Session 1

Chairman
Professor Stephan Ladisch

Rapporteurs
Dr. Tony Chu
Dr. Francoise Basset

HOW VIRUSES INTERACT WITH CELLS

Overview - Professor Jeff Almond

Introduction

20 different families of animal viruses are recognised or proposed. Viruses represent a diverse group of intracellular parasites with a range of tissue trophism and interaction with host cells. The pattern of reaction within the host depends on the localisation of the virus within the host; its' specific interaction with the cell and the immunological response it engenders.

Localisation of Virus

Viruses exhibit selective trophism to various tissues. Factors that determine this are:

1. Access to cells and target organs.
2. Presence of specific receptors on cells
3. Factors which restrict replication intracellularly

Specific trophism is exemplified by the Polio virus - this is an Enter virus which gains access via the gastrointestinal or respiratory tract. The virus replicates at the primary site with viraemia and replication in lymphoid tissue. The virus specifically localises to the motor neurons in the spinal cord via the nerve endings and leads to paralysis.

Cellular Receptors

Viruses possess attachment proteins (VAR) which bind to specific receptors on the surface of various cell types. In some cases these receptors have been molecularly characterised.

Virus	Receptor
Polio 1 - 3 Rhinovirus HRV14 EBV HIV	Ig Superfamily ICAM - 1 CR2 on B cells CD4

The Canyon theory suggests that for some viruses the VAP is a cleft or canyon into which the cell receptor fits. As the VAP is a cleft, it is protected from an antibody response which is directed to the surrounding antigens. Changes in these antigens result in serotype differences.

Interaction of VAP with the cell receptor may cause functional changes in the cell by blocking receptor function or preventing expression of the receptor on the cell surface.

Following binding of the virus to the receptor, most, but not all, viruses penetrate the cell using a classical mechanism.

Interaction of the virus with the receptor induces a coated pit, vesicle formation and fusion to an early endosome. Inside the endosome the pH falls leading to a conformational change in the viral envelope proteins revealing a fusigenic domain, which fuses with the membrane of the endosome with release of viral genome into the cytoplasm.

Restriction Factors

A number of factors will influence the ability of a virus to replicate in a given tissue. An example is Polio virus, where a single point mutation at the 5' end profoundly affects its ability to replicate in neural tissue.

Different isolates of Polio virus will grow well in tissue culture, and isolates inoculated into mouse brain will normally grow well. However, in isolates of the virus with a single point mutation of C to U at 472, there is no growth. It is possible that the single point mutation causes a disruption of the secondary structure of the viral RNA.

Different Outcomes of Infection with the Same Virus

There may be a different response to a virus in different individuals as exemplified by Hepatitis B infections. Infection with this virus in most leads to disease and spontaneous recovery, however, the disease may become fulminant and then resolve or lead to death of the individual. The virus may be chronically produced, leading to cirrhosis of the liver and death, or carcinoma of the liver may occur before death. The virus may also be persistently produced with no disease or liver damage, i.e. a healthy carrier state.

Rare Outcome of a Common Viral Infection

This is one possible way in which LCH could be linked to a common virus. One such example of this phenomenon is measles.

Following measles, up to 50% of individuals show evidence of virus in the brain. In 1 in 300,000 individuals, after 3-13 years, subacute sclerosing parencephalitis develops. In such individuals, the virus is often defective and virus spreads from cell to cell, not by release of virus and dissemination. This spread is in spite of a strong immune response against the virus, including a strong hypergammaglobulinaemia against most virus proteins.

Persistent Infection with Viruses

Viral nucleic acid may be present free as subviral particles which may be linear or plasmid. Others may integrate randomly into the host genome, either intact as in retroviruses, or disrupted, as in papillama virus.

There are also instances where the viral genome integrates in a chromosome in a site specific fashion. Such a virus is the adeno associated virus, which integrates into chromosome 19 and remains latent until the cell is co-infected with adenovirus or herpes virus, which allows replication and release of both viral types.

Unconventional Viruses

An example of a class of unconventional infectious agents are those composed of a filamentous single protein and known as **prions.** These agents, e.g. scrapie, cause spongiform change in the grey matter and amyloid plaque deposition in the brain. Prion rods are host proteins and no specific nucleic acid has been identified associated with these infectious agents. These are very protease resistant and resistant to UV irradiation. In scrapie they are found in brain and spleen. The genes coding for prion proteins are present in normal unaffected individuals. There are 3 hypotheses as to how prion rods are formed:

1. That an as yet unidentified prion nucleic acid is present, which on infection, subverts the host mRNA coded for by the prion gene and

causes the formation of the scrapie prion protein form rather than the native form.

2. That the agent acts on a second host gene (Prn-i), which controls the rate of infectivity of the prion protein and acts on the prion mRNA to give rise to the scrapie form.
3. That the scrapie protein directly causes the conversion of normal prion protein to the scrapie form.

Prions are able to switch species as can be seen in England with bovine' spongiform encephalopathy developing in cattle fed on sheep offal, which contained the scrapie prion rods. With species switches, there is a long initial incubation period, but thereafter the prion rods are specific for the new host. Prion genes are highly conserved - mouse / sheep show a 60 -70% homology.

In Humans, there is a familial form of prion rod infection. This is an autosomal dominant disease, which is identical to Creutzfeldt - Jakob disease, called Gershmann - Straussler syndrome. In affected subjects, the sequence of the prion gene shows a consistent change with a mutation of proline to leucine at 102.

Retrovirus - Cell Interactions - Professor Robin Weiss

Retroviruses are envelope RNA viruses which use reverse transcriptase to divide in vertebrate cells. Although very similar at the molecular level, their natural history and means of transmission is highly polymorphic.

Retroviruses can cause a diverse range of diseases in a variety of vertebrates, from fish to mammals:

Leukemia, lymphoma, sarcoma, carcinoma, pankleukopenia, anaplastic anaemia, haemolytic anaemia, immunodeficiency, auto immune disease, arthritis osteopetrosis, encephalitis, paralysis, slow neuropathy and pneumonia. Retroviruses are probably derived from a broader class of elements which were not initially infectious - transposons - involved in genetic exchange within cells.

As with other viruses, retroviruses bind to specific cell receptors - HIV to CD4. The viruses carry their own reverse transcriptase and are diploid, carrying two sets of RNA. With replication, they undergo a form of "meiosis" as the DNA provirus synthesised in the cell is haploid. Mutation and recombination during reverse transcriptase leads to considerable polymorphism. Within the host cell, the DNA provirus integrates randomly into the host chromosomal DNA, with the same orientation as the original RNA. RNA transcribed acts as both messenger and

genome.

Consequence of Infection

1. Infection may become latent with the integrated genome becoming silent.
2. May get gene expression - viral protein expression and production of virons - without compromising the cell.
3. Virus may be cytopathic leading to cell death.
4. Virus may be oncogenic, leading to cell transformation.

Integration of retroviral genome into gamete DNA has led to passage as a Mendelian set of genes. Some sets of heritable viral genes represent a complete genome and can be activated by mutagens to produce infections virus. Theoretically, retrovirus could pick up host genes and transfer them to another host or species.

Human Retroviruses

These cause long term persistent infection and may be occult:

1) Endogenous genomes
2) Foamy viruses - may be linked to autoimmune thyrioditis or may cause disease:
3) Leukemia viruses HTLV 1 & 2
4) AIDS - HIV 1 & 2

Oncoviruses

Only 5% of those infected with HTVL 1 express disease.

HTLV 1	Adult T cell leukemic
	Tropical spastic paraparesis
	Immunodeficiency.
HTLV 2	T cell hairy cell leukemia?

Tropical spastic paraparesis may be more frequent in adults where HTLV 1 is acquired by blood transfusion. In Japan, ATL occurs in adults (usually > 40 years) only in those infected as infants.

Lentiviruses

HIV	AIDS related complex
HIV - 2	- West African strain.

Transmission of these human retroviruses show differences.

	HIV 1	HTLV 1
Blood	Cells, plasma	Cells
Sex	Male Female	Male Female
Foetal	Yes	Probably not
Milk	Cells, fluid	Cells

How do retroviruses cause tumourogenesis

1) A defective retroviral genome could "capture" a cellular oncogene. Both complete helper virus and defective genome are then needed to create new virus and maintain infectivity. Integration of the captured gene into host cell will lead to proliferation because of the effect of the virus promoter regions on the oncogene leading to tumourogenesis.

2) If the retroviral gene is integrated next to a cellular oncogene its promoter may lead to transcription of the oncogene. This mechanism of oncogene activation resembles that in cells not infected with retrovirus where chromosome trans-locations may put a promoter next to a transactivating sequence or cellular oncogene.

3) In HTLV 1, the virus encodes a transactivator gene, TAX - a 40,000 mw nucleic protein - which has a positive feedback on its own long terminal repeat which turns on viral replication. TAX may also act on host genes quite separate from proviral genes, i.e. on genes coding for IL2 R, GM-CSF and C-fos oncogene - which further promotes cell growth.

4) In HIV-1, 2, there are several genes analogous to TAX which stimulate or downregulate viral replication. Some of these act indirectly through host genes. Viral gene products can act as internal "cytokines". If LCH were shown to be clonal, then a gene activation mechanism by a virus or by mutation or chromosome translocation would be a possible pathogenic mechanism.

How viruses interact with the immune system - Professor Peter Beverley

Viruses can interact with the host immune system leading to symptoms and pathological changes characteristic of a disease. As an example, HIV was chosen,

as it is probably the best studied virus in the world, it has profound effects on the immune system and the Langerhans cell is a key player in the immune system (and is involved in AIDS: decrease in the number of LCs, CD4 present in the phenotype of LCs).

The virus can:

1. Interfere with cell surface receptors.
2. Subvert cells.
3. Induce a powerful immunological response which may affect the cell or host.

Surface Receptors

The cell surface receptor for HIV is the CD4 complex present on helper T cells and cells of the monocyte / macrophage series, including Langerhans cells. It is a 5,000 mw transmembrane molecule consisting of a surface N terminal end, four V domains, a transmembrane section and a cytoplasmic C terminal end.

Evidence that CD4 is the cell receptor:

1. Only CD4+ are easily infected in vitro.
2. Can block infection with anti - CD4 antibodies.
3. CD4 is down regulated in infected cells.
4. CD4+ T cell numbers decrease with disease progression.
5. Transfection of CD4 into resistant human cells renders them susceptible to HIV.

After binding of HIV or gp 120 to cells, CD4 expression is down regulated and antigen specific responses are reduced in vitro.

Binding of gp 120 is on the first V domain of CD4 between 43 and 57. This overlaps with the binding sites for class II molecules to CD4 which spans V1 and V2 domains. HIV thus mimics the effect of anti class II antibodies and inhibits the proliferative response of T cells to antigen presented by class II and APCs.Depletion of CD4 on the infected T cell may be due to cytoplasmic binding of gp 120 to CD4, preventing expression of the antigen on the T cells.

Other Consequences of HIV binding to CD4

HIV binding to CD4 leads to change in phosphorylation within the T cell - not well defined, but probably via tyrosine kinase (LCK). If this is persistently stimulated, this may lead to cell depletion.

Binding of HIV to normal helper T cells will result in the production of a number of cytokines - TNFα, β and IFNγ. Binding of the virus, thus stimulates cells to produce cytokines, which may have effects on other cells and systematically leading to fever, weight loss and toxic syndromes.

Cytokine production stimulates virus replication - especially TNFα. IFNγ has no antiviral effect on this system, but does stimulate viral replication. Viral replication can be inhibited by antibodies to these cytokines.

In the gene of HIV, there are a number of growth stimulator binding sites - some of these are th6 same or similar to the control regions of cytokine and cytokine binding receptor genes. A possible hypothesis is that binding of HIV to 004 leads to phosphorylation, PIP hydrolysis and calcium flux. This leads ultimately to production

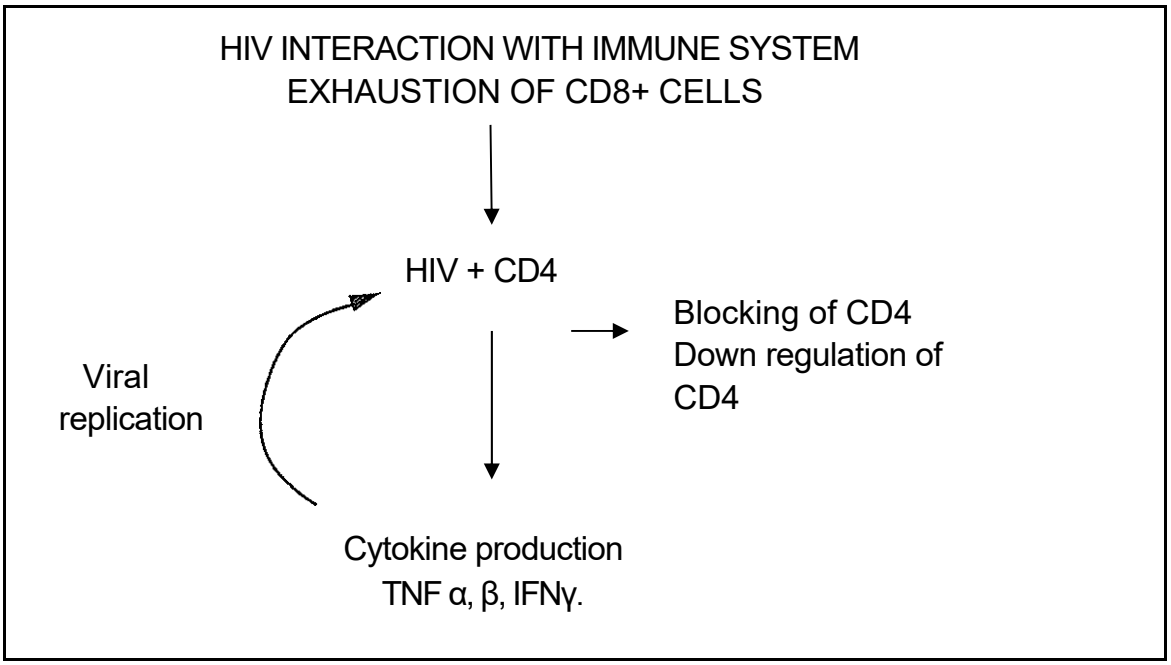
of TNFα which feeds back to the cell and stimulates an homologous site on the HIV gene (NF-KB) which stimulates division of the virus.

Immune Response to HIV

In most viruses, $CD8^+$ cells are responsible for killing of virally infected cells. This is optimally achieved by memory cells, naive cells having a much lower activity. In EBV infections, memory cells represent 1 in 8,000 circulating lymphocytes.

In HIV, fresh lymphocytes from seropositive patients will kill HIV infected cells with high efficiency. When memory cells are examined, they represent 1 in 100 of the lymphocytes. If the fresh lymphocytes are first incubated with HIV positive cells - this system is usually used in cytotoxic T cell assays - the frequency of memory cells falls to 1:3,000 to 1: 16,000, i.e. similar to the level normally seen in EBV.

It is suggested the blood are chronically stimulated and terminally differentiated, so that further stimulation results that HIV is a potent immune stimulator and cells in in cell death.



SESSION II

Chairman
Dr. Tony Chu

Rapporteurs
Professor Blaise Favara
Dr. Valerie Broadbent

Clinical Features of LCH - Dr. Jon Pritchard

Dr. Pritchard presented an overview of the clinical aspects of LCH, touching on epidemiology. The incidence seems to be 3-4 new cases per 1,000,000 children aged less than 15years / year worldwide.

Male incidence was twice that of females, except in Japan, where it is equal. (Lymphomas are 2 - 3 times more common in males than females). Reasons for this are unknown. There was no geographical clustering.

The clinical presentation of the disease may be very variable. To date the aetiology is unknown but scientific data shows that it is not a cancer. Clinical lesions of the

skin, bone and CNS were illustrated.
Dr. Pritchard emphasised the conservative therapeutic approach but acknowledged that this was still controversial. Comparison of the Great Ormond Street approach with that of groups from Italy and Austria / Germany, showed comparable mortality in each study, but the Great Ormond Street regime, has a high frequency of long term morbidity. The need for a good, randomised study was emphasised.

Cutaneous Touch Preparations in the Diagnosis of LCH.
Professor Kozo Nishimura.

Dr. Nishimura presented illustrations of the use of needle scrapings of LCH skin lesions as a first line diagnostic test. Material is suited to special stains but, electron microscopy still requires biopsy. He then showed interesting slides of Japan's registry of paediatric cancer cases between 1979 and 1987:

201 cases of LCH among 12,150 cases of cancer (1 - 2% of children with cancer)

Discussion ensured about the relative numbers of cases of disseminated disease.

Classification	Number of cases
Females	110
Males	90
Letterer - Siwe	86
Hand - Schuller Christian	16
Eosinophilic granuloma	52
Unclassified	47

Epidemiology of LCH - Professor Mark Nesbit

Dr. Nesbit presented data from a questionnaire survey of 177 cases of LCH performed several years ago.

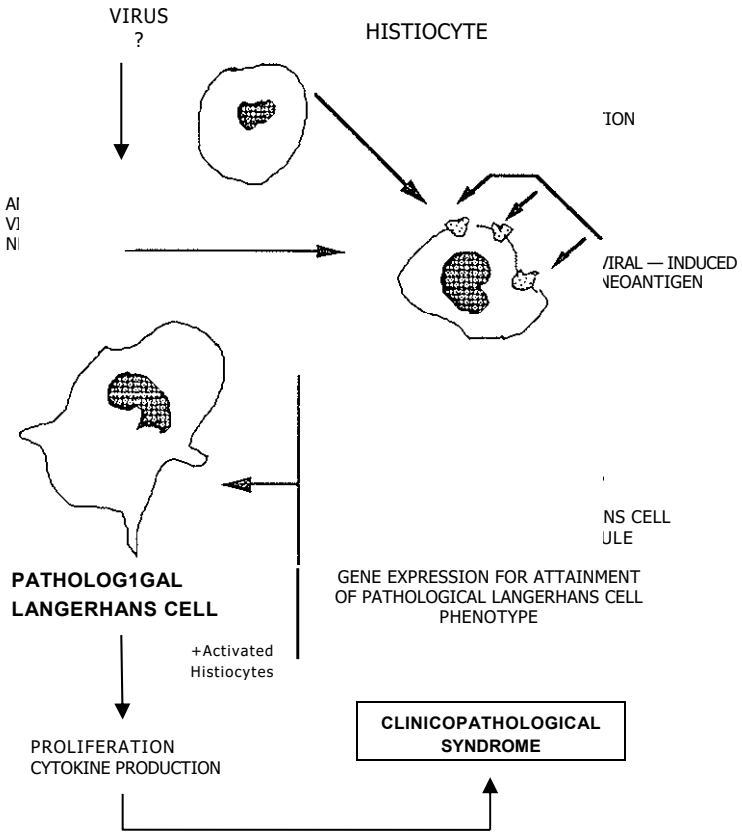
Associations that were provocative included:

a) History of postnatal transfusion
b) Feeding problems
c) Family history of benign tumours.

A fascinating case was presented to illustrate the relationship between LCH and leukemia and lymphomas. A child with T cell acute lymphoblastic leukemia, developed skin lesions that ultimately evolved into characteristic lesions of LCH. He suggested that the T cell leukemia had evolved into LCH. Lively debate followed, in which other hypotheses were presented by Drs. Pritchard and Willman.

Pathology of LCH - Professor Blaise Favara

The pathological aspects of LCH were presented, including the phenotypic features of the pathological Langerhans cell. The presence of multinucleated giant cells in lymph nodes and bone lesions was emphasised. Liver lesions, including non - specific lymphocyte / phagocyte system activation was demonstrated. A scheme of hypothetical pathogenesis featuring the role of virus was presented (see Figure 1).



Cellular Events of EBV Infection - Dr. Kenneth McClain

Dr. McClain presented a review of what is known of EBV infection as it might relate to the symposium topic. EBV infection has:

1. An effect on the immune system
2. An effect on macrophages
3. Induces cytokines that cause macrophage activation.

The immune response to EBV infection includes the production of neutralising antibodies, an increase in NK cells, initiation of T cell mediated cytotoxity, an increase in suppressor T cells and interferon secretion by T cells which will have an effect on macrophages.

EBV Molecular Virology

The EB virus contains a large genome which codes for over 50 proteins. Some of the coded proteins effect cellular transformation, while others are responsible for inclusions seen in rheumatoid arthritis and systemic lupus. The genome has been mapped in reference to protein production of antigens etc.

EBV gains access to the body via the oropharyngeal epithelium, the oropharyngeal epithelium has receptors for EBV which also attaches to EBV receptors on B lymphocytes in the adjacent tissue.

Replication occurs in these sites and viraemia ensues. The virus can then become latent or it can result in other paradigms including non-specific stimulation of B lymphocytes to differentiate and to produce heterophile and other antibodies. Immunological response to EBV is complex. EBV infected B cells are attacked by activated T cells (the "atypical" cells of mononucleosis). Interferon produced by T cells, initially inhibits EBV, but interferon is later produced by the virus. NK cells attack EBV infected cells and destroy them. Overall T cells are responsible for control of the infection.

Transforming Strains of EBV

EBV gains access to the cell via the C3d receptor which is important in cell cycle control. The EBV glycoprotein (gp 350/220) binds to the receptor. The natural progression of B cell growth entails the acquisition of receptors and antigens that are promoted by EBV. UV irradiation decreases the level of activation of EBV infected cells. Virus resides in its latent state in B cells.

EBV infection causes release of cytokines:

1. IL1 by macrophages and NK cells, which leads to activation of T and B cells. This can be blocked by antibodies to IL1. IL1 activation of T cells leads to secretion of IL2 and IFNγ which peaks on day 5 of infection.
2. INFα derived from NK cells and B cells is seen within 24 hours of EBV infection and may act to suppress further EBV infection.
3. EBV infection of Lymphocytes causes release of IL5 that has eosinophil colony stimulating function. Could this be related to the eosinophilia of LCH?
4. Infected Lymphocytes also produce macrophage stimulating factor (CSF 1), which will increase division of monocyte bone marrow precursors. The effect of EBV on lymphocytes can thus be summarised as:

Activation - Progression - Replication - Differentiation mediated by cytokines. Macrophages are activated secondarily and produce a series of monokines.

In Discussion

Dr. Robin Weiss suggested LCH patients should be studied for immunity to EBV.

Dr. Jon Pritchard commented that in congenital cases of LCH it is hard to imagine that EBV could be the cause unless there was transplacental infection.

In Vitro Culture of cells of the myeloid lineage/Tγδ cells. - Dr. Cheryl Willman.

Dr. Willman presented the traditional scheme of haematopoiesis, stating that she felt that there was much more lineage plasticity than was depicted. She described an experimental in vitro liquid (bulk) culture system in which haematopoetic progenitor cells are directed along the different lines of differentiation desired, with cytokines; in this case monocytes - macrophages. Normal monocytic cells in large numbers may be derived from such short term cultures with IL3 and Gm-CSF or CSF - 1 (M-CSF). These cells may assume dendritic morphology under appropriate conditions and may be useful as a target system for virus culture.

B cells infected with retrovirus take on macrophage morphology - an illustration that morphology may, in certain instances, reflect function more than it does lineage.

She described the T cell subset with the γδ form of the T cell antigen receptor as a cell that is capable of attaining a dendritic cell phenotype. This is a small population of cells (<5% of peripheral blood T cells) that are characteristically $CD4^-$ and $CD8^-$. Cells have a homing capacity, strikingly similar to LC. Clonality is easy to determine in this lineage by molecular methods. γδT cells are thought to have an immunological interaction with LC in skin. In the skin, LC cells may support the development and function γδT cells as do the Hassell's corpuscles and dendritic cells in the thymus.

She questioned that these cells may have a role in LCH and speculated that:

1. The skin may be the site of γδT cell differentiation in association with LC and
2. The γδT cells may be a target for virus infection in LCH. Persistent viral infection in these cells may stimulate LC reaction and cytokine release.

If LCH is clonal in some cases, then a small clone of γδ cells could stimulate the most prominent population of reactive (LC) cells as do certain leukemic cell

populations.
She urged that LCH lesions be probed to examine clonality by X-linked RFLP methods.

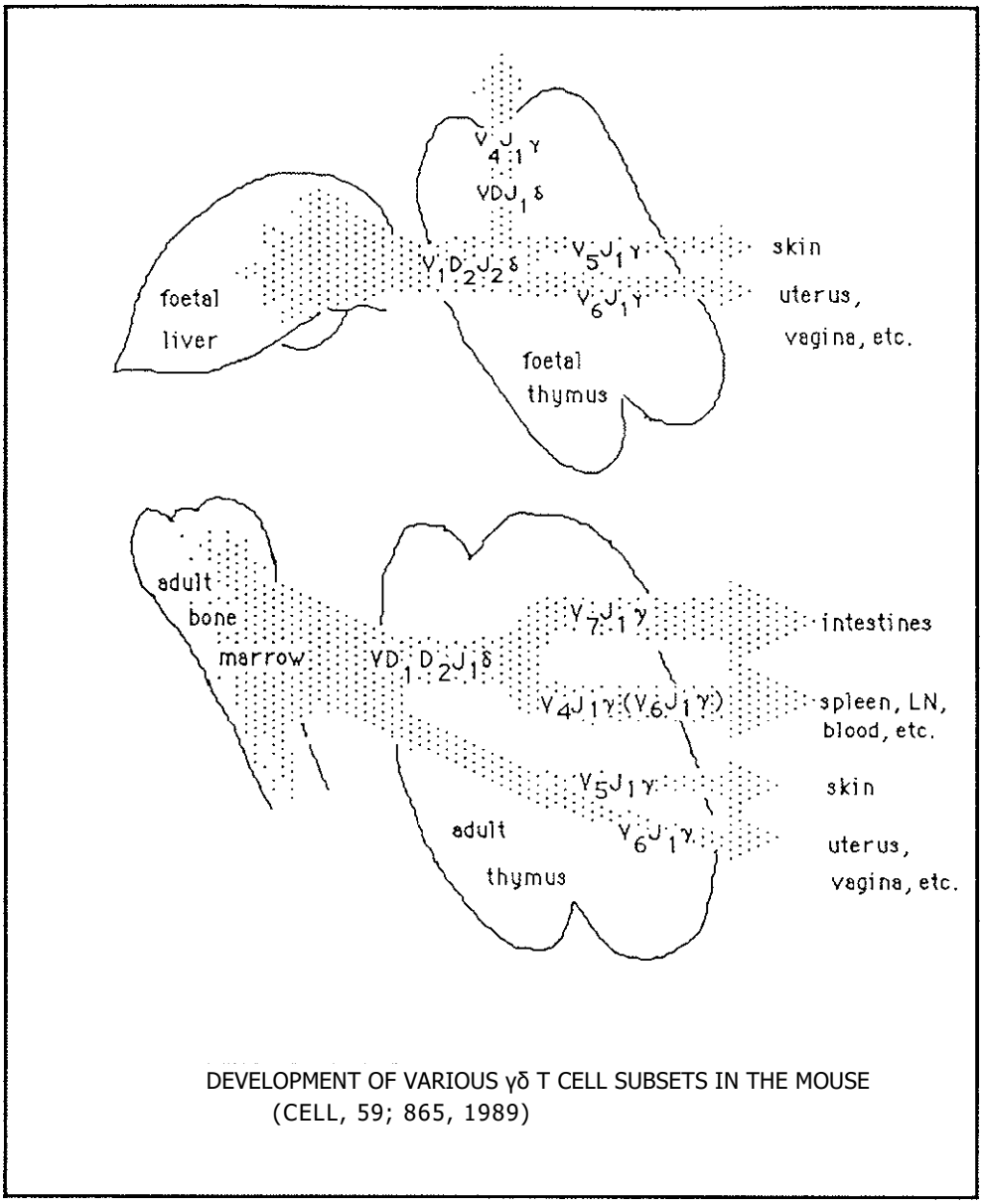
Evidence in support of clonality in LCH:

1. Some cases of "Malignant Histiocytosis" have been found to have clonal populations of γδ T cells, suggesting that these cases are T cell Lymphomas, and
2. Rare aneuploid cell populations from LCH lesions have been recently detected in 2/5 LCH patients with multisystemic disease using flow cytometric techniques. No aneuploid cells were detected in patients with skin lesions only.

The similarity to Hodgkin's disease was mentioned. Reed-Stenberg cells are thought to be clonal lymphoid cells (either T or B cells) that produce the IL5 that causes eosinophilia.

Although γδT cell migration has been confirmed in mice, these studies have not yet been done in humans.

Tony Chu commented on the controversy of γδT cell location in humans - some groups, including his own, cannot demonstrate increased γδT cells in human skin. However, he does find the γδ cells to be increased only in the dermis of LCH cases. Lively debate ensued.



DEVELOPMENT OF VARIOUS γδ T CELL SUBSETS IN THE MOUSE
(CELL, 59; 865, 1989)

SESSION III

Chairman
Professor Blaise Favara

Rapporteurs
Dr. Malcolm Brenner
Dr. Jon Pritchard

Molecular Techniques Used to Investigate Viruses - Dr. Cheryl Willman

Dr. Willman started by pointing out that of the $3.2x10^9$ base pairs in the human haploid genome, only 3-5% constituted single copy genes ($1.5x10^5$ per haploid genome). She described the various techniques available for studying these genes under headings:

a) Detection of immobilised target sequences, i.e. Southern blot and pulsed field gel electrophoresis.

b) Detection of point mutations, i.e. RFLP's ASO probes, RNA cleavage methods and denaturing gradient gel electrophoresis.

c) Gene amplification techniques, i.e. Polymerase Chain Reaction (PCR). She pointed out the limitations of these methods. Automated systems for Southern transfer are less efficient than traditional stacking (capillary transfer) methods, especially when only small amounts of DNA are present, and she used examples of B cell and T cell gene rearrangements as an illustration of this method. In the case of PCR, she stressed the importance of strict quality control, to avoid contamination from the environment and reagents.

She described in detail the use of X chromosome - derived RFLPs to determine clonality of cells of diverse lineage. (Refs: Science 1985:227:642 and Cancer Res 1987:47:4806). X chromosome polymorphisms can be detected in about 95% of US females if 2-4 different X - linked probes are used. Having established heterozygosity, using one enzyme, e.g. Barn H1, a methylation sensitive restriction enzyme (such as Hpa II) is used to determine which chromosome is methylated and thus "active".

Allele - specific oligonucleotide (aso) probes: - There is considerable geographical variation in the frequency of specific mutations in single-gene diseases, such as cystic fibrosis. In UK/US the commonest CF point mutation has an incidence of 70-

80%, whereas the incidence was only 40-50% in Spain and Italy and 30% in Ashkenazy populations. It is possible that there may be an LCH-predisposing gene. This potential must be taken into account when studying the incidence in various populations.

Can Viruses Cause LCH? - Dr. Ken McClain

Dr. McClain began by posing the question, "Can viruses cause LCH?" and reviewed available evidence in the literature. This literature search revealed just one positive paper in an obscure Rumanian journal by Athanasiou, reported in 1970. Biopsy material from three patients with "LCH" was subjected to "phenolic extraction" and then injected into mice, which were then said to develop lesions similar to those observed in the patients. After passage through mice their sera produced a syncytial / cytopathic effect on tissue culture cells. The phenolic extraction method and the nature of the injected material were not clearly described. No other reports were found suggesting a transmissible agent either in vivo or in vitro.

He displayed a skull film of a patient with HTLV 1 and hypercalcaemia that showed multiple lytic lesions, superficially resembling LCH. Dr. Favara commented that such lesions do not contain lymphoma. They seemingly are the result of paracrine cytokine effects that activate osteoclasts. Their patchy rather than diffuse nature could not be explained. Dr. Pritchard commented that LCH lesions produce PGE2 and IL1 (Nezelof et al.). It is known that LTR's from HTLV 1 can induce the expression of other genes, e.g. cytokine genes.

A computer search was then described which sought homology between growth factors for monocytic cells and viral DNA. A number of homologies were found, e.g. γIFN and the CMV splice sequence for integration and a possible homology with M-CSF and pseudorabies / rubella / HSV II. Dr. Brenner drew attention to the homology between EBV lytic cycle gene and IL 10 (a pre B cell growth factor). He concluded by describing collaborative work with the Halifax group - a study of biopsy material from 8 cases of LCH (6 bone samples and 2 nodes). Controls included hyperplastic nodes and lymphoma. In 8 of 8 cases, in-situ studies showed hybridisation with probes to HTLV 1 but to none of the other probes used (HIV, CMV, EBV, HHV6, Adenovirus). With PCR, using a HTLV 1 gag-pol primer, HTLV 1

primers yielded amplified fragments in one patient (LN specimen: frozen). PCR results from paraffin blocks were inconclusive. The PCR products were not confirmed by hybridisation. Controls also showed some PCR background that could not be explained. He was cautious about interpreting these early results. Confirmation, using other techniques, more material and other controls is needed. In discussion, it was concluded that HTLV 1 was highly unlikely for clinical and epidemiological reasons, but an HTLV like virus could be operative.

Dr. Willman commented that HTLV 2 may be endemic to the U.S.A. in the S.W. Indian population; many of the study set were Hispanic Americans, which could explain the results. There may also be cross-hybridisation with a novel retrovirus. The findings were, none the less, of interest to the group.

Discussion of Methods - Professor Jeff Almond and Professor Robin Weiss

Professor Almond reminded us of the variety of "slow virus infections: and he viewed LCH as being more likely to be due to this than to a hypersensitivity reaction to a single-hit virus (acute). He holds this view despite the fluctuating course of the disease. Although this is not typical of slow viruses, Aleutian mink disease also fluctuates and Weiss pointed out that a disease caused by a lentivirus in horses also fluctuates (Equine haemolytic anaemia).

It was concluded that if LCH was virally induced it might be by:

a) A common virus that is pathogenic only in genetically predisposed persons, or

b) by a rarely detected, but ubiquitous virus, with little host restriction.

The neurotrophic nature of slow viruses was explained by (a) the immune-privileged status of the CNS, or (b) the fact that CNS cells have a lower turnover rate, thus enabling non-cytolytic viruses to persist.

The non-infectivity of LCH patients to siblings or immunosuppressed patients was noted as being evidence against a viral etiology.

Methods of Investigations - Dr. Malcolm Brenner

Using molecular hybridisation techniques of lower stringency, one could screen for viruses efficiently since highly conserved 5' sequences can be found in different families of picornaviruses. Conserved sequences are present in other viral families. Only 30% homology is needed for cross reactivity under these conditions.

The lack of primate models was confirmed. Dr. Bassett described the nude mouse work with LCH by Dr. Nezelof, who found that xenografts of LCH did not "take" i.e. were rejected.

Strategy for Search for Causative Viruses in LCH -

Serology - Patients' serum and LCH tissue sections: Positive binding of antibody could be detected with FA methods. High backgrounds may be a problem due to FC receptors on monocytes / LC.

E M - Looking for viral particles.

Tissue culture and co-cultivation methods - looking for cytopathic effects.

Hybridisation - Using viral sequences at low stringency in situ and with PCR.

Animal inoculation - Primates and nude mice.

Proposals for research should be developed by the Nikolas Symposia. Pilot studies are needed before the usual funding sources will support such work.

Malcolm Brenner then briefly summarised the meeting to date. He suggested that a viral aetiology of the disease was the most satisfactory starting hypothesis for planning future investigation. Whether or not this was a persistent virus infection or a "one hit" infection followed by an auto-immune process was unclear.

None the less, the observation that the patients with severe or progressive disease generally benefited from immunosuppressive therapy (i.e. chemotherapy) implied that a large component of the disease process was a consequence of immune system "over reaction". It now seemed critical to identify which cell in the disease was primarily abnormal, since this would focus on attention on aetiology, pathogenesis and treatment. The primary target cell could be the LC, γδ8 T cell, αβ T cell, or surrounding tissues in the lesions - e.g. skin or bile duct epithelium. As discussed last year, demonstration of clonality in any of these populations would be helpful in understanding the disease process and in directing future investigations and therapeutic intervention.

It was also important to follow the strategies suggested by Jeff Almond and Robin Weiss to detect novel viruses or defective viruses, such as EBV-het or paramyxoviruses in the affected tissues. This, however, was likely to be a longer term project which would be simplified if the target cell for investigation could be better defined. He then discussed pathogenesis and said that, as suggested last year, it looked very much as though the release of cytokines such as TNF, IL1 , 1L6, PDGF and IL8 contributed both to the constitutional symptoms of the disease and to bony/skin lesions.

He proposed to look at cytokine levels in the blood of patients responding to cyclosporin before, during and after treatment. While this would say nothing about the underlying process, manipulation of cytokine release or availability could help to prevent re-establishment of an autocrine/paracrine inflammatory loop following a pulse of immunosuppressive therapy. Similarly, studying cytokine proteins/messages in situ would help to understand which cells contributed to which aspects of the disease. This was a reasonable straightforward task which could help in the design and evaluation of future treatment.

The discussion then closed with suggestions for ways in which the two minor (clonality, cytokine production) and one major virus presence) topics could be organised and financed.

SESSION IV

Chairman
Professor Giulio D'Angio

Rapporteurs
Professor Stephan Ladisch
Professor Mark Nesbit

Clinical Problems of the Central Nervous System in LCH - Dr. Valerie Broadbent and Professor Mark Nesbit.

The objective of the session was to provide a perspective on the clinical spectrum of CNS disease and particularly to the possible link between viral infection and CNS symptomology. This hypothesis is suggested by the general lack of classical pathological findings of LCH in the affected tissue of CNS.

Dr. Broadbent discussed 8 children with diabetes insipidus (DI) who were evaluated for growth as well as by CT's, MRI's and insulin tolerance tests. Five of the eight had growth failure and two of the five had panhypopituitarism. Seven of eight had abnormal CT's, four had a CNS mass lesion, two of these four (those with panhypopituitarism) who had thickening of the pituitary stalk. Three others had bone lesions contiguous with the hypothalamic pituitary area. The MRI's did not add any further information to the CT's.

In discussion the feeling was that the all failure to grow is not due to growth hormone deficiency. With respect to radiographic studies, CT's are thought to be

more useful for detecting mass lesions while MRI's will better detect white matter abnormalities (i.e. gliosis).

Six further cases of a neurological disorder consisting of cerebellar signs (e.g. dysarthria, tremors, dysmetria) and spastic quadreparesis were discussed. Table 1. outlines some general parameters on these patients.

The aetiology of the clinical complex of neurological findings is obscure. It should be differentiated, however, from CNS involvement with massive LCH cell infiltration of the CNS. The very indolent yet progressive course raises the possibility of a slow virus as a cause. The only virus which is known to cause a similar symptom complex is HIV infection. The pathological findings of the CNS include infection of the microglial cells, with resulting further damage by cytokine release. Measles (SSPE) infection was suggested as a possible agent but, this has a neuronal not a microglial involvement.

While the pathogenesis of this complex of CNS findings has not been elucidated, it, in fact, has certain parallels to the involvement of another organ system - the liver. Hepatic involvement with LCH has always been puzzling. Despite significant liver dysfunction, hepatic biopsies generally do not show LCH infiltration. Rather, they show the hepatic equivalent of CNS gliosis-fibrosis and cirrhosis.

There are several potential pathophysiologic mechanisms which could be a cause for the findings in these two organ systems.

They are:

1. Slowly progressive viral infection
2. Toxic / non infectious products of infection (e.g.) viral capsid antigens)
3. Production of sclerosing cytokines (e.g. platelet derived growth factor).

Age at diagnosis	Organ involvement at Diagnosis	Treatment duration	Length of time from diagnosis to neurological symptoms	Pattern of neurological signs and symptoms	Radiological and other findings
8 years	bone (skull) DI	none	10 years	cerebellar signs dysarthria ataxia progressive spastic quadreplegia	CT - demyelination of cerebellum and cerebellar cortex cortical atrophy
2 years	bone (skull) mastoid	radiation (600 RAD)	5 years	cerebellar signs dysarthria and progressive spastic quadreplegia cranial nerve deficits	MRI - demyelination of cerebellar peduncles
8 months	skin spleen bone marrow	combination chemotherapy 6MP prednisolone cytoxan(2years)	1.5 years	learning disabilities progressive spastic diplegia	CT - ventricular enlargement MRI - generalised atrophy
2.5 years	bone (skull) DI skin	prednisolone/velban (2years)	5.5 years	cerebellar signs - horizontal nystagmus and progressive spastic quadrepligia - mild central VII nerve paralysis	MRI - progressive cerebellar and periventricular demyelination biopsy - cerebellum – showed no evidence of LCH - activated microglia cells and gliosis of white matter Purkinji cell axon swelling
12 years	lung bone (skull and spine)	prednisolone/velban (1 year)	12 years	dizziness and gait weakness muscle wasting parasthesias right > left	none
4 years	bone (skull and other) spleen liver	velban (2 years)	7 years	gait disturbances cranial nerve findings	biopsy - mass lesion of occipital parietal area histiocyte infiltration with no LCH cells found

Table 1. CNS Complications of LCH