Langerhans Cell Histiocytosis: Dendritic Cell Plasticity

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The Nikolas Symposia

The mission of the annually held Nikolas Symposium is to find a rational cure for Langerhans Cell Histiocytosis (LCH) (Beverley et al., 2005). It is sponsored by Paul and Elizabeth Kontoyannis, whose son Nikolas developed LCH in infancy but has survived his battle with this disease. The symposium is an interactive forum of basic scientists and clinicians who discuss different aspects related to the disease, and attempt to apply this information towards an improved understanding and treatment of LCH. A particular focus is the biology of the dendritic cells to which the Langerhans cells belong. Although LCH is a rare disease, the organizers believe that the research stimulated by this symposium will not only improve our understanding of LCH, but also our understanding of normal dendritic cells as well as other disorders in which dendritic cells are involved.

Introduction

Langerhans cell histiocytosis (LCH) is a rare disease in which an uncontrolled accrual of cells with Langerhans cell (LC) characteristics occurs (Beverley et al., 2005; Laman et al., 2003). LCH is of unknown etiology and occurs in various clinical forms in a broad age range from the newborn to the elderly but peaking between 1-4 years of age. The annual incidence in the pediatric age range has been estimated at 2-5 per million per year. A central question is whether LCH cells develop owing to an intrinsic proliferative defect of LCs, or whether the disease is reactive, resulting from environmental triggers (e.g. smoking) and leading to aberrancies in dendritic cells (DC). LCH primarily presents as a lesional disease, either with single or multifocal lesions in different organs. Skin and bone are most frequently affected. In these lesions, LCH cells are invariably present, which are characterized by CD1a, Langerin and S-100 expression and the presence of Birbeck granules (Favara et al., 1997; Geissmann et al., 2001). However, other cell types also contribute to the LCH lesions and are thought to have a profound influence on the biological behavior of the LCH cells.

Adaptability to environmental conditions is inherent to the DC nature of LC, and therefore the theme of this year's meeting was "plasticity of DC". In the introduction on the pathology of histiocytoses, **dr. Malone** illustrated the various forms, which can be largely divided depending on their DC or macrophage nature. DC histiocytoses are LCH and the less frequently occurring juvenile xanthogranuloma (JXG), in which the affected cells bear phenotypic resemblance to dermal DC ($FXIIIa^+$ $CD1a^-$ $S100^-$). Remarkably, JXG patients have a twenty times enhanced risk to develop juvenile chronic myeloid leukemia, which suggests that common denominators between these diseases exist. Histiocytoses related to macrophages are the macrophage-activation syndrome or hemophagocytic lymphohistiocytosis (HLH), which can occur in association with infections or autoimmune conditions, but is also known in a familial form. The affected cells in the Rosai Dorfman syndrome express overlapping features between DC and macrophages ($S100^+$, $CD1a^-$, $CD68^+$). Some aspects in the diagnosis of LCH are genuinely characteristic for the disease. Often, LCH lesions are epitheliotropic (e.g. bile ducts in liver), and lesion composition is organ-dependent and may evolve depending on the stage of the disease. Plasticity of the aberrant histiocytic cells is illustrated by a number of clinical findings. Although rare, some

patients may develop LCH and JXG concurrently, or the latter as sequel to LCH (Hoeger et al., 2001). Also, LCH may trigger local or systemic activation of macrophages, leading to hemophagocytic syndrome in a significant part of LCH patients (Favara et al., 2002). Furthermore, LCH may occur simultaneously with Rosai-Dorfman syndrome, or may evolve from so-called non-LC histiocytosis, which does not fulfill all diagnostic criteria for LCH. Together, these findings reinforce the notion that the biological variation of histiocyte-related diseases exceeds the current diagnostic boundaries, and demonstrate the plasticity of the involved cell type.

DC origins and functional heterogeneity

Dr. Shortman discussed the diversity and development of DC populations in the mouse. In general, two main DC types can be distinguished: plasmacytoid DC (pDC) and conventional DC (cDC). Among the latter, different subtypes can be identified in spleen, differing in their expression of CD4 and CD8 molecules. Additional populations of DC can be recognized in lymph nodes; these DC derive from tissue DC, including LC. It appears that the plasticity among different DC subtypes is limited: pDC mature into cells with cDC features upon activation, but different cDC subtypes are not precursors of others. Local proliferation of cDC may contribute to the maintenance of the population as 3-5% of cDC proliferate under steady state conditions.
The various DC subsets show remarkable functional differences. For instance, they display characteristic expression profiles of Toll-like receptors (TLR), enabling them to recognize distinct microbial stimuli. High level type 1 interferon (IFN) production is a selective feature of pDC. Furthermore, cross-presentation of antigens, i.e. presenting exogenous antigen peptides on MHC class I molecules, is a constitutive capacity of CD8+ DC, while other DC can only do so when appropriately activated. In addition, DC differ significantly in the ability to produce IL-12p70 or different inflammatory chemokines.
DC may develop from different progenitors, myeloid or lymphoid, although undistinguishable mature cells can be derived from both origins. A lymphoid background of DC is especially noticeable in thymic DC, where approximately 70% of the major CD8+ subpopulation shows IgH gene rearrangements. The development of DC from both myeloid and lymphoid progenitors occurs via a common Flt-3+ precursor (D'Amico and Wu, 2003). Apparently, these develop into DC-restricted precursors, as such cells could be demonstrated in spleen. These CD11c^{int}, CD43+, F4/80low, MHCII- cells of intermediate density represent 0.3% of total splenocytes. Although phenotypically similar, they are not monocytes as the DC precursors are unresponsive to M-CSF. Monocytes probably function as DC precursors only under specific, inflammatory conditions. In vivo, the DC-restricted precursors give rise to all conventional DC types, but not pDC. Interestingly, this population also develops in Flt3L-stimulated BM cultures (Naik et al., 2005), thus enabling in vitro approaches of lineage relationships, also in human.

Dr. Liu elaborated on the functional differences between DC subtypes, in particular pDC and cDC in human. PDC are major type 1 IFN (-α, -β, -ω) producers as up to 50% of their mRNA may encode type 1 IFN in an anti-viral response. However, the capacity of pDC to produce IL-12p70 is limited in comparison to cDC. The functional specialization between these DC subsets is established at different levels. First, DC differentially express receptors recognizing microbial stimuli. Monocytes (pre-cDC) express Toll-like receptors (TLR)-2, -3, -

4, -5, -6 and -8, while pDC only express TLR-7 and -8 (Ito et al., 2005a). This limited TLR repertoire explains why pDC do not respond to bacterial products. Furthermore, DC differ in the expression of signal transduction pathway components. For instance, pDC are unique among hematopoietic cells in high level IRF-7 expression, enabling their high level of type 1 IFN production (Liu, 2005). Together these notions explain why an identical trigger, e.g. CpG oligonucleotides, may stimulate cDC to strong IL-12 production, while pDC respond with high level type 1 IFN production.
Another level of complexity exists, since the same DC subset may stimulate T cell responses into different, polarized directions depending on the activation stage of the DC. Dr. Liu showed that thymic stromal lymphopoietin (TSLP, IL-50) is produced by activated epithelial cells and skews DC to stimulate Th2 responses. This is mediated by inducing OX40L expression by DC, which thus leads to the differentiation and stimulation of inflammatory Th2 cells producing IL-4, -13 and TNF-α, but, surprisingly, no IL-10. The involvement of these effector cells in allergic inflammation has been recognized before (Soumelis et al., 2002). Interestingly, OX40L appears to down-regulate IL-10 production not only in Th2, but also in Th1 cells. Thus, the view emerges that Th cell responses may not only be divided into Th1 (IFN-γ) or Th2 (IL-4, -13) responses, but that from either subset inflammatory or regulatory states can be distinguished. These are characterized by the absence or presence of accompanying TNF-α or IL-10 production in the inflammatory or regulatory states, respectively. OX40L production by DC, potentially stimulated by TSLP-exposure, drives the inflammatory T cell response (Ito et al., 2005b). TSLP-exposed DC not only play a role in Th cell polarization, but also in homeostatic proliferation of naïve T cells (Watanabe et al., 2004). In the thymus, TSLP-DC strongly stimulate proliferation of CD4+8- (medullary) thymocytes, which now develop into CD4+25+FoxP3+ regulatory T cells with immunosuppressive activity. In the human thymus, Hassall's corpuscles are a major source of TSLP and thus play an instructive role in this DC-mediated Treg differentiation (Watanabe et al., 2005).

As indicated before, different models exist for the development of DC types as they may derive from different precursors via distinct lineages, or, alternatively, through environmental instruction of similar precursors. **Dr. Manz** indicated that, in earlier studies, he and his colleagues had found that both CMP and CLP can generate both IPCs and conventional DCs (Chicha et al., 2004; Manz et al., 2001). Since Flt3L -/- mice have a strongly reduced development of both types of DC, this cytokine is probably non-redundant in DC generation. Flt3 (CD135), the receptor for both membrane and soluble forms of Flt3L, is expressed by early hematopoietic progenitors, but on mature cells only maintained on DC. (Karsunky et al., 2003). In vivo treatment of mice with Flt3L stimulates expansion of all $Flt3^+$ stages including the different DC types. By retroviral transduction, human Flt3 could be overexpressed in mouse cells that either (CMP, CLP) or not (MEP; megakaryocytic, erythrocytic progenitor) expressed endogenous Flt3. Interestingly, enforced expression of Flt3 in MEP now endowed these cells with the ability to develop into functional DC (Onai et al., 2006). At the molecular level, overexpression of Flt3 in MEP instructed gene transcription profiles affiliated with IPC/DC- and GM- development, including STAT3, PU.1, and G-/M-/GM-CSFR. The direct association between DC development and Flt3 expression was confirmed by pharmacological inhibition of Flt3 signaling via application of SU11657, a selective inhibitor of class III/V receptor tyrosine kinases, which strongly inhibited DC development in vitro and in vivo. Treatment of mice with SU11657 strongly stimulated circulating Flt3L levels, indicating the existence of a regulatory loop. Thus, a model surfaces

suggesting that DC development is regulated on demand: cells expressing Flt3 are licensed to develop into DC and may do so, depending on the available Flt3L levels. Inhibition of Flt3 signaling to downregulate DC development might be an interesting therapeutic option for multiple DC-related diseases. In this respect, preventive administration of SU11657 annulled the development of EAE in a mouse model.

Recent studies performed by **dr. Merad**'s laboratory indicate that LC, in contrast to some other DC populations, are maintained locally under steady state conditions, independent of circulating precursors (Merad et al., 2002). LC are radioresistant and BrdU labeling experiments in mice indicate that about 30% of LC proliferate in 4 weeks time. During early development, seeding of LC occurs before birth, as transplantation of foetal liver or BM cells to newborn mice does not generate donor-derived LC. In contrast to the steady state situation, skin injury, for instance induced by high level UV irradiation, recruits circulating LC precursors. Recruitment is mediated via local production of CCR2-binding chemokines in the skin and influx of Flt-3+CCR2+ cells from the circulation. A recent publication from dr. Merad's group indicates that these may be Gr-1+ inflammatory monocytes that give rise to LC in an M-CSF-dependent manner (Ginhoux et al., 2006). On a per cell basis, early myeloid progenitors (CMP) have a higher potential to reconstitute LC than the more differentiated granulocyt-macrophage progenitors (GMP) (Mende et al., 2006), but this may be related to the higher proliferative potential of CMP. Interestingly, also common lymphoid progenitors (CLP) show some degree of LC reconstitution after UV-mediated depletion. The recruited skin-infiltrating cells differentiate into LC precursors, which proliferate in situ and persist in the skin through life. While syngeneic or congeneic BM transplantation does not cause replacement of LC, due to their radio-resistance, this does occur when allogeneic BM is transplanted and allo-(i.e. host-) reactive T cells from donor origin are present (Merad et al., 2004). Probably, this is related to the inflammatory situation elicited by local stimulation of alloreactive T cells by remaining host LC. Removal of these host antigen presenting cells by UV treatment prevents the development of skin graft-versus-host disease. Although these studies were performed in mice, similar rules might apply for the maintenance of LC in human skin. This was indicated by a retrospective study of skin biopsies from sex-mismatched BM transplant patients. In a minority of cases, up to 100% of host LC appeared to remain in the skin several months after transplantation.

Cellular and molecular biology of DC

Presentation of antigens by DC starts with acquisition and processing of these molecules. In the first part of his presentation, **dr. Mellman** discussed the possibility raised in recent literature that endoplasmatic reticulum (ER-) membrane might be involved in particle phagocytosis (Gagnon et al., 2002). Using a variety of techniques, however, dr. Mellman and colleagues obtained no indication of fusion of ER with plasma membrane, nor could a contribution of ER membrane material to forming or maturing phagosomes be demonstrated (Touret et al., 2005). Although both immature DC and macrophages have a high capacity to internalize material from their environment, they appear to differ significantly in handling exogenous protein antigens. Typically, macrophages have a high level of lysosomal enzymes, enabling them to clear peripheral tissues from foreign substances by digesting them locally. Immature DC similarly have a high lysosomal enzyme content, with the exception of lysosomal proteases, in particular cathepsins (-L, -B, -S, -D), which are present only at low

levels (Delamarre et al., 2005). This allows DC to transfer protein antigen to draining lymph nodes for presentation. In different models, it was shown that relative stability of a protein antigen contributes strongly to its immunogenicity.
Subsequently, a putative role of E-cadherin in LC maturation was discussed. As adhesion molecule, E-cadherin is involved in LC-epithelial interaction. Simultaneously, E-cadherin-mediated adhesion is involved in intracellular signaling via its link to the transcriptional activator β-catenin. E-cadherin is also expressed by cultured DC and causes clustering of the cells. Physical disruption induces phenotypic maturation, which coincides with nuclear translocation of β-catenin. A similar nuclear translocation occurs upon physiological Wnt signaling, which inhibits GSK-3β-mediated phosphorylation of β-catenin, thus preventing it from degradation. This is mimicked pharmacologically by the GSK-3β inhibitor SB216736. In line with the previous results, GSK-3β inhibition induces a similar degree of DC maturation as cluster disruption. DC maturation induced by β-catenin signaling differs significantly from maturation induced by microbial factors, since only the latter is accompanied by strong pro-inflammatory cytokine and chemokine production. Together, these findings suggest that emigration of LC from the skin in the absence of a "danger" signal leads to generation of “semi-mature” DC, which stimulate tolerance (Lutz and Schuler, 2002). In contrast, in the presence of microbial stimulation fully mature DC develop, which are immunostimulatory.

In his presentation, **dr. Rollins** indicated that chemokines play a decisive role in the migration of leukocytes. The chemokine system consists of 4 families, divided according to their molecular structure. A high level of functional complexity exists since multiple ligands may bind to the same receptor, while, on the other hand, multiple receptors may bind the same ligand (Rot and von Andrian, 2004). For example, the important monocyte chemokine receptor CCR2 binds MCP-1, -2, -3, and -4. Despite this redundancy, a unique role of MCP-1 can be observed in inflammatory processes, as inferred from phenotypic aberrancies observed in MCP-1 -/- mice. Studying the involvement of chemokines in LCH, dr. Rollins et al. observed in 24 LCH lesions co-expression of CCR6 and CCR7 by LCH cells (Fleming et al., 2003). These results are at variance with those obtained by Annels et al., who observed no CCR7 expression in LCH (Annels et al., 2003). Interestingly, coexpression of CCR6 and CCR7 was also observed in lesional cells of Rosai Dorfman syndrome and hemophagocytic syndrome. In breast cancer, a high tumor MCP-1 content correlates with poor clinical outcome. Is this related to mononuclear cells infiltrating the tumor? This was studied in a BALB-HER2/neu mammacarcinoma model in mice on MCP-1-proficient and -deficient backgrounds. Interestingly, the mononuclear cell infiltrates of the tumors developing in these mice did not differ between the different backgrounds. Yet, in MCP-1-deficient mice the tumor appearance and growth was delayed, leading to improved survival. This effect appeared to be directly related to tumor cell responsiveness to MCP-1, produced by both infiltrating host mononuclear cells and the tumor cells themselves. These findings are probably relevant for the human situation as application of neutralizing antibodies to MCP-1 or antagonists of CCR2 inhibit proliferation of MDA-435 breast carcinoma cells. Taken together, these observations reinforce the notion that chemokines may influence tumor growth by multiple mechanisms, both directly and indirectly via infiltrating mononuclear cells (Rollins, 2006).

The nuclear factor (NF)-κB signaling pathway plays a crucial role in the activation and maturation of DC in response to inflammatory triggers. The NF-kB family comprises 5

members: RelA/p65, p50, cRel, RelB and p52. In his presentation, **dr. Beg** pointed out that DC are rather unique in the fact that they may express all 5 members of the family simultaneously. Despite the importance and expression of these factors, absence of individual proteins in gene-mutant mice does not influence DC development profoundly. However, combined absence of cRel and p50 strongly affects IL-12p40 production by DC and CD40L-mediated enhanced survival. Micro-array and subsequent immunophenotypic analysis of LPS-stimulated BM-derived DC from cRel/p50 double-KO and control mice indicated that also induction of other molecules important in T cell stimulation (CD40, CD80, CD86, IL-2, 4-1BBL) was strongly impaired. This involvement of the NF-kB pathway in DC costimulatory molecule expression was confirmed in experiments in which IKK function was inhibited pharmacologically. In contrast, costimulatory molecule expression by BM-derived (M-CSF-stimulated) macrophages (BMDM) appeared to be regulated differently. LPS stimulation of BMDM showed a much lower induction of CD40, -80, and -86, possibly related to a significantly decreased expression of cRel and RelB by macrophages compared to DC. While the NF-kB pathway played a predominant role in DC costimulatory molecule expression, in macrophages this was mainly induced via the type 1 IFN route. With regard to the expression of inflammatory cytokines, DC and macrophages behaved similarly, as induction in both appeared to be regulated primarily via RelA. Thus, the unique immune stimulating capability of DC may be related to their broad use of NF-kB family members (RelA, cRel and RelB) in response to microbial triggers, in contrast to the macrophage proinflammatory response, which relies significantly on RelA only. Also in migration of LC, cRel appeared to play a decisive role as stimulated LC emigration was strongly impaired in cRel -/- mice, related to the inability to up-regulate CCR7 and down-regulate E-cadherin. Although little is known about the NF-kB pathway in LCH, the high cytokine production suggests that LCH cells may show strong RelA signaling. Therefore, pharmacological inhibition of NF-kB might thus beneficially affect the cytokine storm.

Micro-array technology has enabled the characterization of DC gene expression profiles induced by different stimuli. These studies have indicated that such responses vary widely and are partially trigger-specific, influencing both adaptive and innate immunity. In his presentation, **Dr. Hacohen** elaborated on the efforts of the "RNAi Consortium" to link gene expression to complex functional processes. Their approach aims to develop and optimize methods for high-throughput mammalian loss-of-function genetic screening. To this end, functional genome-scale libraries of short hairpin (sh)RNAs for human and mouse gene knockdown are constructed (Moffat et al., 2006). These libraries are lentivirus-based, allowing also study of many non-dividing cell types. After successful transduction, the vector integrates in the genome and interference RNA is continuously expressed, causing functional inhibition of the host cell gene. In April 2005, the libraries contained ~43,000 vectors (March 2006: 104,000 vectors), targeting each of 22,000 human and mouse genes with multiple constructs. In a pooled screen approach, multiple viruses can be used to infect target cells. Upon selection and isolation of functionally affected target cells, the respective gene can be identified by PCR or micro-array. Alternatively, arrayed screens can be used in which target cells are infected with virus clones. Functional aberrations can then be screened per well, identifying directly the respective gene. Evidently, this approach requires a robust high throughput screening for the cellular function of interest, based on plate reader technology, high throughput flowcytometry, or imaging using automated microscopy. An example of such an assay is flowcytometric analysis of phagocytosis of labeled E.coli by DC.

Interestingly, this revealed that inhibition of SHIP-1 function significantly increased the phagocytic activity of cells. On the basis of this preliminary screening, it is expected that approximately 1-3% of the genome is involved with DC phagocytic function.

Under both normal and pathological conditions, cells of the mononuclear phagocyte lineage may fuse to form syncytia, such as osteoclasts in bone, or multinucleated giant cells (MGC) developing in microbial infections or other chronic inflammatory conditions. **Dr. Servet-Delprat** indicated that presence of such MGC in bone tissue may lead to increased bone resorption, as observed in LCH or Paget's disease. Although the precursor cells of osteoclasts are only partially characterized, it was previously thought that osteoclasts and DC comprise distinct developmental lineages. Recently, however, dr. Servet-Delprat and colleagues showed that human monocyte-derived DC could be stimulated with M-CSF and RANKL to fuse into MGC (Rivollier et al., 2004). Interestingly, already the mononuclear monocyte-derived DC expressed tartrate-resistant acid phosphatase (TRAP). The DC-derived MGC expressed TRAP, αVβ3 vitronectin R, cathepsin K and osteolytic activity, and thus have all characteristics of osteoclasts. Exogenous conditions strongly influence MGC formation and activity as synovial fluid from rheumatoid arthritis patients (RASF) stimulated fusion of DC into MGC, but these expressed no osteolytic activity. Yet, in the presence of permissive factors M-CSF and RANKL, RASF strongly stimulated osteolysis by DC-derived, but not by monocyte-derived MGC. Thus, the view emerges that DC-derived MGC may represent a distinct class of cells with separate regulatory mechanisms. Time-lapse microscopy of developing DC-derived MGC indicated that cell fusion occurs through "molecular doors", which are present only at few, selected sites at the MGC surface. DC-derived MGC represent very mobile cells with phagocytic and bactericidal capacity, when appropriately stimulated. They express MHC class II, but do not stimulate naïve T cell proliferation. These MGC have a limited life span in vitro (3-4 d.) and die by apoptosis.

New developments in LCH

Bone lesions represent the most frequent lesion type in both restricted and extensive forms of LCH. Among these, the skull is most frequently affected. **Dr. Egeler** investigated the multinucleated giant cells (MGC) that frequently inhabit the LCH lesions next to LCH cells, T cells, macrophages and eosinophils. Immunohistochemical studies of LCH lesions indicated that MGC in LCH express genuine osteoclast markers, such as TRAP, cathepsin K and the vitronectin R αVβ3 (da Costa et al., 2005). Typically, these markers are also found on MGC in non-bony lesions, such as skin or lymph node. Such MGC probably derive locally from fusion of mononuclear cells, including LCH cells, as both lesional $CD1a^+$ and $CD68^+$ cells express RANK, while lesional $CD4^+$ T cells, or even LCH cells themselves, may express RANKL at high levels. In addition, also other cytokines stimulating MGC formation, such as M-CSF, TNF-α, IL-1, and IL-6 are present. Thus, the pathogenic scenario may be that LCH cells, in an autocrine and paracrine fashion, stimulate development of activated osteoclasts, assisted by locally activated T cells. Specifically, RANK - RANKL interaction may be fundamental in LCH as it stimulates both osteoclastogenesis and DC survival. Focal bone resorption in LCH is thus probably related to the function of these LCH-related MGC. This notion provides a rationale for the reported therapeutic efficacy of bisphosphonate treatment in an LCH patient (Farran et al., 2001).

An association has been described between Langerhans cell histiocytosis (LCH) and acute leukaemias (Egeler et al., 1998), but the biological basis for this association is unknown. The work presented by **dr. Feldman**, one of the Artemis fellows, provides evidence that both diseases may, in some cases, represent different phenotypic manifestations of the same neoplastic process. In two paediatric patients with LCH and precursor T-cell lymphoblastic leukaemia/lymphoma (T-ALL/LBL), the LCH cells and T-ALL/LBL cells shared identical rearrangements of the T-cell receptor gamma (TCR-g) gene, suggesting a clonal relationship between the two neoplasms (Feldman et al., 2005). In some aspects, these cases deviate from classical LCH, since generally TCR rearrangements are absent in LCH (Yu and Chu, 1995). Furthermore, in these cases LCH was locally aggressive and the pathological specimens showed cytological atypia. Yet, they cannot be characterized as Langerhans cell sarcoma, since multi-organ involvement and cellular anaplasia were absent. Probably, a gradual scale of malignant deviation exists, in which these cases represent intermediate stages. A rational explanation for finding clonal relationships between neoplastic cells of distinct hematopoietic lineages is so far lacking. Recent work suggests the possibility of lineage switching ("transdifferentiation") between B-lymphocytic cells and macrophages (Xie et al., 2004), and a similar situation might exist involving T cells and myeloid cells. Hypothetically, these patient cases may therefore represent links between T cells and Langerhans cells. Elucidating the signaling switches that control lineage plasticity in such cases may shed light on the molecular basis for Langerhans cell differentiation and identify critical molecular targets for novel LCH therapies.

Dr. Bechan, also an Artemis fellow, aims to develop a more effective, less toxic therapeutic and diagnostic approach that will benefit patients with LCH. This is effectuated by targeting the CD1a molecule expressed by lesional LCH cells. CD1a is also expressed on cortical thymocytes and normal LC, but precursor cells for these do not express CD1a, thus making it a promising immunotherapeutic and diagnostic target. Potential advantages of targeted immunotherapy are that the monoclonal antibody will bind selectively to lesional cells, leading to more effective treatment with fewer and less severe adverse side effects as well as, possibly, a shorter duration of therapy compared to conventional, chemotherapy-based treatment. Proof-of-principle in this respect has been provided by using conventional monoclonal anti-CD1a antibodies (Kelly et al., 1994). However, treatment had to be discontinued due to adverse immunologic reactions associated with the non-human origin of the antibody. In a novel approach, reported by dr. Bechan, a human spleen phage display library was used to select CD1a epitope binding regions with appropriate specificity. Six clones were identified that recognize extracellular domains of CD1a, and the positive binding sequences were cloned into a human IgG1 backbone resulting in complete, fully human monoclonal antibodies. In general, the produced mAbs had good binding specificity and high binding affinity for different epitopes of CD1a. From the selected panel, mAb CR2113 appeared to have the most preferable properties, since it mediates both complement-dependent and (NK) cell-dependent cytotoxicity. However, mAb CR2113 binding did not induce apoptosis. Since, mAb-CD1a complex is internalized at 37°C, this offers the possibility to label the mAb with toxic substances to increase its efficacy in therapy. Future studies are directed at showing the usefulness of the antibodies for imaging and therapeutic purposes in pre-clinical animal models. If successful, this mAb may be applied not only in LCH, but also in CD1a-positive leukemia or in prevention of skin GvHD after allogeneic transplantation.

In general, LCH is considered a non-familial, multifactorial disease, although familial recurrence has been reported occasionally in identical twins (Arico et al., 1999). To assess whether a genetic component may be present in LCH, **Dr. Aricò** initiated a study comparing disease concordance rates between mono- and dizygotic twins. Combining literature findings with data obtained from various surveys, it appeared that among monozygotic twins with LCH there was a very high concordance (92%), while also dizygotic twins showed a strongly increased concordance rate (15%). Furthermore, several cases were found in which LCH was diagnosed among relatives, and it is probable that at least 1% of LCH patients have another familial case. This frequency highly exceeds the normal statistic occurrence of LCH in the general population and strongly suggests the involvement of genetic factors. A few candidate genes were studied for the presence of polymorphisms among patients and controls. Interestingly, the CD45 C77G polymorphism, associated with altered immune function, occurred in 7.3% of children with LCH, in contrast to 0.5% in controls ($p = 0.012$) (Boxall et al., 2004). Further frequency deviations were found between patients and controls in polymorphisms in E-cadherin, mannose-binding lectin and TNF-α genes, in particular in those patients with early onset and multisystem disease. From the analysis of other cytokines it appeared that the genetic characteristics of patients with single- and multisystem disease differed significantly, as polymorphisms in IFN-γ, IL-4 and IL-1β genes were only associated with single system disease (De Filippi et al., 2006). Together, these findings may represent just few of the many genetic variants affecting cellular functions that, alone or in association with other genetic variants, predispose to LCH. In addition to these polymorphisms, various previous studies suggested increased genetic instability in LCH patients, especially those with multisystem disease (Murakami et al., 2002; Scappaticci et al., 2000). Such instability leads to loss-of-heterozygosity, chromosomal breaks and poliploidy. Taken together, the question arises whether LCH should be considered a syndrome in which different pathogenic mechanisms may lead to similar clinical outcomes. In comparison, for hemophagocytic lymphohistiocytosis (HLH) this has been recognized before, and awareness of this notion has proven very fruitful in improved diagnosis and treatment of the disease (Arico et al., 2002).

Epilogue

In the summation, initiated by **dr. Beverley**, it was concluded that significant steps have been made in our understanding of DC, and LC in particular. LC belong to the most flexible, plastic cell types among leukocytes, although their plasticity decreases as the cells mature. Still, many basic questions remain concerning the developmental and functional relationship between LC and other DC, as well as concerning the regulation of their proliferative and proinflammatory activities. It is unclear so far to what extent developmental programs of DC or LC are inherently determined, or induced by different microenvironmental factors.
Given the multitude of unsolved questions in basic DC biology, it is no surprise that translating the basic findings to improved understanding and treatment of LCH remains a challenge. Little is known about putative genetic aberrations underlying LCH, while LCH may be even more diverse and complex compared to HLH, for which pathogenesis a blueprint is now emerging. Even the fundamental question whether the LCH cell is the main pathogenic cell in LCH is not answered unequivocally so far. The improved humanized mouse models that become available should revive the attempts to maintain LCH cells in vivo and re-address this question. Furthermore, the full picture of the LC differentiation

pathway in LCH patients is still lacking as only limited studies have been performed on presumed precursor populations in peripheral blood. The relative availability of patient peripheral blood cells, compared to the scarcity of lesional tissue, offers a good opportunity to approach putative genetic and functional deviations in the lineage.
In view of this meeting's theme, a very relevant question is: what are the implications of LC/DC plasticity for LCH? Does the heterogeneity of the disease phenotype reflect different stages? And, might the plasticity of the cells offer possibilities for therapy, for instance by inducing maturation of the cells to a non-proliferative stage? The current wealth of available tools to study the genetic program of cells can and should be applied to elucidate these questions in LCH and identify novel targets for directed therapy. In this respect, functional analysis and pharmacological inhibition of specific kinases in LCH cells might offer potent possibilities for innovative therapeutic intervention.

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