SESSION 1: Basic Science 1

Origin of Langerhans cells

The putative precursor cell of Langerhans cells originates from the bone marrow. The nature of this cell is unknown but a small percentage of CD1+ bone marrow cells has been identified and if bone marrow cells are grown in methylcellulose "monocytic" colonies, which are CD1+ and CD11+, can be identified.

A small percentage of CD1+ cells have also been identified in peripheral blood. These cells are morphologically monocytic and on electron microscopy have dense bodies in the cytoplasm. If cultured with GM CSF these cells appear to proliferate and may reach up to 8% of the total cells present. The circulating CD1+ cells represent approximately 1% of peripheral blood mononuclear cells in adult blood and can reach 6% in cord blood. These cells are DR+, CD11+ and CD14+. Such cells seem to be increased in the peripheral blood in severe burns patients, after thoracic duct drainage and in HIV-infected patients.

The factors responsible for the maturation of Langerhans cells are unknown and it is as yet unclear whether Langerhans cells divide *in situ* in the skin or are replenished from a circulating pool of precursor cells. Studies in the guinea pig have shown that Langerhans cells in the skin incorporate tritiated thymidine, suggesting that they actively divide and studies using bromodioxyuridine have shown that 6% of Langerhans cells present in the skin are in S phase.

Langerhans cell ultrastructure

The hallmark of the Langerhans cell is the trilaminate Birbeck granule which is 33 - 42 nM in thickness and has a central crystalline core. The cell has a number of organelles and is obviously capable of protein synthesis but shows no evidence of phagocytosis. LCH cells show pseudo-junctional structures between individual LCH cells. Some LCH cells show intranuclear Birbeck granules and increased mitotic activity and also show "worm-like" structures in the cytoplasm. These worm-like structures are seen in other cell types as well as LCH cells, are half the thickness of Birbeck granules with no central lamination or periodicity, and may represent rough endoplasmic reticulum.

Langerhans cells are present in most stratified squamous epithelia including the suprabasilar layer of the epidermis at a density of 400-900 cells/mm^2. Langerhans cells have also been described in oral mucosa, uterine cervix and vaginal epithelium, guinea pig oesophagus, sheep stomach, human rectum, in low numbers in the conjunctiva and peripheral corneum, trachia, bronchus and alveolar lining, in liver in patients with hepatitis and in urinary bladder in Vitamin A-deficient rats where epithelioid metaplasia has been induced.

In the bronchus Langerhans cells have normal morphology and Birbeck granules are present. Epidermal Langerhans cells are decreased in number in viral warts, sarcoidosis, graft-versus-host disease, AIDS and other immunodeficiency syndromes and are increased in number in vitiligo, actinic reticuloid, vaccinia sites, contact allergic dermatitis and mycosis fungoides.

Langerhans cells are also found in some extraepithelial sites; perivascularly in the dermis and in lymph nodes, thymus, spleen and pulmonary alveolar interstitium.

In the alveolar mucosa, Langerhans cells are increased in smokers and cells derived from bronchial lavage of smokers show 1 - 1.5% Langerhans cells. It is interesting that in Dr. Basset's series of adult patients with LCH of the lung, 85 - 90% were smokers.

Markers for Langerhans cells

Ultrastructurally the hallmark of the Langerhans cell is the Birbeck granule. Enzyme histochemistry shows that Langerhans cells are ATPase positive and NSE positive. Cytoplasmic antigens present in Langerhans cells include 5100, vimentin and the new Lag antigen which is reported to label Birbeck granules. Surface markers of Langerhans cells include the CD1a and c molecules, CD4, HLA Class 1 and 2, certain receptors such as FcIgE receptors, C3b receptors and adhesion molecules including ICAM-1 and LFA-3. The functional nature of the Birbeck granule is still unknown. It may however be related to receptor-specific endocytosis since, if Langerhans cells are labelled with gold tagged anti-CD1 or anti-DR, Birbeck granules are formed from the cytoplasmic membrane which incorporates the gold granules.

CD1 is a complex present on cortical thymocytes which consists of three sub-units of different molecular weight, CD1a-49kD; CD1b - 45kD and CD1c-43kD, which are associated with ß2 microglobulin. In Langerhans cells only CDla and CD1c are present.

As the majority of Langerhans cells used in vitro are derived by trypsinisation of normal human epidermis, it should be noted that CDla is modified by trypsin treatment which cleaves the CD1a molecule into a lower molecular weight protein of 27kD. OKT6 only reacts with the native 49kD protein and therefore if freshly trypsinised cells are examined for OKT6, this may give an artificially low density of CDla molecules on the cell surface.

CD1a molecules are actively synthesised by the Langerhans cell and are re-expressed on the cell surface after one hour's incubation. MHC Class 1 molecules are present in very low density on Langerhans cells, 10 times less than Class 2 molecules. The CD1 molecule is structurally similar to MHC Class 1 molecule and it may well be that CD1a expression by Langerhans cells is linked with the low expression of MHC Class 1 molecule.

MHC Class 2 molecules are increased in density after culture and if cultured without cytokines for 2 hours, show marked up-regulation. This is further up-regulated by γ interferon. The CD4 molecule (the HIV receptor) is present in low density in normal Langerhans cells but is up-regulated with activation of Langerhans cells and this may be related to γ interferon or prostaglandin production. The CD4 molecule is however sensitive to trypsin and the epitopes recognised by OKT4a and Leu-3a are cleaved by trypsin and these are the sites which represent the HIV receptor. The sites recognised by OKT4 are not affected.

Rapporteur : A. CHU

SESSION 2 Basic Science 2

Functional Properties of Langerhans Cells

Two major classes of function were considered, the production of cytokines and the processing/presentation of antigen. Experimental studies to modulate cytokine production have shown that sunlight (and specifically UVB) will increase IL6, IL7 and GMCSF production by epidermal cells. Since UVB does not penetrate, the skin is most likely the source of circulating high levels of IL1 and 6 after UV irradiation.

Among the various cytokines which are now known, including the interleukins, colony stimulating factors, growth factors and interferons, it is IL-1 which is clearly produced by LC, as it is in general by cells of the monocyte / macrophage lineage. Conversely, LC are affected by cytokines, in that in culture, keratinocytes, which release IL1, IL6, GMCSF and TNF, and are associated with LC in situ, improve surface antigen presentation (though not processing) of cultured LC. Overall, the keratinocyte may possibly be considered the nurse cell for the LC, and attempts to propagate LC should take this into consideration (although co-culture of LC and keratinocytes has not resulted in LC proliferation).

To study the other major aspect of LC function that is known, antigen processing/presentation, a major obstacle remains - the inability to either induce LC to proliferate or to efficiently separate LC from other cells in the epidermis. An approach, in progress, is to attempt to fuse an LC (lesional, preferably) to a normal bone marrow-derived histiocyte cell line, to then be able to study functional characteristics. Short of success in this approach, the circulating dendritic cells can be studied, offering some information. (These cells comprise the "large cell" subset of the peripheral blood monocyte population).

Finally, initial studies using patient-derived LCH cells suggest that severe (but not mild) disease is associated with decreased antigen presentation capability.

Rapporteur : Prof S LADISCH

SESSION I : Applied Science I

Normal and abnormal interactions of Langerhans cells

Evidence from bone marrow transplant recipients demonstrates that skin Langerhans cells in man are donor-derived and suggests that these cells are part of a circulating common progenitor cell population in peripheral blood.

Studies of BMT recipients also suggests that LC per se cannot initiate a DTH response even in the presence of circulating T cells:- other components of the immune system, in particular other specialised antigen presenting cells, must also recover.

These, and other observations, suggest a schema for the normal sequence of LC interaction and maturation.

After emerging from BM into peripheral blood, cells from the common antigen presenting cell subset migrate into skin, either following interaction with cell bound receptors or by following a cytokine gradient. Once in tissue, further interactions occur to trigger differentiation into a granule containing LC. After exposure to antigen the LC leaves the skin and travels in the afferent lymphatics to the lymph node. It is in the lymph node that primary antigen presentation occurs. T cells (NK cells and ? B cells) then leave the node and enter the skin - perhaps because a "transepithelial" lymphoid subset has been selected or instructed by the LC.

In the skin T/NK cells are able to encounter fresh antigen on remaining LC and interact either in an MHC restricted or an MHC unrestricted manner:- Once LC are activated by interaction with lymphocytes, expression of a higher density (and perhaps higher affinity) of cell adhesion molecules will allow contact to be made with effector lymphocytes by an MHC unrestricted mechanism.

Activation/proliferative signals may then be exchanged between the LC and the surrounding lymphocytes.

If this schema is correct, then LCH can be viewed as an abnormal migration of the "common progenitor" pool of antigen presenting cells (APC). Either tissues secrete cytokines or express adhesion molecules inappropriately so that APC migrate and partially differentiate, or the APC themselves respond inappropriately to physiological signals.

The questions to be addressed therefore include -

1) What are the signals that govern APC migration into and out of the tissues?

2) Is there evidence for a (clonal) abnormality in the common precursor pool of APC in this disease?

3) What are the lymphocytes surrounding/infiltrating the lesions? Do these contribute to maintenance of the lesion? Are they held nonspecifically by bell adhesion molecules?

The clearest candidates for regulating APC movement and capacity to recruit lymphocytes are the cytokines, including IL 5 ("eosinophilic granulomas") and IL 8, and the production and the cellular source of the agents in normal skin and LCH should be further investigated, particularly using in situ techniques.

If abnormal quantities or sites of cytokine production can be identified, then one immediate therapeutic implication is that infusion of monoclonal antibodies to these cytokines could interrupt the cycle and abrogate the lesions.

Rapporteur: Prof M NESBIT

SESSION 2: Applied Science 2

Leads to pathogenesis: Prof. Peter Beverley

The Langerhans cell is an antigen-presenting cell that originates as a precursor cell in the bone marrow and gets to the skin via the blood. What induces the Langerhans cell to migrate to the skin is unknown but the accumulation of abnormal Langerhans cells (LCH cells) in tissues other than the skin may reflect an abnormality of either the antigen-presenting function of the Langerhans cell or to the trophic factors that influence the cell. An important factor, in investigating LCH would be to determine whether the LCH cells were clonal, which would imply an abnormality of the antigen-presenting cell itself, rather than the trophic factors. If it is not an abnormality of the cells, which factors are involved in inducing migration of the precursor cells into the skin, and are these normal in the disease?

A major problem in investigating LCH cells and Langerhans cells is the lack of the cell line to work with. It is possible that Langerhans cells or LCH cells could be immortalised using oncogenes with permissive temperatures so that the oncogene itself could be turned off and allow normal activity of the cell.

The problem with looking at Langerhans cell clonality is the lack of markers of clonality. One marker that could be looked at would be the X gene.

Another paradox that needs to be examined is that of whether Langerhans cells and LCH cells can divide, as histologically, Langerhans cells show little or no cell division, i.e., mitoses, but the cells label with Ki-67 which shows that they are an S-phase. The use of monoclonal antibodies against activation antigens and also maturation antigens on the cells would give clues as to whether these cells were capable of division within the skin or whether they were terminally differentiated.

It would also be important to look at other cells present in the infiltrate of patients with LCH, as there is a possibility that the LCH cell is a bystander and that other cells are important cells as far as pathogenesis goes. In this regard T cells should be extracted from lesional tissue to examine for a clonal population.

Rapporteur: Prof D KOMP

FINAL SESSION

The group dealt with issues one or more of the attendees believed were left unresolved. It was decided that:

1) The pathology panel of the Histiocyte Society (H.S) would be available for central review of ultrastructure preparation.

2) Each institution should attempt to bank samples of blood and solid tissues (normal and abnormal?) from LCH patients.

3) A review article be prepared for publication every 5 years detailing progress in the study of Langerhans cells (LC) and the histiocytoses. This might be a stand-alone volume or a supplement to an established periodical, e.g. MPO.

4) No report for publication be prepared covering this meeting.

5) The Yearbook be prepared and distributed as before.

6) Any epidemiological (e.g. smoking as an environmental factor) component of any clinical study contemplated should have expert guidance in the preparation as well as the execution. The University of Minnesota group offered its services.

7) Human skin transplants in nude mice hold promise as a model for the study of normal histiocyte physiology and the study of LCH cytohistologic dynamics, clinical pharmacology et al. D Schmitt, who made the suggestion, was identified as the potential project leader to work with T Chu et al. as the basic science co-ordinator of the H.S.

8) The morphology and biochemistry of the LC granule (LCG) is a promising field of research. Funds for such an undertaking could be derived from the I.H.A. if and when they become available, e.g. to support a post-doc. working in an excellent biochemistry laboratory. Morphologic studies could be in the form of measuring total length of the LCGs in samples of LCH patients, correlating these data with clinical outcomes.
T. Chu was again asked to pursue these suggestions.

9) Transfections of histiocytes, perhaps from touch preparations from LCH skin lesions, might lead to histiocyte cell lines. Again, the matter was referred to T. Chu.

10) M. Brenner suggested there might be trophic factors for histiocytes similar to those in the IL-8 group that affect monocytes and he would be willing to study this.

11) Any elaboration of the description of the LC should not be published or distributed widely, but should be kept for reference and use by the members of this working party and few others.

H.S. Business

12) A central and permanent Statistical Office should be identified as soon as possible. Recommendations were made by some that a similarly centralised Secretariat should strongly be considered. T. Chu is to seek to identify the first of these centralised offices; and T. Chu and J. Pritchard to consider the feasibility of the second. Recommendations to be made to the H.S.

13) Draft proposals for (a) a method of grouping LCH patients with a view toward clinical trial applicability and (b) a randomised clinical trial, were presented by V. Broadbent and J. Pritchard respectively.
Considerable discussion ensued and these useful ideas were referred back to the H.S. Clinical Panel for further consideration. Meanwhile, the detailed data collection scheme could be piloted by the cadre of institutions represented, to work out imperfections prior to H.S.-wide implementation. The same could be done for the other two clinical projects if the instruments can be worked up and tested by the time of the Halifax meeting.

.14) A central body should be designated the responsible organisation if these Workshops are not to be a function of the H.S. It was agreed that the I.H.A. and its Steering Committee (or one suitably designated by the I.H.A) would be a suitable body for this purpose.

15) The next Workshop will focus on Virology as it might help elucidate LCH opacities, and that Hodgkin's disease authorities should be invited to attend. This is because of similarities between the two diseases struck some of the attendees.

16) The next meeting tentatively would be held in May, 1990 in Greece, at a time that does not conflict with the ASCO/AACR meeting, religious holidays or other conflicting dates.

Rapporteur: Prof G. D'ANGIO