Langerhans Cell Histiocytosis: A hematopoietic stem cell disorder?
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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 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 in which an uncontrolled accrual of cells with Langerhans cell (LC) characteristics occurs (Laman et al., 2003; Beverley et al., 2005). 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 LCs, 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). Nevertheless, LCH is considered to be a sporadic disease, rather than inheritable. In the previous symposium, dr. Aricò has presented evidence 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 genetic instability in LCH patients, especially those with multisystem disease (Scappaticci et al., 2000; Murakami et al., 2002). Together, these notions are very reminiscent of malignant and pre-malignant deviations identified in virtually all hematopoietic lineages. Since the fundamental pathogenic aberrations in these conditions are found at the stem cell level, this inspired to focus on the question whether LCH might represent a disorder of hematopoietic stem cells as this year's theme.

In the introduction on the pathology of the disease, 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, DC and macrophages differ in their anatomical location, immunophenotype as well as functional aspects such as phagocytic capacity and lysosome quantity. 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, akin to Hodgkin's lymphoma lesions, but plasma cells are absent. Difficulty in diagnosis may arise due to the evolution of lesions, with ac-

companying loss of LCH cells, or in association with other diseases. For instance, a "reactive" LCH may occur in lymph nodes adjacent to Hodgkin's disease or carcinoma (see also the summary of the presentation by dr. Goodlad, below). Aberrant cells in the histiocytic disease juvenile xanthogranuloma (JXG) are thought to originate from dermal DC, which have a distinctive phenotype ($FXIIIa^+$ $CD1a^-$ $S100^-$). 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 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. This illustrates the need for genetic markers to show a similar derivation of these cells and raises the question whether precursor cells might be present that function as lesional stem cells.

DC and LC differentiation: in vivo and in vitro model systems

The developmental pathway of LC and their separation from other myeloid lineages is only partially understood at present. **Dr. Geissmann** focused on early stages of DC and macrophages in bone marrow and peripheral blood using knock-in mice that express green fluorescent protein (GFP) transcribed from the promoter of the fractalkine receptor (CX3CR1) (Jung et al., 2000). Especially cells of the monocytic lineage are tagged by this marker. The earliest detectable GFP-positive precursor cells in the bone marrow appear to be part of the granulocyte-macrophage progenitor pool expressing c-kit/CD117 in addition to CX3CR1, but no other lineage markers (Fogg et al., 2006). These cells represent clonal precursors of monocytes, macrophages and DC, in vitro as well as in vivo, and are therefore labeled macrophage-dendritic cell progenitors (MDP). DC developing from these cells are only partially monocyte-derived and DC may thus also derive from a separate route of differentiation of MDP. Among the circulating monocytes derived from these precursors, distinct stages can be identified that differ in the expression level of GFP (Geissmann et al., 2003) and may represent different stages of maturation (Sunderkotter et al., 2004). Interestingly, these monocyte subtypes appear to have different functions as only GFP^{lo} monocytes accumulate at sites of acute inflammation. Using in vivo imaging of mouse ears, dr. Geissmann showed that some of the GFP^{hi} cells are sessile in the dermal microenvironment, while others migrate in- and outside small vessels with appreciable velocity. Tissue injury induced the acute extravasation of GFP^{hi} monocytes as the initial host response. This raises the possibility that GFP^{hi} monocytes function as patrolling cells in search of tissue damage. When detected, these scouts may initiate the ensuing inflammatory response.

Dr. Geissmann also showed recent findings in tissue specimens of LCH patients, aimed at the analysis of proliferating cells in LCH lesions. Using Ki-67 as a proliferation marker, only 1-2% of LCH cells appeared to be labeled. In contrast, many $CD4^+$ T cells present in the lesion were in cell cycle. A remarkably high percentage (~ 30%) of $CD4^+$ T cells were regulatory T cells as indicated by the expression of the transcription factor FoxP3. It is tempting to speculate that these regulatory T cells contribute significantly to an immunosuppressive environment that facilitates the accumulation of aberrant LC in the LCH lesion.

Dr. Romani reviewed the developmental biology of LC *in situ*. From a series of earlier and more recent studies in human and mouse skin the picture emerged that, under steady state conditions, LC are not continuously replenished by circulating precursors, but locally maintained from precursor cells that seed the skin shortly before birth. Neonatally developing LC acquire their characteristic markers locally in the sequence: ATPase / CD45, MHC class II, Langerin, CD205 (Romani et al., 2003; Tripp et al., 2004). This view is supported by the finding that freshly isolated dendritic leukocytes obtained from newborn skin lack several of these markers, but will gain them over time in culture. Mouse knock-out studies have indicated that several humoral factors are essential for the development of LC: both the macrophage growth factor M-CSF as well as TGF-β appear to be indispensable (Borkowski et al., 1997; Ginhoux et al., 2006). Interestingly, in LCH patients the circulating levels of M-CSF are significantly elevated (Rolland et al., 2005). (*PL note:* Also TGF-β and its receptor are abundantly present in LCH lesions (Kannourakis and Abbas, 1994; Schouten et al., 2002)). A selective chemokine, BRAK (CXCL14), that is constitutively expressed in epidermis and other epithelia attracts monocytes and may be important for the retention of LC in their

epithelial location (Schaerli et al., 2005). Under steady state conditions, LC appear to be self-maintained through local proliferation, while after injury these cells are replenished by circulating Gr-1^{hi} monocytes (*note: corresponding to the previously mentioned GFP^{lo} monocytes in CX3CR1-GFP-transgenic mice*) (Merad et al., 2002; Ginhoux et al., 2006). The epidermal immigration is then primarily guided by CCR2-binding cytokines such as CCL2 and -7 (MCP-1, -3). Regarding the functional role of LC in skin immunity, recent studies have challenged the classical paradigm that LC are the primary stimulating cells responsible for the induction of protective immunity. It is now realized that LC may also play an important role in the maintenance of T cell tolerance while other skin DC subsets may be involved with the stimulation of anti-microbial immunity (Steinman and Nussenzweig, 2002; Allan et al., 2003). Results from recently developed mouse models, in which LC have been selectively depleted, have left an unclear picture in this respect as contrasting results have been obtained in different laboratories. Removal of LC has either shown no effect (Kissenpfennig et al., 2005), a limited reduction (Bennett et al., 2005), or even an enhancement of skin-induced immune responses (Kaplan et al., 2005). Hopefully, further insight into these models, also stimulated by presentations and discussions at the next 17th Nikolas symposium, may shed light on these controversies.

A sophisticated mouse model to study *in vivo* development of human leukocytes was presented by **dr. Blom**. This model makes use of gene-targeted mice that lack adaptive immune cells and NK cells by deletion of the genes encoding Rag-2 and the common γ chain of the receptor for multiple cytokines, incl. IL-2, -4, -7, -9, -15, and -21. The defective development of endogenous immune cells allows the reconstitution with human stem cells and thus generates a mouse with a humanized immunized system, the so-called HIS mouse (Gimeno et al., 2004; Legrand et al., 2006; An et al., 2007). Optimal conditions for engraftment are present during the first week of life of these mice, and CD34$^+$ human fetal liver or cord blood cells are typically used as a source of donor stem cells. A few weeks after transplantation, in a percentage of recipient mice stable reconstitution is observed not only with human T-, B- and NK-cells, but also human monocytes, conventional DC and plasmacytoid DC are present. In addition to endogenous mouse LC, also some human LC are found, and therefore this novel model might give an opportunity to revive attempts to propagate human LCH cells *in vivo*. Furthermore, it enables the *in vivo* study of the molecular requirements of human LC development and function. In dr. Blom's laboratory, this model was used to study development of plasmacytoid DC, and in particular the role of the Ets-family transcription factor Spi-B. This factor was previously shown to be required for plasmacytoid DC development (Schotte et al., 2004). Human CD34$^+$ progenitor cells were first transduced with a lentiviral vector that mediates knockdown of Spi-B expression by RNA interference before injection into newborn Rag2/γc mice. Development of plasmacytoid DC in the HIS-mouse appeared to be strongly impaired when Spi-B expression was ablated in CD34$^+$ cells, while development of B cells and monocytes was normal or even slightly enhanced. These findings clearly indicate that Spi-B is specifically required for development of human plasmacytoid DC.

Dr. Acha-Orbea presented his promising results aimed at generating a mouse model of histiocytosis. This is based on the DC-specific transgenic expression of the viral large T oncogene using the CD11c promoter. In two independent transgenic mouse lines with different transgene expression levels the animals are healthy until the age of 3-4 months and 6-12 months, respectively. In younger mice, the DC show a normal distribution and immunological function. Thereafter, the mice become ill and start developing features of human multisystem histiocytosis. They show massive infiltration of DC in spleen, liver, thymus and bone marrow. These cells remain non-activated and retain full capacity to mature into professional antigen presenting cells as indicated by up-regulation of MHC class II and co-stimulatory molecules as well as secretion of the expected cytokines such as IL-12 and IL-6, after activation with microbial stimuli. *In vitro*, these transgenic DC efficiently stimulate naive T cells. Subtype analysis of the affected DC showed that preferentially CD8$^+$ DC are transformed. Interestingly, these blood-derived DC share many feature with aberrant histiocytes in LCH, such as S100 and Langerin expression. From diseased mice, DC cell lines can be derived eas-

ily, which can be maintained growth factor-independently *in vitro* and express all features of freshly isolated DC. Furthermore, these cells can be adoptively transferred into healthy recipients and cause similar pathological features in 1-2 months. Given the remarkable resemblance with human multi-system LCH, characterized by malignant clinical behavior caused by accumulation of pathologically non-malignant DC, it is of great importance to elucidate the changes in gene expression between normal DC, transgenic DC in healthy mice and the changes occurring during transformation. The increasing loss in cellular regulation along this pathway is probably explained by the accumulation of additional mutations in the large T-expressing DC. Studies assessing the molecular changes are currently underway, supported by a research grant from the Histiocytosis Research Trust. This interesting approach received an enthusiastic audience, although it was argued that the main organs affected in the mouse model, i.e. spleen, liver, thymus, bone marrow, did not fully match those in human multi-system LCH, as this manifests itself especially in bone, skin and lymph nodes, in addition to the mentioned visceral organs.

An alternative approach to model human hematological disorders was presented by **dr. Fairchild**. He made use of mouse and human embryonic stem (ES) cells, which can be maintained indefinitely as untransformed, pluripotent cells *in vitro* due to their unlimited self-renewal capacity. Furthermore, they are relatively accessible for genetic modification, in contrast to primary DC, which are inherently resistant in this respect. Thus, ES cells represent tractable candidates to dissect the genetic basis of complex diseases, including LCH. To guide the generation of DC, ES cells are first stimulated to generate complex three-dimensional structures, known as embryoid bodies, which mimic early development of the yolk sac. In these, the first stages of hematopoietic differentiation can be detected. Exposure to cytokines such as GM-CSF and IL-4, known to stimulate DC differentiation from bone marrow- or peripheral blood precursors, stimulates the development of ES-derived myeloid DC (Fairchild et al., 2000; Fairchild et al., 2005). Comparison of ES-derived and bone marrow-derived DC by global genome expression profiling indicates that these cells show a high degree of similarity. Furthermore, ES-derived DC have similar capacities to process exogenous antigens, to stimulate T cells in an antigen-specific manner and to migrate *in vivo*. Importantly, genetic modification of the parent ES cells permits the universal expression of the transgene without any observable adverse effect on the function of derived DC. The future possibility of exploiting somatic nuclear transfer from LCH cells to generate ES cells bearing the genotype associated with this disease may provide a powerful model of pathogenesis.

LCH studies by Artemis fellows

Dr. Costa, one of this year's Artemis fellows, presented her exciting work in search of consistent chromosomal abnormalities in LCH. If present, this would provide final proof that LCH is a neoplastic disease with a genetic basis, rather than a reactive process to unknown triggers. However, to date only a few studies provide support for this view, while various reports are conflicting or inconsistent (Betts et al., 1998; Murakami et al., 2002). The latter might stem from technological issues or from the presence of highly variable chromosomal defects. In the present study, a first challenge was to isolate LCH cells from frozen and paraffin-embedded archival LCH material. This was achieved by careful pre-treatment of tissue specimens followed by cell sorting of Langerin- or CD1a-positive cells. Simultaneously, these LCH cells could be evaluated for DNA content, and thus for putative aberrancies in ploidy. So far, LCH cells from 13 solitary as well as 3 multisystem lesions, including bone, skin and lymph node were all found to be diploid, as far as can be concluded from this relatively insensitive method. The extraction of genomic DNA from the sorted LCH cells is currently being optimized to enable genome-wide screening by array comparative genomic hybridization (aCGH). This method, using >3500 BAC (bacterial artificial chromosomal) clones, will allow reliable detection of chromosomal abnormalities such as interstitial deletions, non-reciprocal translocations and amplifications with high resolution. A first pilot experiment in this direction indeed showed abnormalities in the genetic profile of CD1a+ lesional cells, which were not present in the inflammatory CD1a-negative cells. The latter finding raises the possibility that somatic mutations, on top of genetic aberrancies, may be involved in LCH pathogenesis. It furthermore urges to analyze normal tissue from LCH patients in parallel

with affected, lesional tissue. The aCGH study is currently being extended to a higher number of LCH lesions and the results will be confirmed with higher resolution array.

The other Artemis fellow, **dr. Goodlad**, analyzed tissue from LCH patients who carried the disease in association with other lymphoproliferative disorders. Previously, proliferations of Langerhans cells (LCs), morphologically indistinguishable from LCH, have been described in association with a variety of other tumor types, most commonly malignant lymphoma and lymphoblastic leukaemia (Egeler et al., 1998). It has been argued that in some instances of acute leukaemia LCH arises as a complication of chemotherapy, or that the two conditions are associated by chance. There is also evidence to suggest that both neoplasms may arise occasionally from transformation of a common stem cell or that lineage switching occurs within a single neoplasm (Magni et al., 2002; Feldman et al., 2005). However, it also remains possible that, where LCH and a haematological malignancy are concurrent and present at the same site, the former is not a true neoplasm but an exaggerated response to the lymphoid tumor. To distinguish between these possibilities, dr. Goodlad performed in depth analysis of five recent cases of LCH in the context of other lymphoproliferative disorders (Christie et al., 2006). These include two cases of mycosis fungoides and one of cutaneous B-cell pseudolymphoma. Two patients were female, allowing clonality analysis of the accumulating LC using laser capture microdissection and the HUMARA (human androgen receptor) assay, based on the clonal inactivation of one of both X-chromosomes. The results show that the LC forming discrete nodules in a case of cutaneous B-cell pseudolymphoma and a case of Hodgkin's lymphoma are polyclonal. This suggests that, at least in a proportion of cases, the aggregates of LC occasionally identified within other lymphoproliferative lesions represent a reactive proliferation rather than a potentially aggressive second neoplasm.

Molecular aberrancies in hematological disorders

As outlined above, the notion that inherited and/or acquired genetic mutations are the basis for the etiology and pathogenesis of LCH is gaining support. **Dr. Willman** initiated the discussion on how to approach the exploration of these presumed molecular defects in LCH, and to confirm finally whether LCH is or is not a neoplastic disorder. Following the determination by her and dr. Chu's laboratories that LCH is in principle a clonal disease (Willman et al., 1994; Yu et al., 1994), little progress has been made to elucidate the genetic abnormalities and pathways that underlie this disease. There have been rare reports of cytogenetic abnormalities in LCH and LOH. However the LOH studies were plagued by a lack of paired controls and interpretation is problematic as these LOH/SNP changes might have been polymorphisms.

Dr. Willman presented her current in-depth analyses of genetic and molecular changes in acute leukemia (Bhojwani et al., 2006; Radich et al., 2006; Wilson et al., 2006). These multicenter studies are aimed at identifying molecular signatures that typify leukemias with different risk for relapse after initial therapy. Such identified molecular pathways might also generate targets for therapy. In childhood acute lymphoblastic leukemia, patients with different chromosomal aberrations in their leukemic cells are known to have significantly distinct relapse rates. It is unclear, however, why patients in a certain risk class do or do not relapse. Therefore, gene expression profiles of cells, isolated at diagnosis, have been compared retrospectively between relapsing and non-relapsing patients. From these analyses, a list of molecular markers is obtained that highly significantly correlate, either positively or negatively, with failure or complete remission in a 4 year period. Interestingly, classical clinical parameters, such as age of onset, sex and karyotype, have no added value to the newly developed molecular risk classification. On the bases of these findings, a highly improved prediction can be made at diagnosis, which can determine the intensity of the therapeutic regime.

These findings are now the basis of study in a consortium of laboratories that aims at identifying molecular pathways in high risk ALL in children. These studies are integrating whole genome SNP/LOH studies using Affymetrix SNP arrays to determine copy number abnormalities in ALL cells using paired leukemia cell DNA and normal DNA from the same patient, which are critical for such studies (Relling, Downing, St. Jude Children's Hospital); gene expression profiling of ALL samples using new high density Affymetrix arrays (U133

and Human Exon Chips containing 1.5M genetic elements measuring expression and alternative splicing of all exons) accompanied by modeling new genetic groups and pathways involved in high risk ALL (Willman, UNM); and micro RNA expression (Croce, Ohio State Univ.). Integration of these large data sets are identifying novel molecular lesions in high risk ALL in children. The integration of these large data sets will lead to informed sequencing of chromosomal regions implicated to be involved in the disease and the identification of novel therapeutic targets.

Dr. Willman believes that a similar approach should be undertaken in LCH and that only through such studies will the determination of whether LCH is or is not a neoplasm be made. Regardless of the outcome of these studies (i.e., whether LCH is or is not a neoplasm), the data generated would provide tremendous new insights into the disease and potentially identify new therapeutic targets. Critical for these studies will be the accessioning and sharing of highly characterized tissue samples from LCH patients along with matched DNA/blood controls. This will require the development of a committed consortium of investigators. Dr. Willman recommended that such an effort commence immediately.

In his presentation, **dr. Layton**, highlighted the clinical features and underlying molecular defects in a number of clonal hematological stem cell disorders which analysis might be exemplifying for the study of LCH. These included hypereosinophilic syndrome (HES), paroxysmal nocturnal hemoglobinuria (PNH) and multiple myeloma. In general, HES is caused by mutations, in particular translocations, involving the A-chain of the PDGF-receptor (Gotlib et al., 2006). As a consequence, a constitutively signaling tyrosine kinase is encoded, leading to the proliferative advantage and accumulation of eosinophilic granulocytes. Treatment with the tyrosine kinase inhibitor imatinib leads to complete remission in the majority of patients, although additional mutations have been identified that cause imatinib resistance. The defect underlying PNH is the failing formation of a glycosylphosphatidylinositol- (GPI)-anchor in a clone of hematopoietic cells (Parker, 2007). This anchor links a subset of cell surface molecules, including CD24 and the complement inhibitor CD59, to the cell membrane. The enigmatic selective advantage of cells derived from a hematopoietic stem defective in GPI-synthesis might be explained by the hypothetical existence of a cytolytic autoimmune response against the GPI anchor or one or more GPI-linked molecules on normal cells. In multiple myeloma, patient prognosis directly correlates with the level of bone resorption and this again associates with the ratio between the soluble mediators sRANK-ligand and osteoprotegerin (OPG). Interestingly, recent studies in LCH have indicated that the sRANKL/OPG ratio is similarly useful as indicator of osteolytic activity (Ishii et al., 2006).

Dr. Levine elaborated the notion that LCH might represent the DC variant among the myeloproliferative disorders, which, amongst others, include polycytemia vera, essential thrombocytemia, hypereosinophilic syndrome as well as chronic myeloid and myelomonocytic leukemia. In these myeloproliferative disorders, cells with a relatively normal maturation accumulate due to disturbances in pathways regulating cell proliferation and survival. Such in contrast to myelodysplastic syndromes in which cells show hampered maturation. In both conditions, cells may eventually obtain further mutations and progress to acute myeloid leukemia. In recent years, important breakthroughs have been established in understanding the molecular background of virtually all myeloproliferative disorders. It is very remarkable that in all of these, mutations have been identified that cause constitutive activation of tyrosine kinases involved with essential cell signaling processes (Campbell and Green, 2006). In addition to the *PDGFR-A* mutation in HES, mentioned above, and the well-characterized *BCR-ABL* mutation in CML, other activating mutations have been found involving kinases such as PDGFR-B, c-kit and, most recently, Jak-2. Interestingly, the latter kinase appears to be mutated in different diseases involving multiple lineages. A rationale for this diverse appearance may be found in the observation that coexpression of a homodimeric type I cytokine receptor, such as the erythropoietin R, the thrombopoietin R, or the G-CSFR, together with the mutated Jak-2 kinase is necessary for transformation (Lu et al., 2005). The involvement of activated kinases as common molecular principle in pathogenic deviation provides excellent therapeutic opportunities using kinase inhibitors such as imatinib (Gleevec). Re-

cently, imatinib application in an LCH patient with brain involvement appeared to improve clinical symptoms (Montella et al., 2004). This finding clearly urges to search for molecular deviations in kinases involved in signaling pathways that may cause pathogenic transformation of LC.

Cancer stem cells

Dr. Bonnet highlighted the question how leukemia, in particular acute myeloid leukemia (AML), is maintained in vivo. Elucidating the nature of the target cell that undergoes leukemic transformation is essential for both the understanding of the leukemogenic process and for the design of effective therapies. As a model system, she made use of engraftment of cells, purified according to phenotype, into NOD/SCID mice or NOD/SCID mice that also lack β2 microglobulin. Successful transplantation will cause outgrowth of leukemic cells that in all aspects resemble those in human patients. From normal stem cell sources, such as bone marrow or cord blood, it could be established that only the earliest stem cell populations, being $CD34^{-\text{ or }+}$ $CD38^{-}$, will generate long term engraftment. When leukemic cells are used for transplantation, only about 60-70% of leukemias will propagate *in vivo*. The ability to engraft NOD/SCID mice appears to be an inherent characteristic of the leukemic stem cells (LSC), irrespective of their frequency or homing capacity to the bone marrow of recipient mice (Pearce et al., 2006). Remarkably, engraftment typically increases with poorer prognosis. The LSC have extensive proliferation and self-renewal capacities, in contrast to the leukemic blasts forming the bulk of tumor cells (reviewed in (Bonnet, 2005)). In a particular case only 2% of leukemic cells were $CD34^{+}$, but LSC were exclusively present in this population and represented only 1 in 10^6 to 10^7 total leukemic cells. Since the phenotype and functional characteristics of these LSC may be fundamentally distinct from the vast majority of leukemic cells, it is of great importance to evaluate the effect of novel therapies not only on the bulk of tumor cells, but in particular on the LSC, since these are essential in maintenance of the tumor.

Malignant stem cells appear to play a role not only in the myeloid leukemia, but in the maintenance of most if not all proliferative disorders. **Dr. Jones** elaborated on LSC in different malignancies, in particular leukemias of lymphoid nature. From a clinical point of view it is enigmatic and frustrating that, despite the introduction of a large variety of novel anticancer therapeutics with shown clinical benefit of tumor eradication, the improvement of survival rates has been limited over the last two decades (Huff et al., 2006). Dr. Jones showed that the tumor-maintaining LSC in different types of acute lymphoblastic leukemia, in multiple myeloma as well as in Hodgkin's lymphoma all have phenotypic properties and drug sensitivities highly distinct from the bulk of tumor cells. Typically, the phenotype of LSC corresponds to stages preceding the developmental stage of the main tumor population. In multiple myeloma, which typically manifests itself in the bone marrow, it was shown that stem cells can also be found in the circulation. In general, drug resistance is higher in stem cells as these are typically quiescent, have a high level of ABC transporters, exporting drugs from the cytoplasm, and also express high levels of drug-detoxifying enzymes. Therefore, bulk tumor reduction that is traditionally taken as a clinical endpoint for the evaluation of novel drugs is an insufficient criterion. More important for tumor recurrence and related patient survival is the effect on proliferation and survival of tumor-maintaining stem cells.

Summation and conclusions

In the summation, **dr. Steinman** and **dr. Beverley** reviewed the highlights of this year's symposium and initiated the discussion on which issues were mostly timely to approach in LCH. The cellular composition of the LCH lesion remains an important source of information on the clinical behavior of the tumor. It is relevant to note that, in addition to the local cytokine storm that is elicited, $CD25^{+}$ $FoxP3^{+}$ regulatory T cells are relatively abundant. Together with the immature nature of the LCH cells, these generate an immune tolerant environment that is permissive for tumor development. Stimulated maturation of LCH cells might render these cells immunostimulatory and initiate an autoimmune response against the tumor. Such experimental therapies should ideally be devised and evaluated using *in vivo* models in which human LCH cells can be propagated. So far, experiments in this direc-

tion were unsuccessful. The recent development of improved models, such as the Rag-2/γc knockout mouse, might stimulate renewed efforts to transplant LCH cells. In this respect, it should be realized that an LCH stem cell might exist that is phenotypically distinct from the typical $CD1a^+$ $Langerin^+$ LCH cell that constitutes a major component of the lesion. Also, such stem cells might be circulating and not be restricted to the lesions. To provide a niche in the skin of recipient mice, it might be considered to deplete resident mouse LC using UV irradiation.

The recent advances in the understanding of the molecular alterations in other myeloproliferative diseases inspire to search for mutations in LCH, in particular involving tyrosine kinases. Relevant candidates in this respect are those that are known to be important in normal LC/DC generation and function, especially c-fms (M-CSFR, CD115), c-kit (CD117) and Flt-3/Flk-2. The notions that in some cases inheritable genetic deviations may underlie the transformation of LC, or that the acquisition of a molecular defect may have occurred at the stem cell level and thus occur in all hematopoietic cells derived from this single cell imply that a wide range of cells and tissues from the patients need to be compared to detect putative molecular aberrations.

It was generally felt that the current state of technology was highly adequate and should be applied for the detailed molecular analysis of LCH at the genomic and transcriptomic level. Obstacles to do so are primarily the highly limited availability of patient material and the logistics of the analytic process rather than scientific and technological in nature. To manage these challenges, it was proposed to initiate the foundation of an international consortium that could manage the collection of patient samples and distribute these among the participating laboratories. Involvement of patient organizations, facilitating easy material collection and giving incentives to participating clinicians were seen as important requirements for success. While fresh or frozen material from LCH lesions is very important, but scarce, the analysis of normal cells from blood and other tissues from LCH patients already enables the search for inherited genetic deviations or somatic mutations acquired at the hematopoietic stem cell level. Dr. Beverley was willing to investigate the possibilities to found such a consortium. Hopefully, this effort will be successful and expedite LCH research at the molecular level and thus increase our insight into this enigmatic disease.

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