991, the Histiocyte Society's LCH 1 trial started. It involves a pulse of high dose steroids (methylprednisolone - all patients) and a randomized comparison between vinblastine and etoposide, each for 6 months. All patients with multisystem disease and some

with single-system disease are eligible. Outcome measures include;

a) response rate,

b) recurrence rate and

c) incidence and type of "late effects" especially diabetes insipidus. Already (5/92) >90 patients have been recruited. There is a tissue storage programme.

Dr Chu described the disease in adults. He pointed out that "definitive" LCH has been described in adults of all ages, even the elderly, but that the incidence was unknown, partly because adults tended to be referred to "organ specialists" e.g. dermatologists, respiratory physicians and not to oncologists. There was some difference in emphasis in terms of organ involvement, adults having proportionately less involvement of the liver, spleen and a higher percentage of lung involvement. The treatment approaches were similar in adults and children. Dr Chu has noted impressive, all be it anecdotal, responses to azathioprine in several of his patients.

SESSION II

CLINICAL NEUROLOGY AND PATHOLOGY

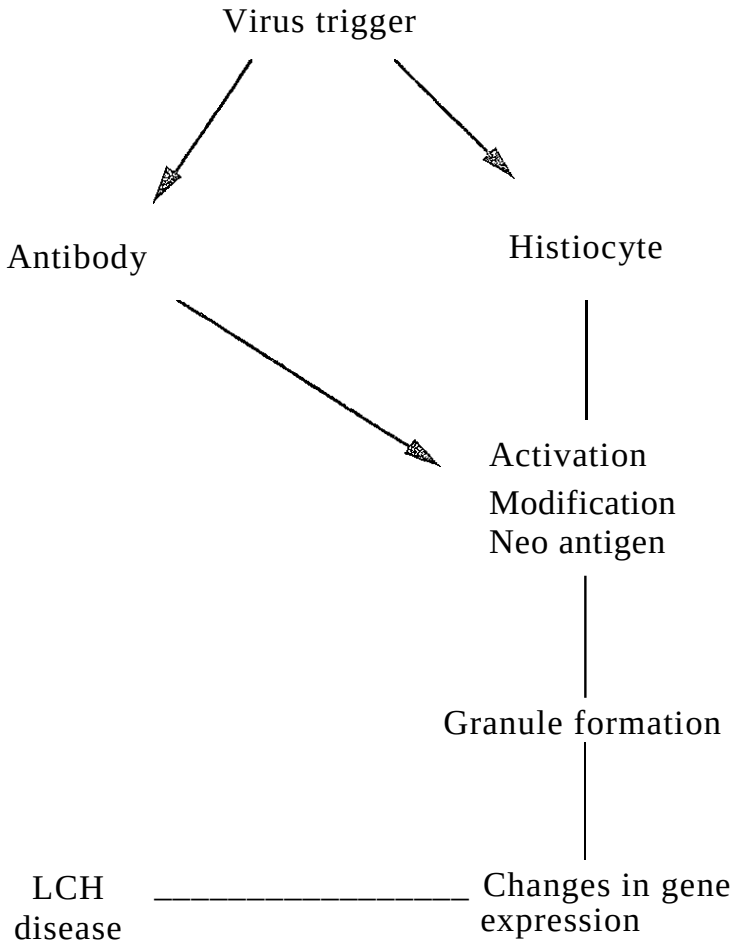
Chairman & Rapporteur Professor Peter Beverly

Review of the Histopathology of LCH and an analysis of the clonality of the lesions - Professor Blaise Favara

Professor Favara briefly reviewed the features of LCH lesions and discussed some of the problems raised by the histology. The first difficulty encountered is in defining the disease but it is now widely accepted that although lesions may be pleomorphic, containing granulocytes, plasma cells, lymphocytes and giant cells, the presence of unequivocal Langerhans cells is essential. Histiocytes have variable morphology and may be difficult to distinguish from LC. Various cells may be S 100, CD1a or Birbeck

granule positive though the giant cells generally lack S 100 or CD 1 a. LC are thought to originate from monocyte-like cells in the blood. The lesions contain proliferating cells as indicated by Ki67 and PCNA staining. Studies of DNA content indicate that the lesions are diploid. Attempts to grow LC in vitro or nude mice have been unsuccessful.

The pathology of the lesions is indicative of cytokine effects, including fibrosis and gliosis. In the liver greatly enlarged Kupffer cells are seen. There is a clinical association with Hodgkins and non-Hodgkins lymphoma and perhaps leukaemia. Professor Favara suggested an aetiological scheme for the evolution of LCH.



Professor Favara reported on Dr Willman's study of clonality in six cases of LCH using Southern blotting of restriction fragments generated with methylation-dependent restriction enzymes. All cases were female and from the Southwestern USA.

cases were female and from the Southwestern USA. 4/6 were HTLV II+.

All six cases were apparently clonal. No oligo-clonal Ig gene or TCR rearrangements were detected.

In discussion this was thought to be very important data but several questions were raised. Had control material from the patients been analysed? Had clonality studies been performed on more than one lesion from a single patient? Could clonal analysis be performed on sorted CD1a cells?

Immunohistopathology of LCH - Professor Peter Isaacson

Professor Isaacson briefly summarised earlier immunohistological results. LC have been described as CD1a+, CD2+, weakly CD5, 6 and 7+, CD68+ and possibly also express lysozyme.

The present study included both frozen and paraffin material. LC were found to express CD 1 a, were 84% CD68+ and usually showed cytoplasmic staining with CD2 (100%) and CD3 (80%). They lack B cell antigens. The majority of LC lacked lysozyme though occasional cells were weakly positive. Macrophages and eosinophils in the lesions were positive.

5-10% of LC expressed PCNA and in 3 cases where frozen and paraffin material were both available there was a good correlation with Ki67 expression. This is a similar proportion to a low grade lymphoma.

When the APAAP method of staining was used a high background was found. Surprisingly an antibody to placental alkaline phosphatase (PLAP) stained LC. Staining was stronger in frozen sections.

The results can be summarised as showing that LC have a macrophage-like phenotype with cytoplasmic CD2 and CD3, they

are proliferating and contain placental alkaline phosphatase. It was suggested that measurement of serum PLAP might be a measure of disease activity.

Cytokine mRNA in LCH lesions - George Kannourakis

Dr Kannourakis reviewed his work on detection and quantitation of cytokines produced in LCH. The pleomorphism of the lesions raises the question of what controls entry of cells to them. Cytokines are likely to be important. CSF's regulate survival of progenitors in vitro, proliferation, differentiation and activation of mature cell function. In GM-CSF transgenic mice, there is excessive entry of macrophages into tissue.

Assays

Bioassays. Conditioned medium of lymphocytes from LCH tissue supported growth of erythroid, myeloid and mixed colonies, suggesting the presence of IL-3 and GM-CSF. This was confirmed with blocking, antibodies. CSF production by sorted CD3+ cells does not require in vitro stimulation.

Detection of Message

In situ hybridisation for mRNA has been developed but only preliminary data is available as yet. For Northern blotting or PCR, RNA degradation is a major problem, perhaps due to release of RNase in inflamed sites.

PCR analysis for IL-1-6, GM-CSF, TNFa and IFNy has been carried out.

IL-4 is strongly positive although serum IgE is not raised. GM-CSF is positive and an extra band is seen on Southern blotting the PCR product. This is in the coding region and only seen on LCH samples. IL-3 was not detected by PCR but the primers may not have been optimal.

In situ anti-sense oligo probes showed IL-3 and IL-113 with rare IL-4+ and GM-CSF+ cells. TNFa was positive on the giant cells.

A synthetic giant riboprobe containing 10 cytokine primers has been constructed. This will in future allow quantitation of PCR amplified c DNA.

Review of Brain Histopathology - William. Hickey

Dr Hickey reviewed the histology of 5 cases of LCH he had obtained. He discussed 3 cases in detail.

Case 1. Died of pneumonia after a 16 year history. The brain showed thickening of the pons but no obvious focal lesions. Histologically two types of lesion were seen. 1. Perivascular infiltrates of large eosinophilic cells and lymphocytes. The large cells were S100+ and CD68+. 2. Xanthomatous lesions containing foamy histiocytes laden with neutral lipids. Areas of demyelination and astrocytosis were seen.

Case 2. A 60 year old male who died after a 2 year history of neurological symptoms. Autopsy showed multiple small tumour masses some LCH-like but others with the appearance of large cell lymphoma.

Case 3. After a CT scan showed brain lesions, a biopsy was performed showing eosinophilic cells and lymphocytes. The cells were Vimentin +, S 100+, Mac 387+, MB2+ and CD68+. Birbeck granules were not seen by EM.

Dr Hickey has also studied turnover of brain macrophages in Fl to Parent rat radiation chimera. Donor cells are seen in perivascular areas. In 90 days 50-70% of meningeal, 30-40% of perivascular and 1% of parenchymal macrophages are turned over. Perivascular macrophages share some properties with LC such as high MHC class II, macrophage markers and S 100.

Central Nervous System Disease in LCH - Nicole Grois.

Dr Grois reviewed the clinical data from 9 patients with LCH CNS disease, 7 male and 2 female. All patients had disseminated LCH with skeletal involvement and skull lesions. In 3 patients diabetes insipidus (DI) was present up to 5 years before diagnosis of LCH. In 2 patients DI developed 2 and 5 years post diagnosis.

A variety of neurological symptoms were detected including ataxia in 7/9, hyper-reflexia 6/9, tremor 5/9 and psychomotor retardation 5/9. Symptoms did not correlate well with magnetic resonance imaging findings. Brain biopsy was performed 7 times in 5 patients and revealed non-specific atrophy in 3 and normal brain in 4.

Treatments post diagnosis of CNS disease included x-rays, steroids, VPI 6 and cyclosporin. Outcome did not appear to be well correlated with treatment.

Dr Grois also reviewed the literature on 72 LCH patients with CNS disease. Fourteen had acute symptoms of raised infra-cranial pressure and pathology characteristic of LCH. This subgroup did well after treatment. Fifty-eight showed insidious onset of whom 75% developed DI, often long after diagnosis. Forty had autopsies, with cerebral and cerebellar lesions predominant. Perivascular histiocytic infiltrates and granulomas were frequent, with demyelination, gliosis and loss of Purkinje cells.

These findings raise questions relating to the aetiology, assessment and treatment of CNS LCH.

SESSION III
EXPERIMENTAL NEUROLOGY

Chairman & Rapporteur Professor Blaise Favara

Analysis of macrophage function *in situ* in tissue
-Dr S. Keshav

Morphological and chemical properties of macrophages are site dependent. Macrophages in different sites have different morphology and different biochemical properties.

Macrophages respond to different cytokines in two basic ways;

I Thl group causes activation, up-regulation

2. Th2 group results in other functions- down regulation

In-vivo studies of cytokines

Choices of methods are limited and include:

. Collection of cellular secretory products which is not practical for the most part.

2. Localization of proteins but this has limitations as you cannot tell target from source as the material may be phagocytosed.

3. mRNA localization which is the best means of identifying the source or "maker" of the cytokines.

Lysozyme is a good marker of macrophages.

Alveolar macrophages are the only ones that make lysozyme without being activated. Others require activation.

Newly recruited macrophages are lysozyme+, "old" resident ones are negative.

Activation of recruited macrophages causes lysozyme production but activation of resident macrophages is not associated with lysozyme production.

IL 1, 1L6, TNF and MCP- l (monocyte chemotactic peptide) are relevant cytokines. For example MCP- I presents first in early granuloma formation.

Macrophage activation is tissue site dependent.

Tannic acid injected in CNS causes a microglial reaction after a latent period of 2 days. At the same time there is recruitment of macrophages from the bone marrow.
The lesion of LCH may therefore reflect morphology that varies with the tissue site of the lesion.

Dynamics of Macrophages in the Central Nervous System- Dr Linda. Lawson

Macrophages populate the normal central nervous system (CNS) in large numbers but have a "quiescent" phenotype imposed upon them by the CNS environment. Modern immunohistochemical methods have made it much easier to detect these cells and it is clear that they are a major dial population in the steady state and major contributors to reactive gliosis. In normal brains, the microglial population is heterogeneous in morphology and distribution, responding to environmental cues which are not obviously related to neurotransmitter systems, vasculature or developmental cell death.
In rodents, numbers and heterogeneity are maintained into old age. Although resident microglia are stable and long lived cells we believe that the maintenance of the population entails both low level proliferation of resident macrophages as well as slow recruitment of monocytes from the blood. This migration occurs despite an intact blood brain barrier, as does the specific recruitment of larger numbers of monocytes to an inflammatory stimulus in the brain parenchyma. Turnover of macrophages in the normal brain is rather slow compared to peripheral organs (especially lymphoid organs) but the duel origin of new cells is a common feature of macrophage populations elsewhere in the body.
The labelling indices of microglia in different regions of the brain parenchyma were broadly similar but there are subpopulations of microglia in the CNS, in the choroid plexus and neurohypophysis, which have different properties and dynamics. The choroid plexus contains two populations of macrophages on either side of the blood/CSF barrier. Both populations express some of the antigens which are typically down regulated on microglia, including MHC

II. Stomal macrophages (blood side) proliferate some ten times faster than microglia of the brain parenchyma. In contrast new epiplexus cells seem to be supplied solely by migration. It is tempting to speculate that the choroid plexus might be a major site of traffic of cells. It certainly appears to be easier to induce inflammatory recruitment of leucocytes into the choroid than other regions of the brain.

The neurohypophysis contains a subpopulation of microglia with a slightly less restricted phenotype than the microglia of the brain and which turn over at a faster rate. These cells are particularly interesting because that respond to increases in secretory activity of the neurohypophyseal neurons by transiently increasing proliferation.

Migration of macrophages into the CNS potentially opens this compartment to the invasion of activated or infected cells; the most well known example would be 1-IIV-1 infected monocytes. We are continuing to study the factors which normally restrict both the phenotype and turnover of microglia in the normal brain as well as those which mediate alterations in proliferation and recruitment.

Microglia in "S" phase are morphologically unaltered and they divide with processes extended.

SESSION IV

THERAPY IN LCH

Chairman and Rapporteur — Dr Tony Chu

Review of past and present therapy - Prof Stephan Ladisch.

A brief overview of the evolution of treatment of LCH can be divided into early, late and recent therapeutic approaches. The diversity/heterogeneity of expression of what we call LCH, ie, single lesions, multiple system involvement, organ dysfunction, greatly impeded systematic evaluation of patient outcome. Lahey (1962) contributed substantially to our understanding of the

disease by demonstrating I) the prognostic importance of organ dysfunction and age to the diagnosis and 2) the benefit of specific therapy. Survival in an untreated group of children with multisystem disease was 100% while that in a treated group was 70%. Lack of true controls as well as other factors made this study, although a pioneering one, not conclusive.

Subsequently, (1980) retrospective stratification and outcome analysis (survival) of a number of studies demonstrated the validity of stratification (table 1) and clearly suggested the need for prospective studies to determine the potential value of chemotherapy.

Table 1

GROUP	SEVERITY	SURVIVAL
A	All LCH excluding mono-ostotic	71%
B	Mild - polyostotic	98%
C	Moderate - soft tissue without organ dysfunction	76%
D	Severe - soft tissue with organ dysfunction	37%

In the 1980's, treatment approaches to LCH have generally paralled the severity of the disease, e.g. local treatment for isolated lesions and intensive chemotherapy for multisystem disease. The most recent and large scale prospective study, which incorporated patient stratification and uniform treatment approaches is that of the German/Austrian group, DAL HX-83.

DAL HX-83 used vinblastin, VPI6 and steroids with oral 6-mercaptopurine or methotrexate as an option. Treatment was given for 12 months. Patients were stratified into 3 groups according to the clinical disease, le, A - multisystem disease, B - disseminated disease but with no organ dysfunction, and C disseminated disease with organ dysfunction. Results of the trial are given in table 2.

Table 2
DAL HX-83

	A	B	C
Number	28	57	21
Initial resolution	89%	91%	67%
Recurrence	15%	18%	19%
Mortality	0	2	8

Median time to initial resolution was 4 to 6 months. Morbidity was low. Diabetes insipidus was observed in 6% patients.
The suggestion from this study was that early aggressive therapy possibly reduced the incidence of morbidity in these children.

Future directions include randomised, prospective treatment protocols for multisystem disease (LCH1) and experimental approaches to treatment of severely affected patients who do not achieve resolution of disease by treatments such as that of LCH1.

Trimethoprim monotherapy in LCH - Dr Fotini Tzortzatou Stathopoulou.

The efficacy of trimethoprim therapy in LCH was noticed by serendipity when it was used as a broad-spectrum antibiotic in the treatment of otitis in children with LCH. It was noticed that the drug ameliorated or induced resolution of the manifestations of LCH in different organs. These observations led to an open study in which 12 children from 9 months to 12 years were treated for 2 to 12 weeks with 10-15mg/kg/day of trimethoprim in divided doses. The results of the study are shown in table 3.

Table 3
Results of an open study of trimethoprim in LCH

No of Patients	Disease	Result
2	Localised - parotid and thyroid	Swelling resolved in 3 weeks. Disease free for 2 years
5	Bone	Reduction of adjacent soft tissue swelling within 4 weeks. No change on X ray. 4 now recurrence free, I recurrent disease
4	Multifocal: spleen, lymph node, bone, ear, gum	Liver, Skin and ears cleared, 2/4 gums cleared.

In one child with multisystem disease, hepatomegaly reduced and liver function tests normalised after 4 weeks of treatment.

Trimethoprim thus appears to be an effective treatment in some patients with LCH. This drug has been reported to have a beneficial effect in Wegeners granulomatosus. The mechanism behind its action is open to speculation but may be related to an anti-folate or immunomodulatory effect.

Topoisomerases as novel targets for anti-cancer drugs - Dr Paul Smith.

There have been major advances in the field of anti-cancer drugs in the last two decades which have heralded significant improvement in the prognosis of a number of malignancies (table 4).

Table 4

Malignancy	Present cure rates	Cure rates in 1955
Acute lymphocytic leukaemia	75%	0%
Testicular tumours	90%	0%
Hodgkins disease	80%	0%

One class of anti-cancer drug are the topoisomerase inhibitors.

Chromosomes are compact and ordered structures. Fibrils of DNA run in and out of a protein complex or matrix and are thus restrained. Topoisomerase II is an enzyme that is located on the DNA at the base of the chromatin loop domain (fig 1).

The enzyme binds to the DNA covalently via tyrosine molecules. It acts by forming a temporary gate in the double stranded DNA through which an intact helix can pass but prevents a complete break in the DNA strand. First one side of the gate opens and allows the helix of DNA to enter and this gate then closes. The opposite gate then opens to allow the DNA through and this then closes.

The level of topoisomerase II in the cell is related to the cell cycle. The level is low in G 1, increases in S with a further rise in late S phase and an increase in G2. After mitosis, as the cell cycles into G 1, proteolysis reduces the level again.

The enzyme can be trapped on the DNA as a cleavable complex by topoisomerase inhibitors. The term cleavable refers to the ability of strong protein denaturants to reveal that the trapped enzyme sequesters a DNA double strand break in its complex. The trapped enzyme acts rather like a sleeper on a railway line and prevents separation of the two strands of the DNA at that point and thus prevents transcription and replication.

A list of known topoisomerase inhibitors is given in table 5.

Table 5
Inhibitors of DNA Topoisomerases

Class of drug	Examples	Enzyme inhibited
Coumarins	Novobiocin	bacterial gyrase
	Coumermycin Al	euk topo II
Quinolones	Chlorobiocin	reverse gyrase
	Naladixic acid	bacterial gyrase
Anthracyclines	Oxolinic acid	T4 topo
Anthracenediones	Adriamycin	euk topo II
Acridines	Mitoxantrone	euk topo
	Amsacrine (m A MS A)	euk topo II
Ellipticines	2-Me-9-0H-E+	euk topo II
Actinomycins	Actinomycin D	euk topo I and II
Epipodophyllotoxins	VP 16 213	euk topo II
Alkaloids	Camtothecin	euk topo
Minor groove ligands	Hoechst 33342	euk topo I and II

The level of topoisomerase II within a cell differs from one cell type to another, some cells being under producers and other overproducers. Increasing the level of topoisomerase II production by the cell will make it more sensitive to topoisomerase inhibitors. This can be seen in human breast carcinoma cells, where an increase in cell growth can be induced by oestrogen which results in increased DNA damage by VP 16 and increased cytotoxicity (fig 2).

Figure 1

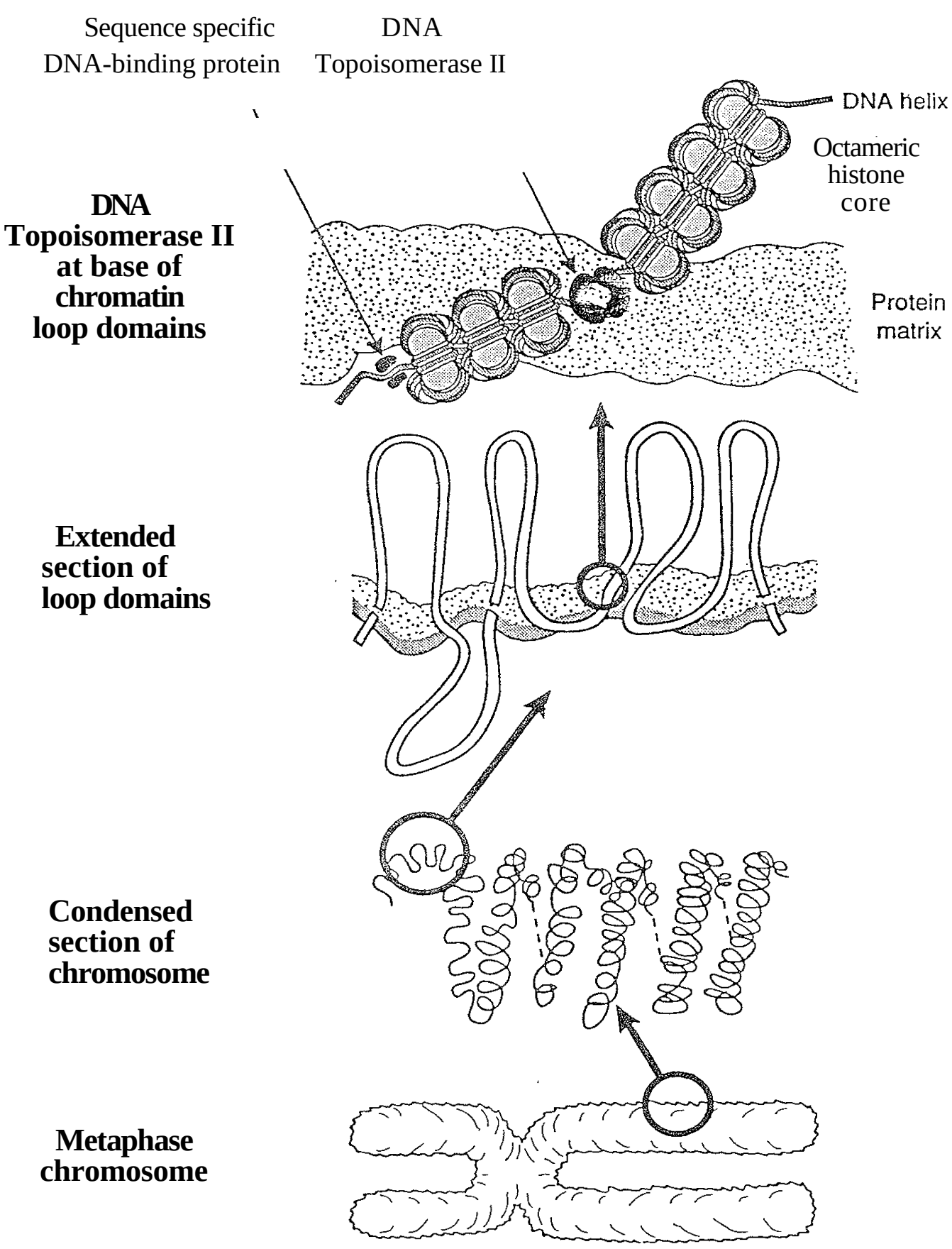
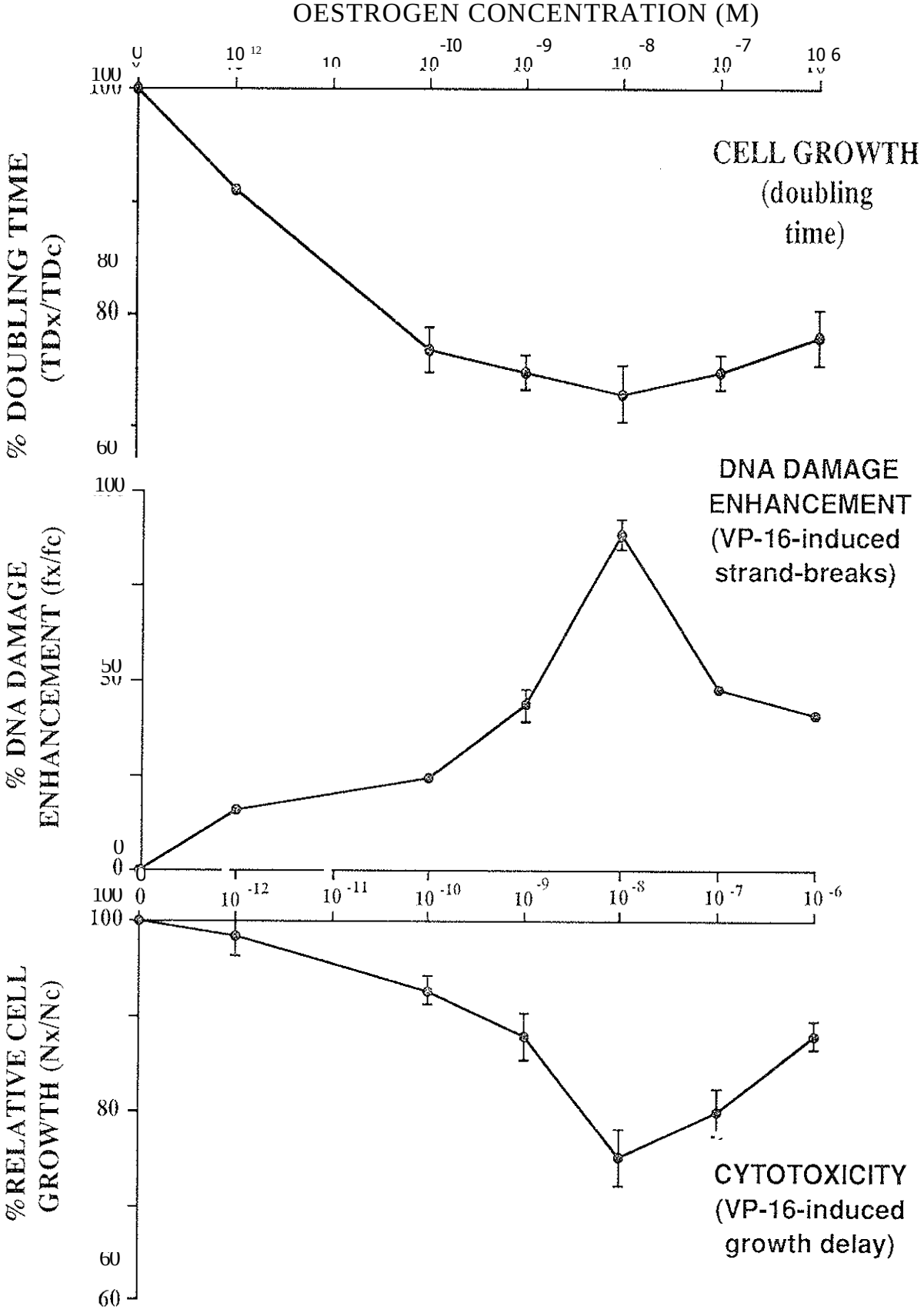

Sequence specific DNA-binding protein
DNA Topoisomerase II
DNA helix
Octameric histone core
Protein matrix
DNA Topoisomerase II at base of chromatin loop domains
Extended section of loop domains
Condensed section of chromosome
Metaphase chromosome

Fig 2
Effect of oestrogen on growth and drug activity



There is some evidence now that topoisomerase inhibitor can modulate nuclear topoisomerase II. In small cell lung carcinoma, VPI6 in vitro has dose dependant effects on the cells growth pattern. At low doses, VPI6 bunches cells in mid and late S phase and G2. At high doses, VP16 traps cells in S phase and at very high doses, VP16 freezes cells in S phase. 24 hours after treatment with VPI6, the small cell lung carcinoma cells are trapped in G2 where expression of topoisomerase II is high. Treatment now with high dose VP16 will result in a high killing rate.

The effect of such drugs on cells can be monitored using single cell electrophoresis. In this method, cells are suspended in agar. If the cell is killed it will release DNA into the agar which can be drawn into rockets by electrophoresis. This is a good method of monitoring cell response to therapy.

There has been recent concern over the induction of secondary malignancies in patients receiving VP16 or teniposide containing chemotherapy for haematologic and solid malignancies (table 6). This is of particular concern in LCH given that conservative and prolonged VP 16-containing regimens have proven effective in most cases.

It is possible that the risk of secondary leukaemias may relate to DNA topoisomerase II playing an active role in the generation of endogenous DNA damage and genetic instability. Sperry et al (Proc Natl Acad Sci USA, 1989, 86:5497) have shown that a family of A+T-rich sequences termed matrix association regions, mediate chromosomal loop attachment and that several regions both specifically bind and contain multiple sites of cleavage by topoisomerase II. A dysfunction of such a region could lead to the genetic instability required for neoplastic progression.

Table 6

Epipodophyllotoxin-related leukaemias (Whitlock et al 1991)

Primary diagnosis	No.	ANLL	VP 16 Tenip	of Alkyl Agent	ANLL type	11g23 abnorm
GCT	1		E	N	M5	N
NSCLC	4		E	N	M4 (1)	Y
					M5 (2)	1 /2
					NS (1)	N Y
GCT			E	N	M5	0/2
ALL	12		T	5112	MI (2)	213
					M2 (3)	Y
					M4 (1)	4/4
					M5 (4)	
					M7 (1)	
					NS (1)	ND
SCLC	3		E	Y	M2 (2)	ND
					M5 (1)	Y
					NS (I)	ND
NB	1			Y	M5	
SCLC			E	Y	NS	N
NHL	1		E		M4	N
SCLC	3		E	Y	M² (2)	ND
					M5 (1)	ND
SCLC	1		E	Y	M4	ND
SCLC	1		E	Y	M4	N
SCLC			E	Y	NS	ND
SCLC	1			Y	M5	ND
SCLC	2		E	N	M4 (1)	2 /3
					NS (1)	
ALL	3		T		M4 (1)	

GCT: germ cell tumour, NSCLC: non-small cell lung cancer, ALL: acute lymphocytic leukaemia, SCLC: small cell lung cancer, NB: neuroblastoma, NHL: non-Hodgkins lymphoma, ANLL: acute non-lymphocytic leukaemia.

The unusual features of leukaemia occurring in epipodopyllotoxin treated patients compared with those induced with alkylating agents are:

1. the latency period for ANLL is significantly shorter (33 mths vs 58-73 mths)
2. Often clear monocytic or myelomonocytic features
3. Chromosomal abnormalities (11q23).

The main issues for VP16 in the treatment of LCH are:

1. what is the target population in LCH, is it the LCH cell or another cell type.
2. What features of LCH cells can provide a therapeutic advantage for the eradication of LCH cells - do LCH cells express high levels of topoisomerase II or can they be induced to express high levels of the enzyme in response to cytokines
3. Will dysfunction or cytostasis of the target population suffice.
4. Is there any evidence of drug resistance
5. Is there any evidence of modulation of sensitivity according to the tissue environment
6. Can we manipulate target cells to become more sensitive to the topoisomerase inhibitors.

Work that urgently needs to be performed is to look at the site of action of VP I6 to see whether the LCH cell is the target population, or whether other cytokine-producing cells are being selectively destroyed leading to loss of tissue targeting of LCH cells. If it is the LCH cell, we need to know more about the cell cycle kinetics of the LC and LCH cells and their their enzyme profiles.

Profile of Antimalarial drugs - Vera Stecher.

Quinine and the synthetic quinine derivatives are anti-inflammatory agents which are used in the treatment of malaria but also in autoimmune diseases, viral infections, hyperlipidaemia and thromboembolism.

Quinine has been known to the Western world since 1630:

HISTORY OF ANTI-MALARIAL DRUGS IN AUTOIMMUNE DISEASE

1630	Jesuit Priests discover "Quinine" which the natives called "Fever bark".
1800	Quinine spread through Europe by the Jesuits for use in febrile illness.
1890	Quinine used in discoid lupus erythematosus but high doses required.
1915's	Germans develop Quinacrine as a synthetic anti-malarial since quinine unavailable during World War 1; this had better anti-inflammatory properties.
1 940	Chloroquine developed as part of anti-malaria research in USA to develop less toxic agents than quinacrine.
1943	Payne reported benefit of Chloroquine in both rheumatoid arthritis and SLE at doses 5-10 fold higher than for malaria; benefit reported in up to 75% of patients.
1950's	Wide use of chloroquine at high dose (often 10-15 mg/kg) in RA led to reports of retinal toxicity.
1957	Scherbel reported on the use of hydroxychloroquine (5-6.5 mg/kg) with good benefit and low toxicity.

The action of hydroxycholoroquine on immune responses is due to its activity as a weak base. On entry into the cell it becomes protonated and concentrated in acidic lysosomal vesicles causing a slight rise in pH within the vesicles. This has a major effect in slowing antigen processing and on the efficiency of antigen-receptor complexing. Hydroxychloroquine also reduces production and release of ILI and 1L6 from macrophages which leads to a decrease in ESR, and reduction in the production of acute phase reactants such as CRP. Hydroxychloroquine also decreases release of IL4, 1L5 and 1L6 from T cells resulting in hyperglobulinaemia and decrease in autoantibody production.

Toxicity of hydroxychloroquine is dose related and if used at less than 7mg/kg, there is low retinal toxicity. The mode of action of retinal toxicity is unknown but may be due to the inhibition of breakdown of retinal pigment by tissue macrophages which fill up with undigested pigment.

Patients who are given hydroxychloroquirie need ophthalmic assessment with fundoscopy, slit lamp examination and Amtergrid.

Hydroxychloroquine has a number of other effects on different cell types by the same mechanism of inhibiting lysosomal activity. Specific examples of these are listed in table 7.

Table 7

EFFECTS OF HYDROXYCHLOROQUINE

CELL TYPE	ACTION	RESULT
Macrophage	Inhibit antigen processing	Immune modulation
RPE cell	Inhibit melanin breakdown	Toxicity
Liver cell	Inhibit lipoprotein receptor recycling	Lower cholesterol
Vascular endothelium	Inhibit protein assembly and inhibit prostaglandin synthesis	Anti-coagulant
Platelet	Inhibit release of thrombin	Prophylaxis for thromboembolism

Hydroxychloroquine has an anti-viral effect. This is thought to be related to the importance of the lysosome in uncoating the virus which is then able to replicate. Hydroxychloroquine inhibits lysosomal activity and thus blocks the uncoating of the virus.

Anti-malarials have been used in lymphohistiocytosis in one reported case (Histiocyte Society Annual Meeting 1991, Temporary inactivation of lymphohistiocytosis with quinacrine. Barnard et al). In this report a child with familial lymphohistiocytosis was treated with quinacrine hydrochloride 2mg/kg three times daily for 5 days. Temporary suppression of the disease was achieved but only short-lived response was achieved following a second course.

Bone marrow transplantation - Gareth Morgan.

In 1990, there had been 4234 bone marrow transplants performed in Europe, 2137 allogeneic and 2097 autologous.

The main indication for bone marrow transplantation is the treatment of malignant disease ie leukaemia, but bone marrow transplantation has been used for genetic diseases, thalassaemia, immune deficiency states and aplastic anaemia.

There have been two published reports of successful treatment of patients with LCH by bone marrow transplantation:

Rineden et al, N Engl J Med, 316:733-735, 1987

Stol et al, Cancer, 66:284-288, 1990.

Both patients were male, one 18 and the other 20 years old. Both had multisystem disease and had had multiple treatments. One patient had diabetes insipidus. Donors were HLA matched siblings and engraftment was demonstrated. Both were disease free at the time of the reports.

In addition to these reports, Greinix et al (Bone Marrow Transplantation 10:39-41, 1992) reported four patients, two treated with autologous and two allogeneic bone marrow grafts. One of each of these was alive and well, the other two died as a result of persistent disease.

The main problems with bone marrow transplantation are:

1. Rejection of the graft
2. Graft versus host disease (GVHD)

Both are related to differences in both the major and minor histocompatibility complexes between the donor and recipient. Differences in the minor histocompatibility complexes are more common in unrelated donors.

Some parallel may be drawn between the responses expected in LCH and those seen in immunodeficiency diseases other than severe combined immunodeficiency disease.

In a series of patients with immunodeficiency diseases, 60 patients were treated with identical matched bone marrow transplants and 71 with non-identical bone marrow transplants. Survival over 10 years was worse in the non-identical bone marrow transplants group at 40% compared to 65% in the identical bone marrow transplant group.

Graft rejection and GVHD can be reduced by T cell depletion of the graft but a major problem with this approach is failure of engraftment.

Autologous bone marrow transplants involve reinfusion of autologous bone marrow harvested in disease remission or purged in vitro following conditioning with chemotherapy and radiotherapy. This avoids graft rejection and GVHD.

In LCH, who would benefit from bone marrow transplantation?

1. Aggressive or treatment-resistant LCH with poor prognosis. We need to be able to identify the patients early.
2. Patients need early referral for bone marrow transplantation.
3. HLA matched siblings. MUD - matched unrelated donors or even mismatched related donor should probably be avoided
4. Patients with CNS involvement may be suboptimal.

Total body irradiation may be a problem in children under 2 years of age and other conditioning methods will need to be used.

Antibody therapy - Robert Spooner.

Antibodies can be directed to tumours which express novel antigens. The long plasma half-life of antibody permits maximal uptake at target cells.

For some targets, regional administration increases the speed with which target localisation occurs, whilst leaving blood and normal tissue toxicity unchanged ie. intraperitoneal administration may increase the target dose that can be achieved.

An alternative approach to increase the effect of antibody binding to target sites is to increase not the yield of antibody at the target, but rather to modify the antibody by conjugating it to an enzyme so that targeted antibody/enzyme is capable of converting a non-toxic prodrug to a cytotoxic drug at the target. This approach is called antibody-directed enzyme prodrug therapy or ADEPT, one example is the use of carboxypeptidase G2 (CPG2), an enzyme from the bacterium Pseudomonas. This can be conjugated to a suitable antibody, and after localisation to target and subsequent blood clearance of unbound antibody/enzyme, a relatively non-toxic glutamyl benzoic acid pro-mustard substrate can be administered. At the site of bound antibody/enzyme, this is converted to toxic mustard, leading to local accumulation of large amounts of cytotoxic

In targeting antibodies to specific sites, one problem is failure of rapid clearance from non-target tissues which can result in significant toxicity directed at blood and normal tissues. To further improve the efficacy of ADEPT, a galactosylated neutralising, second antibody, can be administered at the time of maximal target loading, which rapidly clears the unbound antibody/enzyme from the blood stream by the liver.

The rapid clearance of small recombinant antigen-binding molecules (such as single chain variable fragments) should, by analogy with the biodistribution behaviour of proteolytically

derived F(ab')2 fragments, lead to improved target to non-target ratios of bound material. The amino terminal ends of antibodies (variable heavy, VH, and variable light, VL, domains) are those regions of the antibody involved in antigen recognition.

A number of candidate molecules have been examined as targeting agents. CDRs (complementarily determining regions) are regions of high variability within V domain genes which for some antibodies may represent the minimal recognition unit for the antigen. CDRs, however, have a low half life, low affinity and do not retain their configuration.

The dAB is an isolated V domain with a hydrophobic patch normally covered by its partner V domain. Consequently, it is likely to suffer from high non-specific binding, and is therefore unlikely to make a good targeting agent.

The variable domains, Fvs - VL and VH, show variable affinity for its partner, but in the region of Kd = 10-6 M, which is sufficiently low that many Fvs are likely to be dissociated in physiological conditions. Covalently joining the two partners V domains with a flexible linker artificially raises the concentration of available partner domain, and overcomes this problem, producing a stable scFv. Similarly, a Fab-like molecule is stable owing to interdomain contacts between VH and VL, between CH1 and CL and by reducible disulphide bridge between CH1 and CL domains. Of these two stable molecules, the scFv is the smaller, and is the molecule that has been most modified, for example by fusion to toxins. Since scFvs are smaller than IgG molecules and lack Fc functions, they are expected to interact only minimally with cells and molecules of the immune system. Of those molecules tested, they also show potentially useful half-lives characterised by rapid blood clearance.

Novel scFv fusion proteins with predicted rapid clearance from non-target tissues.

Since the amount of antibody that localises to a target may be very small, the potency of any therapeutic agent delivered by that antibody must be high, a rationale that has led to the

development of immunotoxins (ITs). These comprise cell-binding mAbs (or their fragments) conjugated to naturally-occurring toxins (or their toxic fragments). The toxins most frequently used are diphtheria toxin (DT), Pseudomonas exotoxin A (PE), ricin, ricin A chain and a number of RIPs or ribosome inactivating proteins. Encouraging results have been obtained for tumours such as B cell lymphomas, and in graft versus host disease, where the cells are easily accessible to mAbs. The major limitation of ITs lies in the nature of the cytotoxins used, which all inhibit protein synthesis. DT and PE modify elongation of factor 2 and ricin A chain and RIPs inactivate mammalian ribosomes. Since these targets are cytosolic, an IT must be internalised and routed intracellularly so that the toxic moiety can escape from a suitable intracellular compartment and gain access to the cytosol. All targeted cells must therefore bind the IT.

The cytotoxic A chain of ricin has been fused to a scFv, and to isolated V domains. In all cases to date, ricin A chain fusions retain the ability of ricin A chain to inactivate mammalian ribosomes.

Another approach would be to direct cytotoxic cells to the target tissue rather than to direct toxins to the tissue. This is the philosophy behind the construction and expression of a scFv interleukin-2 fusion (SCA-IL-2). SCA -1L-2 protein retains the ability of IL-2 to activate cloned T cells and such a molecule, with cell-type specificity, should thus be capable of activating T cells in the vicinity of the target. Such a strategy, of course, is not dependent upon targeting of all cells.

Increase of retention time at the target.

To accumulate at a target cell, a small, recombinant antigen-binding molecule requires : high affinity so that target can be bound in the low serum concentrations resulting from rapid clearance; or a high avidity, to provide longer residence of bound material at a tumour. In spite of all its attractive features, a scFv is unlikely to be the best possible derivative of an antibody, since it is univalent. Bivalency of a whole IgG molecule has two advantages. The first of these is the level of avidity. If one arm

of an IgG molecule dissociates from the antigen, the other can still maintain contact. The free arm cannot diffuse far and is maintained in a region of high local concentration of antigen, so promoting re-binding. The second advantage is dependent upon the antigen. Antibodies cross-linking some antigens stimulate patching and capping of antigen, leading to increased endocytosis and internalisation of antibody, a feature that should be advantageous for therapy.

Fusion of a scFv gene to a gene encoding the monomer (protomer) of a bivalent (or multivalent) protein might allow expression of fusion proteins with high avidity. If the fusion partner itself possesses suitable enzymic activity (so that a non-toxic prodrug can be administered after localisation to tumour) or binds a ligand with high affinity (so that modified ligand can be aimed at a pre-targeted tumour), then it should be possible to engineer a two-step therapeutic strategy. This also has all the advantages of delaying delivery of the therapeutic reagent, in a form that can be captured by the antibody fragment fusion, until the tumour to non-tumour ratios of previously administered antibody/fusion are optimal and no significant circulating antibody/fusion remains.

Choice of a suitable fusion partner

The final size of the multimeric complex should not be greatly larger than an IgG molecule (ie 150 kD). As a first attempt we aimed for a final size of between 150 - 180 kDa. Since a scFv is approximately 30kD, a dimeric fusion partner could have a subunit size up to 60kDa. The constraints are much higher for a tetrameric molecule, which would need a subunit size of 15kDa or less.

The candidate multimeric fusion partner should have neither interchain nor intrachain disulphide bonds (thus reducing likely misfolding problems), should be reasonably well characterised, available in protein for controls, and should be stable with subunit monomers possessing a high affinity for each other, in case they are expressed in distressingly small quantities. Furthermore, it should be non-toxic and amenable to preparation in a form

suitable for pre-clinical or clinical use or be already in pre-clinical or clinical use. The fusion partner gene should also be cloned and sequenced. Since the preferred expression organism is Escherichia coli, the fusion partner should not depend upon glycosylation for its biological activity. Finally, if possible, the multimeric fusion partner should bind a ligand with high specificity and affinity, or possess a suitable enzymic activity, so that it can easily be made into a molecule for therapeutic intervention.

As a first attempt, streptavidin has been chosen, since it fits these constraints rather well. It is produced by Streptomyces as a homotetrameric molecule of Mr 60,000 (subunit Mr, 15000), that binds four molecules of the water-soluble vitamin biotin with high specificity and affinity (Kd = 10-8M) for biotin. Each subunit has a tightly packed "core% with relatively unstructured amino- and carboxyl-terminal extensions. These tails are thought to contribute to a little-mentioned fact regarding streptavidin - this is its ability to participate in the formation of higher order aggregates. Many commercial forms of streptavidin are extensively proteolised, have lost their unstructured extensions, and form stable tetramers. The mature form of the protein has been the subject of recent research, and is becoming increasingly well characterised and expressed in E. coli and a modified form of the gene is available commercially from British Biotechnology.

A scFv-streptavidin fusion might be expected to exhibit tetravalency for antigen coupled with ability to bind biotin or biotinylated reagents. This latter activity would allow for easy, one-step detection methods, making such a fusion potentially very useful for diagnostic purposes. The expected long tumour half-life of such a targeted fusion and its biotin binding ability should allow for a therapeutically useful molecule.

Immunotherapy review - Peter Beverley.

Immunotherapy has been used to successfully treat diseases resistant to other therapies. One such disease, which has a number of analogies to LCH, is Hodgkins disease. In a report from Falini et

al (Lancet 339: May 16th 1992) Hodgkins disease that had proven to be resistant to conventional forms of therapy was treated using a monoclonal antibody directed against CD30 or Kil, labelled with saporin. This was administered at 0.8mg/kg for 1-2 doses in 4 patients. Tumour reduction of 25 to 100% was noted. In vitro, CD30 is expressed by activated T cells and is present on some endothelial cells and B cells.

In experimental models\ immunotherapy using a recombinant fragment of diphtheria toxin fused to IL2 has been found to be active in vivo and in vitro. In vivo the use of this reagent reduces the onset of diabetes melitus in mice. Its effect is thought to be due to binding of the IL2 to activated T cells with killing of those cells and delay in onset of the disease.

The specific problems of immunotherapy in LCH were discussed. The candidate target molecule is CD1a expressed by LCH cells. This antigen is expressed by normal Langerhans cells, cortical thymocytes and when certain monoclonal antibodies are used, it is also expressed by interdigitating reticulum cells. The loss of these cell populations would not pose a major problem as all would be replenished by CDIa negative precursor cells.

A problem with this approach is nonspecific binding of antibody to sites such as the renal tubule and some neural cells with consequent unwanted toxicity.

SESSION V

Chairmen **Rapporteur**
Professor Peter Beverley **Professor Peter Beverley**
Professor Abul Abbas

Concluding Discussion

Dr Abbas lead a discussion of future directions for work on LCH. He suggested that two areas showed promise.

1. Diagnosis

a) The work on clonality and immunophenotyping was promising but more samples were needed to extend these studies. For analysis of clonality control tissues (eg normal skin or PBMC) were needed. For the immunohistology frozen specimens were important and serum samples for PLAP assay.

b) More work on cytokines in the lesions was required as definition of which cytokines are involved may open up new therapeutic options. He proposed immunohistochemical studies of cytokines to complement the mRNA studies of Dr Kannourakis. CSF and blood cytokines should be examined more systematically and age matched controls will be required.

Work on cytokines should be extended to cover cytokine receptors by immunohistochemistry and at the mRNA level.

c) There was a need to develop prognostic criteria. As yet these are mainly clinical e.g. age, organ involvement, liver dysfunction and response to treatment. Conventional histology does not provide prognostic information but immunohistology might do so, especially measurement of proliferating cells.

2. Treatment

It was suggested that patients failing conventional treatments might be candidates for new treatments. These include bone marrow transplants, antibody-toxin conjugates or perhaps cytokine antagonists. A number of soluble cytokine receptors or antagonists are in phase 1/11 trials. These include IL-1-RA, TNFR, IL-4R.

The place of these in therapy remains to be defined, particularly since it is difficult to decide when a more conventional therapy has failed.

Conclusion. A key issue remains the collection of material and establishment of a registry so that experimenters can obtain samples to work on.