Langerhans Cell Function: Implications for LCH

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The Nikolas Symposia

Finding a rational cure for Langerhans Cell Histiocytosis (LCH) is the mission of the annually held Nikolas Symposium (Beverley et al., 2005). This meeting 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 will also increase our insight into the biology of normal dendritic cells as well as other disorders in which dendritic cells are involved.

Introduction

Langerhans cell histiocytosis (LCH) is a rare disease that is characterized by the uncontrolled accumulation of cells with typical features of Langerhans cell (LC) (Laman et al., 2003; Beverley et al., 2005; Bechan et al., 2006). LCH is of unknown etiology and occurs in various clinical forms in a broad age range from the newborn to the elderly but peaks between 1-4 years of age. The incidence in the pediatric age range has been estimated at 2-5 per million per year. 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 but 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 (Favara et al., 1997; Geissmann et al., 2001).

A central question is whether the aberrant LCH cells develop owing to an intrinsic defect of LC, or whether the disease is reactive, resulting from environmental triggers such as viral infection. Either scenario may lead to aberrant regulation of dendritic cells (DC) and thus give rise to their accumulation. The notion that LCH cells are clonally related in all instances, except most cases of smoking-related lung LCH, argues in favor of genetic dysregulation (Willman et al., 1994; Yousem et al., 2001). Although LCH is considered to be a sporadic disease, rather than inheritable, the notion is increasing that inheritable genetic components may play a more prominent role in the etiology of LCH than previously thought. This was concluded on the basis of high concordance rates observed in twin studies and supported by significant frequency deviations of several gene polymorphisms (De Filippi et al., 2006). In addition to these polymorphisms, previous studies suggested increased genomic instability in LCH patients, especially those with multisystem disease (Scappaticci et al., 2000; Murakami et al., 2002). This was recently confirmed by studying fractional allelic loss in DNA isolated from lesional and non-lesional tissue of LCH patients, which suggested that more extensive and higher-risk forms of LCH have evidence of more mutational events at tumor suppressor genes (Chikwava et al., 2007). Together, these notions are very reminiscent of malignant and pre-malignant deviations identified in virtually all hematopoietic lineages.

To sketch the field from the pathological point of view, Dr. **Malone** illustrated the various forms of histiocytosis. These can be largely divided into two groups depending on their DC or macrophage nature, although the distinction between the two lineages is not absolute. Typically, LCH is the most frequent DC-related histiocytosis, while macrophage-type disorders mostly present as hemophagocytic lymphohistiocytosis. Both involve pathologically non-malignant cells, although the clinical picture can be very severe in both cases. In LCH, the accumulating cells express markers characteristic of LC, such as CD1a, Langerin and S-100. The hallmark of LC, the Birbeck granule, may be absent in many LCH cells, however. In addition, a variety of other cell types is found in LCH

lesions, such as eosinophils, T cells, macrophages and multinucleated cells. Difficulty in diagnosis may arise due to the evolution of lesions, with accompanying loss of LCH cells, and increasing fibrosis. Furthermore, a "reactive" accumulation of LC-like cells may occur in lymph nodes adjacent to Hodgkin disease or carcinoma. These cells, however, appear to be non-clonal in nature and therefore the pathogenesis is probably distinct from LCH (Christie et al., 2006). The flexibility of the histiocytic cells, complicating the pathological diagnosis, is further illustrated by the (infrequent) finding of co-existence or sequential development of LCH and juvenile xanthogranuloma (JXG) (Hoeger et al., 2001). The latter disease originates from dermal DC, which have a distinctive phenotype ($FXIIIa^+$ $CD1a^-$ $S100^-$). Also, LCH may trigger local or systemic activation of macrophages, leading to hemophagocytic syndrome in part of the LCH patients (Favara et al., 2002). In a significant number of biopsies, not all of the lesional LCH cells will express all characteristic phenotypic LC markers, which likely relates to their deviated development and function.

In this year's symposium, specific attention has been given to the latest insights into the development and function *in vivo* of mononuclear phagocytes and LC in particular. Newly developed models of genetically modified mice have now provided a unique opportunity to study these topics in detail. Furthermore, the value of these models for LCH research have been discussed along with exciting new insights into the pathogenesis of LCH as obtained from studies using patient biopsies.

Kaleidoscopic view of the mononuclear phagocyte system using mouse models

The development of mononuclear phagocytes is controlled primarily by the growth factor, macrophage colony-stimulating factor (M-CSF or CSF-1). Notably, levels of this cytokine were previously found to be elevated in LCH patients (Rolland et al., 2005). To develop a universal marker for cells of the mononuclear phagocyte system (MPS) and study this in a transgenic mouse model, Dr. **Hume** and colleagues made use of the relatively specific expression of the CSF-1 receptor (CD115), encoded by the *c-fms* gene. Using control elements of this gene, they produced the MacGreen mice, in which all cells of the MPS express a green fluorescence gene (EGFP) reporter, enabling visualization and isolation of MPS cells in tissues and living animals (Sasmono et al., 2003). Unexpectedly, the transgene is also expressed in granulocytes, although the CSF-1R protein is absent in these cells. This led to the realization that granulocytes are more closely related to macrophages than was previously supposed. Remarkably, stimulation of inflammatory neutrophils with CSF-1 *in vitro* demonstrated that these cells can transdifferentiate into macrophages (Sasmono et al., 2007). Thus, potentially, macrophages in inflammatory lesions might derive from granulocytes as well as from recruited monocytes. More recently, Dr. Hume's group produced a second-generation reporter mouse, the MacBlue mouse (Ovchinnikov et al., 2008). Using these model systems, MPS numbers in peripheral tissues could be manipulated by application of anti-CSF-1R antibody *in vivo*. Remarkably, a profound depletion of mononuclear phagocytes was found from the large majority of sites, including skin, kidney, liver and GI tract. However, the cells of the brain, liver and lymphoid organs were resistant to treatment. The amount of cell death induced by this treatment was limited, so interaction of CSF-1, which is incorporated in the extracellular matrix, with the receptor on macrophages is probably important for their retention in particular sites. Using a newly developed small molecule inhibitor of the receptor's kinase activity, it was shown that CSF-1R signaling plays an important potentiating role in the production of pro-inflammatory cytokines upon Toll-like R triggering of macrophages (Irvine et al., 2006).

Dendritic cell function: studies in genetically modified mice

The current possibility to generate genetically modified mice expressing certain molecules, such as fluorescent proteins or *in vivo* selectable markers, in a cell type-specific manner offers a unique opportunity to study the developmental and functional biology of dendritic cells, and LC in particular (Bennett and Clausen, 2007). In order to distinguish LC from other DC types, several groups have used slightly different approaches to generate transgenic mice that express either EGFP or the human diphtheria receptor (DTR) under control of the langerin (CD207) gene (Bennett et al., 2005; Kaplan

et al., 2005; Kissenpfennig et al., 2005). The latest findings in these different models were presented and discussed at the meeting by drs. **Kissenpfennig, Kaplan** and **Clausen**.

Using EGFP-expressing LC, Dr. **Kissenpfennig** showed that Langerin$^+$ LC are sessile in an immature state in the epidermis of the skin and in stratified mucosal epithelia (Kissenpfennig et al., 2005). Interestingly, under steady state conditions a significant fraction of the Langerin$^+$ DC in lymph nodes appeared to originate from the blood stream, rather than peripheral, epithelial tissues. The blood-derived subset is also present in the spleen and characterized by elevated CD8α expression. Induction of trauma caused enhanced motility of the epidermal LC and their migration towards draining lymph nodes. Under these conditions, also dermal DC emigrated but preferentially to distinct sublocations in the lymph node T cell area. Ablation of LC from the skin by giving diphtheria toxin to Lang-DTR mice appeared to be complete and relatively long-lasting as restoration only started from 7-14 days. Full restoration of the LC population took 6-8 weeks. In lymph nodes both skin- and blood-derived Langerin$^+$ cells were also absent initially after depletion. Interestingly, absence of skin LC in either the immunization phase or the elicitation phase of a contact hypersensitivity response had no effect on the magnitude of the ensuing skin swelling, implying that LC are dispensable as antigen-presenting cells in this immune response.

Using a similar mouse model, Dr. **Clausen** and co-workers obtained somewhat different results, as they found that depletion of LC prior to sensitization caused a small but significant decrease in the CHS response (Bennett et al., 2005), which became more pronounced at a low hapten dose (Bennett et al., 2007). They showed that, in the absence of LC, topical antigen is inefficiently transported to draining lymph nodes, resulting in sub-optimal T cell priming and reduced CHS. In agreement with the data from the Kissenpfennig lab, they concluded that dermal DC can still induce CHS, suggesting redundancy between the two different skin DC populations in this model with a higher antigen dose favoring the contribution of dermal DC. LC have also been implicated as critical mediators of graft-versus-host disease (GvHD) following allogeneic bone marrow transplantation in patients with blood cell malignancies (Merad et al., 2004). Interestingly, expression of a model antigen (membrane-bound ovalbumin, mOVA) by keratinocytes in the epidermal layer of the skin also resulted in GvHD upon transfer of antigen-specific CD8$^+$ T cells (OT-I), suggesting that presentation of OVA by LC may be critical to induce the severe cell death and inflammation characteristic of GvHD (Shibaki et al., 2004). To test this hypothesis, they have used these K14-mOVA on the Langerin-DTR background. Surprisingly, OT-I T cell expansion and induction of GvHD-like skin disease still occurred in the absence of LC. Further analysis revealed that both dermal DC and keratinocytes themselves were capable to prime OVA-specific T cells *in vitro* and *in vivo*, eliminating a unique requirement for LC to induce disease in this GvHD model.

Dr. **Kaplan's** lab used a different strategy to achieve ablation of LC in a mouse model. By driving transgenic expression of the diphtheria toxin under control of the human Langerin gene, LC appeared to be absent permanently from the mouse epidermis (Kaplan et al., 2005). In marked contrast to the previous models of LC depletion, the CHS response in these mice was found to be enhanced rather than decreased. The constitutive vs. inducible depletion of LC and/or the human vs. mouse genetic control elements probably underlie the observed differences between the different models. The absence of LC in these hLang-DTA mice did not prevent rejection of skin grafts differing in major (MHC) or minor antigen expression (Obhrai et al., 2008). Thus, alternative antigen-presenting populations are probably dominant in this respect. Moreover, in the FVB mouse model where H-Y mismatched skin grafts are normally maintained indefinitely, such grafts lacking LC are efficiently rejected. Although some caution is needed since this is unique to the FVB model, these findings suggest that LC in the donor graft are required for long-term skin engraftment. Hence, LC play a regulatory, tolerogenic rather than immunogenic role in skin graft rejection in this setting.

For the interpretation of the results in the different LC-depletion models, it is relevant to mention that several papers have been published recently that shed new light onto the differential findings. In the dermis of mice, a hitherto unknown population of Langerin-expressing cells has been detected, which appears to be unrelated to the epidermal LC (Bursch et al., 2007; Ginhoux et al., 2007; Poulin et al., 2007). These dermal cells originate from the blood-derived lineage mentioned before and are capable of inducing contact hypersensitivity responses against skin-derived antigens.

The existence of this independent dermal Langerin$^+$ population provides a good explanation for the observation that dermal and lymph node Langerin$^+$ cells reappear long before epidermal LC after depletion in Langerin-DTR mice. This return of dermal Langerin$^+$ cells takes place already from day 3 after depletion onwards and the different experimental protocols used for the CHS responses probably contribute to the differential findings by the Kissenpfennig and Clausen labs (Bursch et al., 2007; Wang et al., 2008). However, the importance of hapten dose, in addition to timing of DT application, is demonstrated by reduced CHS at 4 weeks after DT, at a time when all other Langerin$^+$ DC populations have largely recovered from the toxin treatment. Moreover, in the transgenic hLang-DTA mice from the Kaplan lab, the dermal population of Langerin$^+$ cells appears to be unaffected (Bursch et al., 2007), which may explain the lack of inhibition but not yet the observed enhancement in the CHS response.

A mouse model for the histiocytic disorder involving uncontrolled immune activation, hemophagocytic lymphohistiocytosis (HLH), was presented by one of this year's Artemis fellows, Dr. **Jordan**. In the majority of human cases of the familial form of HLH, mutations have been demonstrated in the perforin gene, strongly affecting natural killer cell function. Episodes of systemic immune activation in these patients are often triggered by viral infection. Similarly, perforin-deficient mice have been recognized to develop an HLH-like disease process following infection with lymphocytic choriomeningitis virus (LCMV)(Jordan et al., 2004). The basis for this abnormal immune activation is not known. Using both perforin-deficient and beige mice, which have distinct genetic abnormalities affecting perforin-dependent cytotoxic function, Dr. Jordan and colleagues found that both mouse strains develop exaggerated CD8$^+$ T cell responses with abnormally increased interferon gamma (IFN-γ) production after LCMV infection. This excessive IFN-γ production was not due to an intrinsic abnormality of these mutant T cells, but was secondary to extrinsic host factors. Interestingly, CD11c$^+$ DC appeared to be the critical cell type driving this abnormal IFN-γ production. Besides having an abnormal T cell stimulatory phenotype, DC populations from perforin-deficient mice harbored increased amounts of viral antigen and an increased frequency of T cell-stimulating cells. In search of mechanistic explanation, it was found that *in vivo* inhibition of apoptosis-associated caspases selectively increased the T cell-stimulatory function of DC from wild type, but not perforin-deficient mice. Together, these findings suggest that induction of apoptosis of DC by cytotoxic cells plays an important regulatory role to dampen immune activation after viral infection.

Langerhans cell precursors

For the regulation of Langerhans cell functions, migration of precursor cells and their subsequent development into mature cells is crucial. Control in this regard is determined by the repertoire of receptors for adhesive molecules and chemokines on precursors and expression of their ligands in distinct microvascular beds and peripheral tissues. Dr. **Moser** discussed in detail their recent findings on CXCL14, an ill-defined chemokine also known as BRAK (Kurth et al., 2001; Schaerli et al., 2005). They report that CXCL14 is highly selective for human CD14$^+$16$^-$ peripheral blood monocytes, although another laboratory has described a selectivity for immature DCs (Shellenberger et al., 2004). It is abundantly expressed in normal healthy human skin as well as in other epithelial tissues. These initial findings suggest that CXCL14 may recruit precursors of antigen-presenting cells, such as blood monocytes, to epithelial environments under steady state conditions. Results from a CXCL14 knock-out mouse model, however, indicated that murine CXCL14 is dispensable for the homeostatic recruitment of antigen-presenting cells toward the periphery and for LC functionality (Meuter et al., 2007). To investigate the role of the tissue microenvironment in differentiation of LC, a model of human epidermis equivalents was devised. This consisted of multiple layers of keratinocytes, generated from hair root epidermal stem cells, and fibroblasts, separated by a membrane support. In this skin model, keratinocyte maturation and keratinization was observed. When CD14$^+$ monocytes were incorporated, these cells accumulated in a suprabasal location and expressed markers typical for LC, such as CD1a, Langerin and CD205. Such LC-like cells were in an immature state and could be

induced to mature *in situ* in the presence of DC maturation stimuli such as LPS, TNF-α and microbial stimuli. Interestingly, LC differentiation of monocytes was not stimulated in cultures of freshly isolated dermal fibroblasts or keratinocytes, indicating a crucial contribution made by epidermal equivalents that may involve factors associated with the epidermal architecture and/or differentiated keratinocytes.

Approaching the identification of LC precursors in the mouse model, Dr. **Merad** summarized recent findings on LC development in fetal life, adult steady state and inflammation. Before or shortly after birth, LC precursors, which are $CX_3CR1^+CD115^+$, seed the developing skin. This recruitment appears to be mediated by the selective expression of trafficking molecules, such as the MadCAM addressin and BRAK/CXCL14 and MCP-1/CCL2 chemokines early during development. These molecules are downregulated in adult life, and under steady state conditions the LC population is maintained by local proliferation of a pool of radio-resistant precursor cells (Merad et al., 2002). Upon serious trauma, such as high dose UV irradiation, however, LC as well as dermal DC leave the skin. Then, mentioned adhesion molecules and chemokines are strongly upregulated again and circulating $CCR2^+Ly\text{-}6C^{hi}$ monocytes are recruited into the inflamed skin to develop into functional LC (Ginhoux et al., 2006). The local precursors, maintaining LC in the steady state, possibly reside in the sebaceous gland in the hair follicle, close to the epidermal stem cell niche. Here, CSF-1 is produced, which is essential for LC development. In human skin, there is a low, but significant degree (~2.5%) of epidermal LC in cell cycle, indicating local proliferation of the cells. In agreement with the notion of local maintenance, it was observed that patients undergoing allogeneic hematopoietic stem cell transplantation, retained host LC for prolonged periods of time (Bogunovic et al., 2006; Merad et al., 2007). However, in inflamed skin, host LC as well as dermal DC appeared to be replaced by donor-derived cells from the circulation.

DC function and hematopoietic stem cell transplantation

The role of DC in hematopoietic stem cell transplantation was further elaborated by Dr. **Luznik**. The challenges in transplant immunology are to optimize immune reconstitution, while minimizing graft-versus-host disease (GVHD) and required immunosuppression, and simultaneously retaining a graft-versus-leukemia effect (GVL). These are virtually oxymoronic requirements as allogeneic T cells play an important role in both GVHD and GVL. It appears that immune reconstitution can be significantly improved by separate donor lymphocyte infusion following hematopoietic stem cell transplantation. The antigen-presenting cells that initiate GVHD appear to be only host- and not donor-derived (Shlomchik et al., 1999). Upon MHC-matched bone marrow transplantation in an animal model, a significant proportion of peripheral host DC is maintained and continuously migrates to draining lymph nodes, thus potentially enabling the stimulation of donor lymphocytes (Durakovic et al., 2006). Furthermore, the activation state of these residual host-derived DC critically influences the donor lymphocyte-mediated responses in MHC-matched mixed chimeras as resting DC primarily stimulate regulatory T cells (Treg), while activated (Toll-like R-stimulated) DC preferentially induce effector T cell responses (Durakovic et al., 2007). This was shown by applying the TLR9-agonist imiquimod to the skin and which strongly augmented a GVL effect. The current working model on the basis of these results is that disease activity in GVHD, caused by host DC-stimulated effector T cells from donor origine, is inversely correlated with the presence of regulatory T cells. Thus, this balance between effector and regulatory T cells is determined by the degree of DC activation. In agreement with this, it was found that in patients who had undergone an allogeneic bone marrow transplant, there is a direct correlation between relatively poor immune reconstitution, few Treg and chronic GVHD.

Increasing insights into LCH pathogenesis

Several lines of evidence suggest that LCH is a primary proliferative disorder of LC, and suspected to be a (pre-)neoplastic condition. One of the key findings in such conditions is the shortening of chromosome ends, the telomeres. One of this year's Artemis fellows, Dr. **Bechan**,

discussed her findings on telomere lengths in different LCH patient samples. To that end, telomere lengths were determined in CD1a$^+$ cells in lesions from LCH patients with local, multi-system or systemic disease (Bechan et al., 2008). These were compared with telomere lengths from CD1a$^+$ cells in unaffected skin and reactive lymph nodes, as well as from lesional and lymph node lymphocytes as controls. Interestingly, LCH cells appeared to contain significantly shorter telomeres than LC from normal skin or reactive lymph nodes. This was observed in all subtypes of LCH studied and reflects the proliferative history of the cells. Since extensive telomere shortening generally should induce p53-mediated apoptosis, senescence or terminal differentiation in cells, the current findings suggest that this mechanism is affected in LCH cells. Thus, LCH cells may share mechanisms of telomere shortening and survival with clonal preneoplastic disorders and cancer. In this respect, it is relevant to mention that recently the presence of functional telomerase was shown in LCH cells of several, but not all investigated lesions (da Costa et al., 2007).

In addition to the proliferation of LCH cells, active lesions are characterized by the extensive local production of cytokines and chemokines by LCH cells and by bystanders such as T cells, macrophages and eosinophilic granulocytes. In his presentation, Dr. **Egeler** gave an overview of the current thoughts on their involvement with the disease. Most of the cytokines present in the LCH lesion, such as IL-1α, TNF-α, IFN-γ, and GM-CSF have a pro-inflammatory nature, although also the anti-inflammatory IL-10, produced by LCH cells and macrophages, has been detected (Egeler et al., 1999). In particular, CD40$^+$ LCH cells and CD40L$^+$ T cells are thought to be involved in an amplification cascade with cytokine and cytokine receptor upregulation in auto- or paracrine loops. Also at the level of chemokines and chemokine receptor expression, similar vicious circles can be envisaged. For instance, lesional CD1a$^+$ LCH cells express an immature CCR6$^+$7$^-$ phenotype, explaining their inability to emigrate to draining lymph nodes, as this is primarily guided via the CCR7-binding chemokines CCL19 (ELC/MIP-3β) and -21 (SLC/6Ckine) (Annels et al., 2003). Furthermore, LCH cells produce high levels of CCL20 (MIP-3α), potentially recruiting additional immature CCR6$^+$ DC, as well as CCL5 (RANTES) and CCL17 (TARC), attracting T cells. Several of the lesional cytokines and associated receptors are involved with the formation of multinucleate giant cells (MGC) found in the LCH lesions, which bear characteristics of osteoclasts. In addition to the factors mentioned above, it should be noted that both osteoclastogenic factors RANK and RANKL are expressed by LCH cells, raising the possibility that LCH cells themselves stimulate ánd contribute to the formation of MGC in the lesions (da Costa et al., 2005).

Dr. **Delprat** further elaborated the connection between LCH cells and osteoclastogenesis. Previous studies from her lab had indicated that immature DC, like monocytic cells, can fuse and transdifferentiate into osteoclasts (OC) (Rivollier et al., 2004). Although the classic factors involved in OC formation, M-CSF and RANKL, are present in LCH lesions, these might not be the predominant factors in LCH. Instead, Dr. Delprat showed that patients with active LCH have high levels of IL-17A and RANKL in their serum (Coury et al., 2008). Surprisingly, lesional LCH cells as well as DC generated *in vitro* from peripheral blood monocytes of LCH patients appeared to express IL-17A, in contrast to DC from healthy individuals. IL-17 induced the fusion of immature DC specifically as this was licensed by GM-CSF, but not M-CSF, and further potentiated by IFN-γ, all factors known to be produced in the LCH lesion. The positive interaction of IL-17 and IFN-γ is surprising since these cytokines are known to be counterregulatory at the T cell level. The serum content of IL-17A and RANKL appeared to be correlated in LCH patients but their values did not parallel the disease activity. This might be explained by the presence of anti-IL-17A antibodies that were shown to be present in LCH patients. These autoantibodies might neutralize IL-17 activity. Taken together, these findings suggest an intriguing mechanism, in which autocrine IL-17 production by immature DC might play a central role in the recruitment of inflammatory cells in LCH. In support of this hypothesis, it has been observed that IL-17-transgenic mice generate inflammatory infiltrates in their lungs, consisting of eosinophilic and sometimes multinuclear macrophages (Park et al., 2005). Moreover, IL-17 plays an important role in the induction of mycobacterially induced granuloma formation (Umemura et al., 2007).

The Corinth initiative - an international consortium for molecular LCH research

Since LCH is a relatively rare disease and many of the patients are children, biopsy material for research purposes is extremely scarce. Yet, progress in therapeutic treatment is highly dependent on increased insights into the pathogenic determinants of the disease. The current status of molecular research in general allows detailed screening at multiple levels using limited amounts of material, but a higher level of logistic organization is required to benefit optimally from these technological developments. At the previous Nikolas symposium, **Dr. Beverley** was agreed to investigate the possibilities of founding an international consortium that could manage patient sample collection and distribution among the participating laboratories. At this meeting, details were discussed concerning the practical approach and priorities that could be set. Given the legal issues involved, it was felt that the consortium should comprise two centers, one in Europe and one in the US. Control of the samples should remain with the centers. Of note, a European initiative, originating from the French LCH study group, for tissue banking of LCH and HLH samples involving a network of clinicians has already been started. A putative connection to the Corinth initiative needs to be investigated.

For tissue collection and processing, a meticulous protocol needs to be in place since patients will be seen in a wide variety of centers. The participants at the meeting agreed that, given the putative systemic involvement, not only lesional tissue material should be collected but also control tissues, in particular peripheral blood and buccal smears. Also serum should be stored for future metabolic analyses. In addition, for pediatric patients it would be informative to have access to parental tissue. Purification of LCH cells from, preferably fresh, tissue using a highly standardized protocol can be done at best by a limited number of specialized centers using flowcytometric cell sorting. Then, CD1a+ and CD1a- cells should be separated and compared, together with the control tissues. It was felt that determination of putative genetic deviations by large array single nucleotide polymorphism (SNP) analysis and sequencing of the kinome at the DNA level would be good starting points for a molecular approach by the consortium.

Summation and conclusions

Several studies presented at the meeting raised important issues for the discussion, led by Drs. **Beverley** and **Arceci**, as the findings might shed new light onto LCH pathogenesis. The observation by Delprat and colleagues showing that not only LCH cells but also monocyte-derived DC from LCH patients spontaneously secrete IL-17A, in contrast to healthy control cells, suggests the presence of abnormalities in peripheral blood monocytes of LCH patients. The ability of these mononuclear cells to express IL-17 was also found when disease activity was undetectable and no elevated levels of serum cytokines were observed. Therefore, this suggests a possible intrinsic abnormality at the bone marrow precursor level. Alternatively, however, a so far undetected intracellular pathogen might be involved, stimulating LC IL-17 production, in a predisposing genetic background. Whether microbial infection would cause LC to express IL-17 remains to be demonstrated, however. Since numerous electron microscopic studies have been performed on LCH tissue to characterize affected LC by the presence of Birbeck granules, it is unlikely that a non-viral pathogen is involved if indeed an infectious agent plays a pathogenic role.

Various T cell subsets may play an important role in the regulation of (lesional) DC. The absence of a notable $CD8^+$ T cell response in LCH patients could argue against viral involvement. However, regulatory T cells, which are known to be a predominant subset of $CD4^+$ T cells in LCH lesions and are expanded in patients with active disease (Senechal et al., 2007), might both inhibit anti-viral $CD8^+$ T cell activity and maintain LCH cells in an immature state. Th17 cells probably do not play a significant role, since these are hardly found in LCH lesions.

Inspired by the fruitful application of transgenic and knock-out technology to the DC field, as discussed in this meeting, different animal models for the disease could be devised. For instance, transgenic mice could be generated with constitutive or inducible DC-specific expression of IL-17 under the control of CD11c or Langerin-promoters. Responses of human DC might be studied in the HIS mouse model, which harbors a humanized immune system, although the myeloid engraftment is relatively low.

Taken together, this meeting has shown that state-of-the-art genetically modified mouse models can provide important new insights into the functional and developmental biology of normal Langerhans cells and mononuclear phagocytes in general. The notion deriving from these models that distinct lineages of Langerin-expressing cells exist, raises the intriguing possibility that aberrant LCH cells might derive from the epithelium-independent lineage of Langerhans cells. The preferential localization of LCH lesions in connective tissues and adjacent to bone, and not associated with epithelia, would be in keeping with such a derivation. Furthermore, an exciting new hypothesis for the pathogenesis of LCH was presented. Although many steps remain to be proven, pathological DC-derived IL-17 might be involved in various LCH-related signs and symptoms, such as the prevention of apoptosis of immature DC, the occurrence of a pro-inflammatory cytokine storm and the formation of granulomatous lesions and multinucleate giant cells. The idea that IL-17 production in DC might be stimulated by a microbial pathogen suggests the possibility that an infectious agent, transferred from mother to child early in development, might be involved in LCH. This possibility underlines the importance of a detailed family analysis of LCH patients.

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