# The tolerogenic nature of tumor-associated inflammation: relevance for LCH

*proceedings of the 18th Nikolas Symposium*

*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*

Langerehans cell histiocytosis (LCH) is a rare disease that is characterized by the uncontrolled accumulation of cells with features of Langerhans cells (LC) (Laman et al., 2003; Beverley et al., 2005; Bechan et al., 2006). LCH is a sporadic disease 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. More severe forms of LCH occur especially in younger children, and these cases of multisystem disease tend to have a clinically malignant character. In LCH lesions of both single and multisystem disease, LCH cells are invariably present but other cell types also contribute 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 longstanding, but still unresolved 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. Suggestive evidence exists in support of both scenarios. Briefly, the clonal relationship of LCH cells, their maturation arrest and the high disease concordance rates observed in twin studies and the increased genetic instability argue in favor of genetic dysregulation (Willman et al., 1994; Murakami et al., 2002; De Filippi et al., 2006; Chikwava et al., 2007). In contrast, no consistent genomic aberrations have been observed in an extensive recent study (da Costa et al., 2009), while LCH disease activity and severity appears to correlate with the DC-poietins M-CSF and Flt-3L (Rolland et al., 2005). Furthermore, an IL-17A-dependent monocyte fusion-inducing activity in the serum, but not IL-17A levels as such, associated with disease activity (Coury et al., 2008). Thus, the underlying cause of DC accumulation in LCH still remains to be determined.

In tumor biology, it is increasingly understood that the microenvironment of the tumor, which appears to be inflammatory in nature, plays an important role in the tumor's ability to expand and metastasize. It can be envisaged that a Darwinian selection pressure exists favoring tumors that are capable to shape their environment to their own profit. This concerns factors such as stimulation of vascularization as well as suppression of innate and adaptive immune responses. To focus on the question how this might apply to LCH, the topic of this year's symposium was the inflammatory microenvironment of the LCH lesion and, in particular, its contribution to resist eradication of aberrant LCH cells by the host's immune system.

To outline the pathology of histiocytic 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 far from 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's 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). Cells characterizing the latter disease have a distinctive phenotype ($FXIIIa^{+}$ $CD1a^{-}$ $S100^{-}$) and were previously designated as dermal DC. Recent studies suggest, however, that FXIIIa expression typifies dermal macrophages rather than dermal DC (Haniffa et al., 2009). 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 developmental flexibility and deviated function.

*Macrophage diversity and immune regulation in infection and cancer*

In his presentation focusing on macrophage diversity in *in vivo* mouse models, dr. **de Baetselier** showed interesting parallels between macrophages developing under conditions of chronic parasite infections and in tumor-bearing mice. In both, suppression of adaptive immune responses can be mediated by immature stages of myelomonocytic development, now indicated as myeloid-derived suppressor cells (MDSC; recently reviewed in (Gabrilovich and Nagaraj, 2009)), as well as by mature macrophages that might have been activated differentially by environmental triggers. Macrophages can be polarized by *in vivo* conditions in various directions, but for the sake of simplicity two major stages are distinguished: M1 or classically activated macrophages are stimulated by exposure to IFN-γ and LPS, and M2 or alternatively activated macrophages are stimulated by IL-4 or -13. Depending on the stage of parasitic infection and the nature of the pathogen, M1 or M2 macrophages may predominate *in vivo*, although M2 macrophages are more commonly associated with parasitic infections (Raes et al., 2007). Both M1 and M2 macrophages can be suppressive, but they employ different mechanisms: M1 macrophages produce in particular nitrogen- and oxygen radicals, while M2 macrophages secrete IL-10 and TGF-β. M2 macrophages developing in distinct *in vivo* models have a remarkably stable gene expression profile (Ghassabeh et al., 2006).

Tumor-associated macrophages (TAM) appear to have a predominant M2 phenotype as well, with associated immune suppressive function. Interestingly, stimulating further M2 polarization via PPAR-γ ligands appears to inhibit their suppressive activity (Van Ginderachter et al., 2006). The immature myelomonocytic stages that execute immune suppression, MDSC, develop in both tumor-bearing and in parasite-infected hosts. In tumor-bearing hosts it was shown that these cells are also heterogeneous and can be divided into mononuclear and polymorphonuclear cells (MO-MDSC and PMN-MDSC, respectively). Also their suppressive mechanisms appear to be diverse: although IFN-γ is important in inducing suppression by both, STAT1 activation plays a role in MO-MDSC-, but not in PMN-MDSC-mediated suppression (Movahedi et al., 2008). Taken together, it appears that an array of myelomonocytic stages, either mature or immature, can have immune suppressive activity albeit through distinct molecular mechanisms. Generation of such cells is stimulated by inflammatory conditions, which may occur in infection, but also in tumor-bearing hosts.

**Dr. Pollard** focused in his presentation on the functional consequences of the interaction between tumors and TAM. A pivotal cytokine in the development of macrophages is M-CSF, or CSF-1. Absence of either the cytokine or its receptor has a deleterious effect on the development of the vast majority of macrophage populations. Since macrophages play an important role in tissue remodeling and angiogenesis, their absence leads to abnormalities in organ development, for instance in bone, brain and pancreas (Pollard, 2009). Using breast cancer models in mice, dr. Pollard and co-workers showed that tumors subvert normal macrophage-associated morphogenic functions, facilitating their invasion into normal tissues and egress of tumor cells into blood vessels (Condeelis and Pollard, 2006). CSF-1 appeared to be involved directly, since mammary tumor-specific transgenic expression of CSF-1 in CSF-1-deficient mice stimulated significant expansion of TAM and subsequent tumor progression. Macrophages and tumor cells are in a paracrine loop-relayed chemotaxis involving EGF produced by the macrophages and tumor-produced CSF-1 (Condeelis and Pollard, 2006). Besides mutual attraction between macrophages and tumor cells, also tumor vascularization is crucial for its malignant potential. Also here macrophages play an essential role as they cooperate with tumor cells in

the production of angiogenic factors, in particular VEGF (Lin et al., 2007). Thus, mammary tumor progression depends highly on the interaction between tumor cells, blood vessels and TAM, mediated in particular by the cytokines CSF-1, EGF and VEGF (summarized in (Pollard, 2008). The importance of this notion for human disease is underlined by the finding that levels of these cytokines correlate with poor clinical prognosis.

*Myeloid-derived suppressor cells and regulation of DC differentiation*

**Dr. Bronte** indicated that TAM are thought to develop either from circulating monocytes or from tumor-infiltrating MDSC (Sica and Bronte, 2007), and he discussed in detail the heterogeneity and specific characteristics of MDSC generated under various non-steady conditions. Interestingly, although the mechanisms of suppression mediated by MDSC are not antigen-specific, anti-tumor responses are clearly affected in tumor-bearing mice while responses to conventional antigens are not compromised. As indicated above, MDSC, defined in mice as $CD11b^+$ $Gr\text{-}1^+$ cells with suppressive activity, comprise activated polymorphonuclear and mononuclear cells (Dolcetti et al., 2008). Molecular profiling of the mononuclear population indicated that the cells are distinct from their normal counterparts and bear characteristics of simultaneous IFN-γ- and IL-13-mediated stimulation. Both cytokines cooperate in the induction of the arginine-metabolizing enzymes arginase and iNOS, which are essential for the suppressive function of these cells. Notably, IFN-γ and IL-13 are not only produced by NK(T)- and T-cells in tumor-bearing hosts, but also by MDSC themselves (Gallina et al., 2006). Since CD124 (IL-4Rα) is important for IL-13 signaling, this is also a functional marker of MDSC.

Although MDSC are widely studied in model systems in mice, there is accumulating evidence that also human equivalents exist: a significant fraction of cases of human cancer show selective expansion of either $CD14^+CD124^+$ (monocytic) or $CD15^+CD124^+$ (granulocytic) cells in peripheral blood, which have suppressive activity in *in vitro* assays. These cells presumably use similar suppressor mechanisms as their mouse counterparts, as it has been shown in human prostate cancer that many tumor-infiltrating lymphocytes are present, but they occur in a non-responsive state. Inhibition of arginase and iNOS activity alleviates this suppression (Bronte et al., 2005).

Different tumor-derived factors, such as VEGF, IL-6, IL-10, M-CSF, and GM-CSF are known to play an important role in the development of MDSC (Gabrilovich and Nagaraj, 2009). In addition, these factors are also involved in defective final DC maturation in tumor-bearing hosts (Gabrilovich, 2004). Evaluating the effect of VEGF on hematopoietic progenitor cells using differential display analysis, **dr. Gabrilovich** and colleagues noticed a consistent upregulation of MRP-14, also known as S100A9 or calgranulin B. This protein is abundantly present in immature myeloid cells and forms homodimers or heterodimers with MRP-8 (S100A8, calgranulin A) to form a Ca-binding complex. Upon maturation of myeloid cells both S100 proteins are down-regulated. (Note: LC and LCH cells express another family member, S100A1). Knockout mouse models have shown that S100A9 is dispensable for myeloid cell differentiation under steady state conditions (Manitz et al., 2003). However, mice lacking this protein mounted more potent anti-tumor responses and rejected implanted tumors more readily compared to controls, which was related to a defective development of MDSC (Cheng et al., 2008). Conversely, S100A9-overexpressing transgenic mice accepted tumors at higher rates. In tumor-bearing, non-mutated mice, S100A9 expression appeared to be up-regulated in myeloid precursors and this caused inhibition of DC maturation and accumulation of MDSC, a process that was controlled by the transcription factor STAT3. The S100A9-mediated inhibition of DC maturation was caused by up-regulation of production of reactive oxygen species (ROS). This was shown by the normal maturation of DC in S100A9-overexpressing mice that lacked the gp91-component of NADPH-oxidase. The direct link between S100 expression and ROS production is explained by the previously shown regulation of ROS generation by S100A8/9, which potentiates NADPH-oxidase activation (Doussiere et al., 2001). Taken together, these findings indicate that up-regulation of S100A9 in tumor-induced MDSC is critically important for their accumulation and coincident inhibition of DC maturation, thus dually affecting adaptive anti-tumor responses.

*IDO as mediator of immune suppression*

In subsequent presentation, drs. Mellor and Grohmann highlighted a distinct mechanism of immune suppression by non-lymphocytic cells, which is mediated by the tryptophan-metabolizing pathway dominated by the enzyme indoleamine 2,3-dioxygenase (IDO). Recent progress has shown that tryptophan catabolism is a pivotal means of immune regulation during infection, pregnancy,

autoimmunity, neoplasia and other inflammatory conditions.

**Dr. Grohmann**'s group has shown that IDO-mediated immune suppression involves not only local depletion of the essential amino acid tryptophan, but also the generation of immunoregulatory kynurenines (Puccetti and Grohmann, 2007). These compounds alone can induce apoptotic cell death in T helper (Th) cells. Moreover, the combination of low tryptophan and kynurenines can convert Th cells into regulatory T cells (Treg) capable of suppressing a fulminant autoimmune disease *in vivo* (Fallarino et al., 2006). Plasmacytoid DC show the highest levels of IDO expression, but also conventional DC, granulocytes and macrophages may express this enzyme. IDO activity is the rate-limiting step in tryptophan catabolism; other enzymes of the pathway are more ubiquitously expressed. This implies that other cells may assist in this suppressive pathway by metabolizing excreted kynurenin pathway products, thus generating immune suppressive catabolites, even if they do not express IDO themselves. IDO expression can be induced by different environmental triggers, including IFN-γ and glucocorticoids. For instance, administration of dexamethasone to mice activates IDO through concordant induction of GITR (glucocorticoid-induced tumor necrosis factor receptor) in $CD4^+$ T cells and GITR-L in plasmacytoid DC (pDC). Reverse signaling through GITR-L on the DC induces IDO in these cells. This involves a non-canonical route of NF-κB activation, i.e. activation of p52-relB via NIK- and IKKα-phosphorylation (Grohmann et al., 2007; Puccetti and Grohmann, 2007).

IDO uses superoxide as a 'cofactor' for oxidative cleavage of the indole ring of tryptophan. This superoxide-dependent step in tryptophan metabolism along the kynurenine pathway is blocked in a mouse model of chronic granulomatous disease (CGD) lacking functional NADPH-oxidase ($p47phox^{-/-}$). Pulmonary Aspergillus infection of these mice thus leads to a lethal acute inflammatory lung injury caused by unrestrained $Vγ1^+$ γδ T-cell reactivity, dominant production of IL-17 and defective Treg activity (Romani et al., 2008). Therefore, a paradoxical pathogenic mechanism emerges in which the absence of ROS - quintessential mediators of neutrophil-associated oxidative damage - causes a different form of IL-17-dependent inflammation due to blocked IDO activity. Reversal of the hyperinflammatory phenotype in CGD mice is achieved by replacement therapy with a single s.c. administration of slow-release L-kynurenine, the main IDO product. Effective therapy, which includes co-administration of recombinant IFN-γ to up-regulate other enzymes of the pathway, restores production of downstream active metabolites and enables the emergence of Treg cells. Therefore, the administration of supplemental kynurenines can efficiently counteract pathogenic inflammation driven by IL-17, which results from a dysfunctional IDO mechanism.

**Dr. Mellor** elaborated in his presentation on the IDO-mediated mechanisms of immune suppression that may have beneficial as well as adverse effects for host integrity as they dampen excessive inflammation but simultaneously provide possibilities for immune evasion (Mellor and Munn, 2008). The mouse pDC that are especially IDO-competent constitute a minor subset of phenotypically unique pDC, characterized as $CD11c^{hi}$ $CD8α^+$ $CD19^+$ $B220^+$ $CCR6^{hi}$. These cells express IDO after stimulation with various triggers, such as CD80/86 ligands, TLR9 or IFN-α. Then, $IDO^+$ pDCs and Tregs cooperate to create dominant suppression in some inflamed tissues as these pDC can directly activate Treg in the absence of CD3-mediated stimulation (Sharma et al., 2007). Such activated Treg suppress T cell responses via a mechanism dependent on an intact PD-1 signaling pathway, and expression of PD-L1 and -L2 on antigen-presenting cells. In contrast, Treg that have been activated via CD3/IL-2 stimulation use distinct mechanisms of suppression. The importance of IDO-mediated suppression *in vivo* is demonstrated in IDO-1 KO mice, which display a tumor-resistant phenotype after repeated exposure to phorbol ester following carcinogen exposure (Muller et al., 2008). This stimulant leads to chronic inflammation, which in normal mice is accompanied by the induction of $IDO^+$ pDC creating a tolerogenic environment, both locally and in draining lymph nodes, and thus facilitating tumor growth, perhaps by inhibiting innate immune surveillance mechanisms. This is also relevant for human tumor development, since an abundance of $IDO^+$ pDC in sentinel lymph nodes of melanoma patients correlates with chronic activation of Treg and with poor prognosis. Therefore, IDO-mediated suppression is a putative target for pharmaceutical intervention in cancer patients in general, and investigation into its contribution to LCH pathogenesis is warranted.

*Langerin-expressing DC subsets and LCH pathogenesis*

Progress in LCH research would benefit significantly from an animal model that reflects human disease. In search of such a model, **dr. Acha-Orbea** and colleagues have generated transgenic mice that expresses SV40 large T antigen under control of the relatively DC-specific CD11c promoter (Steiner et al., 2008). Different mouse lines with high and low expression levels of the transgene were generated. Related to this difference, these so-called Mushi mice show early and late onset of disease, respectively, characterized by accumulation of DC causing highly increased spleen and liver sizes and decreased hematocrit values. The affected DC represent a specific subtype with a Langerin/CD207$^+$ CD8$\alpha^+$ CD11b$^{-/lo}$ CD24$^+$ CD205$^+$ phenotype. Given the widespread dissemination of transformed DC, these mice may serve as a model for multi-system histiocytic disease. The pathological picture bears some similarities to human disease as transformed cells are round and have indented to groovy nuclei. However, the cells have less abundant eosinophilic cytoplasm and a high mitotic rate (but no abnormal figures), and lesions show no sign of an eosinophilic infiltrate. Furthermore, the transformed DC are distinct from epithelium-associated Langerhans cells as they lack significant IRF-4 and DC-SIGN expression like genuine LC.

Transformation of DC is associated with SV40 large T-induced changes in gene expression, and to investigate these in detail, DC were isolated from different stages of tumor development and analyzed by gene expression analysis. Amongst the 45101 probes, 574 cancer-related and 245 immune-related changes were found with $p<0.05$. Many but not all immune-related genes (cytokines, chemokines, receptors, TNF and TNF receptor family, signaling molecules) found in LCH are expressed in tumors. Lacking ones are induced upon activation. Surprisingly, also tumor suppressor genes appeared to be up-regulated in transformed DC, in particular the cyclin-dependent kinase inhibitors p16 and, to a lesser extent, p15. Notably, p16 expression has already been described in LCH lesions (Schouten et al., 2002). Immunohistochemical analysis showed that lesional LCH cells also have up-regulated p15 expression (4/5 cases). Furthermore, transformed DC also showed changes in several pro- and anti-apoptotic genes. Investigating this in more detail, dr. Acha-Orbea and colleagues found that in vivo stimulation of DC with TLR3-ligand (poly I:C) in non-transgenic mice caused early activation of the cells followed by increased apoptotic death, leading to a preferential loss of the CD8$^+$ DC subset. This route involved type I IFN. Interestingly, repeated injection of low doses of TLR3 ligands into Mushi mice before they developed aggressive tumors strikingly prolonged their life span. Further analysis of molecular deviations and putative therapeutic approaches in this model may hold promise for developments in human disease.

To stimulate young investigators in their histiocytosis-related research, the Dr. Jon Pritchard Award is granted yearly to one or two researchers or clinicians in the beginning of their career. This year, dr. Ginhoux and dr. Allen were the recipients.

In his presentation, **dr. Ginhoux** described the identification of a new Langerin$^+$ DC population identified in the mouse dermis and other organs. The existence of such a population was suggested by findings in LC depletion models that showed reappearance of Langerin$^+$ cells in skin-draining LN long before return of epidermal LC (Kissenpfennig et al., 2005). This led to the discovery of an independent Langerin$^+$ DC population situated in the dermis (Ginhoux et al., 2007). Using the unique radioresistance of epidermal LC as a tool, BM-chimeric mice were made in which BM-derived cells could be distinguished from host leukocytes on the basis of different CD45 alleles. After reconstitution, most dermis-derived Langerin$^+$ cells appeared to be from donor BM origin, while LC were still from host origin. Migration from the circulation into the dermis was mediated via P-/E-selectins and CCR2-binding chemokines. Like LC, these Langerin$^+$ dermal DC migrate to draining LN guided via CCR7-binding chemokines. Distinguishing features of Langerin$^+$ dermal DC are significant expression of CD103 and low level Ep-CAM, while epidermal LC do not express CD103, but bear Ep-CAM at high levels (Merad et al., 2008; Nagao et al., 2009). Furthermore, Langerin$^+$ dermal DC have a higher proliferative index compared to their epidermal counterparts and do neither rely on TGF-$\beta$ nor on M-CSF for their development (Nagao et al., 2009). Interestingly, a similar Langerin$^+$ population of DC is present in liver, lung and kidney. The existence of this independent Langerin$^+$ population of cells has been confirmed by other investigators (Sung et al., 2006; Bursch et al., 2007; Poulin et al., 2007). As discussed below, the identification of this LC-like population may have significant importance for the understanding of LCH as the affected cells in this disease might as well derive from this distinct

lineage of Langerin-expressing cells.

**Dr. Allen** presented the first analyses of cell-specific gene expression profiles from Langerin/ $CD207^+$ LCH cells and $CD3^+$ T cells purified from lesional tissue of LCH patients. These LCH profiles were compared with those from $CD207^+$ cells isolated freshly from normal human skin (primarily foreskin), while lesional T cell profiles were compared with those from normal tonsil T cells and from peripheral blood T cells isolated from LCH patients obtained at the time of LCH lesion biopsy. In the pathologic LCH cells 29 genes were found to be up-regulated more than 4-fold, and these included some genes previously described as differentially expressed, including RANTES. Additionally, some genes previously not associated with LCH were up-regulated in LCH cells as well and these encoded proteins that regulate lymphocyte activation and migration, such as osteopontin, vanin-1, and LAMP-3. For various markers, immunohistochemical analysis confirmed their expression by LCH cells. Some unexpected findings, however, questioned the commonly held premise that LCH cells represent activated LC that are seemingly arrested in an immature stage of development (Geissmann et al., 2001; Laman et al., 2003): no difference were found with normal LC in expression of chemokine receptors (CCR6 and CCR7), cell surface markers involved in T cell activation (CD40, CD80, CD86), pro-inflammatory cytokines (TNF-$\alpha$, IFN-$\gamma$, IL-1$\alpha$, IL-1$\beta$, LIF, IL-4, IL-10, TGF-$\beta$), genes involved in regulation of apoptosis (Flt-3, Flt3-ligand, bcl-2), or markers of cell proliferation (Ki67, PCNA, p53). Notably, also IL-17A expression was not found to be enhanced in lesional LCH cells, in contrast to recent observations by others (Coury et al., 2008). The gene expression profiles of lesional $CD3^+$ T cells reflected those of activated T cells as indicated by the up-regulated expression of genes such as MHC class II, CD74 invariant chain, HSP70 and CTLA4. In accordance with recent findings (Senechal et al., 2007), FoxP3 expression was significantly higher in lesional T cells compared to circulating T cells from the same patients.

Taken together, the LCH cell gene expression profile observed in these studies is not consistent with the prevailing model of LC as malignant activated-immature DC that independently elaborate a "cytokine storm". To reconcile this with clinical findings, dr. Allen proposed a model in which LCH cells arise directly from circulating premature DC precursors and subsequently recruit activated T cells that contribute to tissue destruction and tumor progression.

The clonality of LCH cells in lesions, and between different lesions, has long been interpreted as indication of malignant transformation of these cells. **Dr. Chu** re-evaluated the significance of these findings, which were first reported almost 15 years ago, on the background of current notions about normal LC biology. Since no lineage-specific clonal markers exist for LC, studies on clonality in LCH have relied on X-chromosome inactivation markers. These are based on the irreversible inactivation of all genes on a randomly selected single X-chromosome in a precursor cell in female individuals (lyonization), implying that all descendants of this precursor will have the same inactivated X chromosome. Of these markers, the human androgen receptor (HUMARA) has proven to be the most reliable and informative for clonal analysis. So far, three studies have been reported on clonality analysis in LCH (Willman et al., 1994; Yu et al., 1994; Yousem et al., 2001). Two studies concerned single system or multisystem pediatric LCH, comprising samples from 13 patients in total, from which 12 could be reliably interpreted (Willman et al., 1994; Yu et al., 1994). Lesional cells in all of these cases appeared to be clonal, and in a single case, lesions from bone and lymph node showed the same X chromosome inactivation. In contrast, clonality analysis of biopsies from pulmonary LCH patients showed clonality in only 7 out of 24 nodules. This supports the notion that pulmonary LCH is a distinct, reactive disease, which is known to be smoking-related.

Important for the interpretation of the pediatric LCH cases is the question whether unaffected LC in normal skin represent a clonal population. After isolation of normal LC from donor skin from various locations, dr. Chu and colleagues found 5 out of 14 normal LC samples to be clonal. This indicates that some areas of skin are populated from single LC precursors and suggests that the situation in human skin is similar to the situation in mice, where LC precursors are seeded in fetal life and maintained locally during steady state (Merad et al., 2002). Thus, finding clonality among LCH cells in a single lesion probably relates to their common ancestry, but does not necessarily indicate neoplastic proliferation of the cells.

*Tumor-associated DC diversity and therapeutic options*

In LCH, as in other tumors, intralesional DC are observed, besides the characteristic LCH cells. In her presentation, **dr. Stoitzner** addressed the nature of the defective function of tumor-infiltrating

DC (TIDC). To this end, she used a mouse model in which B16 melanoma cells, stably transfected with OVA as a model tumor antigen, were transplanted subcutaneously (Stoitzner et al., 2008a). Within the tumors, both myeloid and plasmacytoid DC populations were present, but LC were virtually absent. Most of the TIDC appeared phenotypically immature, lacking significant expression of costimulatory molecules, but about a third expressed a mature phenotype. However, TIDC did not present tumor-derived proteins, although TIDC were clearly filled with melanocytic material originating from the tumor cells. They were unable to induce the proliferation of tumor-specific $CD4^+$ and $CD8^+$ T cells *in vitro* unless OVA peptides were specifically added. *In vivo*, some presentation of tumor-derived antigen to $CD8^+$ T cells was found in the tumor-draining lymph nodes, but no proliferation of $CD4^+$ T cells. This implies an intrinsic lack of T cell help for anti-tumor cytotoxic T cell responses. Together, this suggests that tumor-associated DC in this model have limited antigen-presenting capacity, particularly related to impaired antigen processing.

To overcome tumor-induced tolerance dr. Stoitzner and colleagues elaborated a method of epicutaneous immunization on tape-stripped skin (Stoitzner et al., 2008b). In a prophylactic model, all OVA-immunized mice remained tumor-free when transplanted with B16.OVA cells and showed significant tumor-specific CTL responses. Even epitope-spreading to tumor-specific antigens was noted as immunized mice also showed delayed and decreased growth of B16 melanoma cells that did not express OVA. In a therapeutic setting, where immunization was performed after tumor transplantation, this treatment also showed clinical efficacy. For epicutaneous immunization on tape-stripped skin to be effective, the presence of LC was required, since this procedure failed to induce an anti-tumor response in LC-deficient mice.

**Dr. Anderson** focused in his presentation on the DC as part of the immune environment in various forms of pediatric cancer, addressing the questions how these tumors influenced DC to block immune stimulation and how this immune evasion might be counteracted. One such cancer type, alveolar rhabdomyosarcoma, is characterized by the presence of the PAX3-FKHR fusion gene, which originates from the erroneous fusion of the two mentioned transcription factors genes. Investigating the molecular targets of this tumor-specific transcription factor, dr. Anderson and colleagues found that PAX3-FKHR alters the tumor cells' gene expression profile as a result of a specific interaction between PAX3-FKHR and the STAT3 transcription factor (Nabarro et al., 2005). This results in a dramatic reduction in tumor MHC expression, and an increase in local IL-10 concentrations, which inhibits intra-tumoral DC maturation and numbers of infiltrating neutrophils, macrophages and lymphocytes.

Effective induction of anti-tumor responses requires, besides immune-stimulating DC, also appropriate tumor antigens. Ideally, these are expressed at high levels by tumor cells and not, or only to a limited extent by normal cells. Potential targets that meet these requirements are MYCN, PAX3 and PAX5 transcription factors that are present at high level in rhabdomyosarcoma, neuroblastoma and B cell malignancy. Theoretically, sufficiently elevated expression would allow tumor antigenic peptide loading to MHC class I molecules, and thus surface recognition by cytotoxic T cells. Indeed, it appeared possible for all three mentioned transcription factors to elicit specific cytotoxic T cell responses in model systems in mice and/or in cancer patients (Himoudi et al., 2007; Himoudi et al., 2008b; Yan et al., 2008).

An intriguing novel population of DC, identified in mice, may prove important in the initiation of anti-tumor responses. These cells have common features of NK cells and DC ($CD11c^{int}$ $B220^+$ $NK1.1^+$ $CD49b^+$ cells) and are capable of producing IFN-γ and express tumoricidal activity, hence their designation as IFN-producing killer DC (IKDC). It appeared feasible to generate these cells *in vitro* from BM precursors using GM-CSF and IL-4 (Himoudi et al., 2008a). Transfer of relatively low numbers of these IKDC into mice bearing small, syngeneic tumors led to significantly decreased tumor growth, which depended on the presence of host lymphocytes. It was shown that injected DC migrated to tumor sites and subsequently to tumor-draining lymph nodes, where the cells up-regulated MHC class II molecules indicative of their antigen-presenting capacity. Hence, these IKDC might represent a specific subset capable of evading tumor-associated immune suppression.

*Summation and conclusions*

In the summation session, chaired by **Drs. Arceci** and **Leenen**, many highlights of the meeting were discussed with regard to their relevance for LCH pathogenesis and therapeutic potential. Due to space limitations, only the major topics are included here.

The discovery of the novel, independent Langerin$^+$ population of DC in the dermis and other organs may have important implications for the understanding of the origin of the deviant LCH cells. The prevalent tissue distribution of LCH lesions would be more readily explained by an aberrant bone marrow-derived precursor cell for LCH, such as has been shown for the Langerin$^+$ tissue DC, than the self-maintained epithelium-associated Langerhans cell. Importantly, the independent lineage origin of Langerin$^+$ tissue DC has so far only been demonstrated in mice, but the identification of similar populations in human gut and lung suggests its existence in humans as well (Chikwava and Jaffe, 2004). The few distinctive features of the lineage in the mouse, such as expression of CD103 and absence of EpCAM, might be a lead to their further identification in human and their putative relationship to LCH cells (Merad et al., 2008).

Molecular expression profiling technology, which has been widely introduced in multiple systems, is also now being applied to LCH cells and lesional T cells. While various findings in this study confirmed previous notions, other observations were more difficult to interpret as they do not match with data, which have been obtained from several pathologic studies, demonstrating that the LCH cells are immature, activated, pro-inflammatory cytokine-producing cells. Since the current molecular comparison was made to freshly isolated (foreskin) epidermal LC, it might be important to compare the profiles with those from other sources of normal LC and Langerin$^+$ tissue DC as well. Several markers found to be up- or down-regulated deserve follow-up as literature data suggest important links with immune or cancer pathogenesis. In this respect, it is interesting to note that vanin-1, which is highly up-regulated in LCH cells, has been identified recently as a pro-inflammatory factor, controlling granuloma formation in mouse models (Meghari et al., 2007). Furthermore, expression of vanin-1 is up-regulated, at least in epithelial cells, by IL-17 and Th1 cytokines but not by Th2 cytokines (Jansen et al., 2009).

LCH cell clonality has long been interpreted as an indication that LCH is a neoplastic disorder. The reappraisal of this interpretation, with the finding that normal LC may sometimes be clonal in patches of normal skin, raises the question concerning the etiology of the lesional LCH cell clonality. The search for genetic mutations specific for the LCH cell therefore continues to be an important avenue of research to help solve this question. It remains to be determined what the clonal relationship of normal tissue Langerin$^+$ DC is, if these turn out to be the normal equivalents of LCH cells.

The recently observed association between active LCH and elevated circulating IL-17 levels provides an interesting link with the IL-17-driven inflammation observed in infected NADPH-oxidase-deficient mice (Romani et al., 2008). A question arising from these data is whether physiologic changes leading to IDO absence or dysfunction, such as defective ROS production in LCH cells, might underlie chronic local inflammatory changes. The notion that LCH cells themselves, and not Th17 cells, might be the major IL-17 producers (Coury et al., 2008) distinguishes LCH from the mouse model work. Nevertheless, a putative role of IDO as a suppressive mechanism especially expressed by immune-regulatory pDC should be considered in LCH. Moreover, it has not been reported whether pDC contribute to LCH lesions, nor has lesional IDO expression and function been studied. These are new routes that are readily approachable. Other immune-regulatory myelomonocytic cells also have not been evaluated for a potential role in initiating or maintaining LCH lesions. Macrophages are known to be present, but whether a possible activation and polarization of these cells by environmental signals might lead to their immune suppressive function remains to be shown.

Insight into LCH pathogenesis is crucial for the development of a rational therapy for the disease. In this respect, the unresolved, central question about LCH as neoplastic or reactive, inflammatory disease remains a hurdle that still needs to be taken. Is it necessary to break enhanced immune tolerance mechanisms in order to allow induction of anti-tumoral immune responses, for instance via vaccination? Or are normal immune suppressive mechanisms hampered in LCH, leading to chronic inflammatory reactions? The results presented by the different speakers suggested various therapeutic approaches based upon either scenario. This offers an optimistic outlook for potential therapeutic progress once the pathogenic puzzle of LCH is solved.

*References*

Bechan GI, Egeler RM, Arceci RJ. 2006. Biology of Langerhans cells and Langerhans cell histiocytosis. *Int Rev Cytol 254:1-43.*
Beverley PC, Egeler RM, Arceci RJ, Pritchard J. 2005. The Nikolas Symposia and histiocytosis. *Nat Rev Cancer 5:488-94.*

Bronte V, Kasic T, Gri G, Gallana K, Borsellino G, Marigo I, Battistini L, Iafrate M, Prayer-Galetti T, Pagano F, Viola A. 2005. Boosting antitumor responses of T lymphocytes infiltrating human prostate cancers. *J Exp Med 201:1257-68*.

Bursch LS, Wang L, Igyarto B, Kissenpfennig A, Malissen B, Kaplan DH, Hogquist KA. 2007. Identification of a novel population of Langerin+ dendritic cells. *J Exp Med 204:3147-56*.

Cheng P, Corzo CA, Luetteke N, Yu B, Nagaraj S, Bui MM, Ortiz M, Nacken W, Sorg C, Vogl T, Roth J, Gabrilovich DI. 2008. Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. *J Exp Med 205:2235-49*.

Chikwava K, Jaffe R. 2004. Langerin (CD207) staining in normal pediatric tissues, reactive lymph nodes, and childhood histiocytic disorders. *Pediatr Dev Pathol 7:607-14*.

Chikwava KR, Hunt JL, Mantha GS, Murphy JE, Jaffe R. 2007. Analysis of loss of heterozygosity in single-system and multisystem Langerhans' cell histiocytosis. *Pediatr Dev Pathol 10:18-24*.

Christie LJ, Evans AT, Bray SE, Smith ME, Kernohan NM, Levison DA, Goodlad JR. 2006. Lesions resembling Langerhans cell histiocytosis in association with other lymphoproliferative disorders: a reactive or neoplastic phenomenon? *Hum Pathol 37:32-9*.

Condeelis J, Pollard JW. 2006. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell 124:263-6*.

Coury F, Annels N, Rivollier A, Olsson S, Santoro A, Speziani C, Azocar O, Flacher M, Djebali S, Tebib J, Brytting M, Egeler RM, Rabourdin-Combe C, Henter JI, Arico M, Delprat C. 2008. Langerhans cell histiocytosis reveals a new IL-17A-dependent pathway of dendritic cell fusion. *Nat Med 14:81-7*.

da Costa CE, Szuhai K, van Eijk R, Hoogeboom M, Sciot R, Mertens F, Bjorgvinsdottir H, Debiec-Rychter M, de Krijger RR, Hogendoorn PC, Egeler RM, Annels NE. 2009. No genomic aberrations in Langerhans cell histiocytosis as assessed by diverse molecular technologies. *Genes Chromosomes Cancer 48:239-49*.

De Filippi P, Badulli C, Cuccia M, De Silvestri A, Dametto E, Pasi A, Garaventa A, del Prever AB, Todesco A, Trizzino A, Danesino C, Martinetti M, Arico M. 2006. Specific polymorphisms of cytokine genes are associated with different risks to develop single-system or multi-system childhood Langerhans cell histiocytosis. *Br J Haematol 132:784-7*.

Dolcetti L, Marigo I, Mantelli B, Peranzoni E, Zanovello P, Bronte V. 2008. Myeloid-derived suppressor cell role in tumor-related inflammation. *Cancer Lett 267:216-25*.

Doussiere J, Bouzidi F, Vignais PV. 2001. A phenylarsine oxide-binding protein of neutrophil cytosol, which belongs to the S100 family, potentiates NADPH oxidase activation. *Biochem Biophys Res Commun 285:1317-20*.

Fallarino F, Grohmann U, You S, McGrath BC, Cavener DR, Vacca C, Orabona C, Bianchi R, Belladonna ML, Volpi C, Santamaria P, Fioretti MC, Puccetti P. 2006. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. *J Immunol 176:6752-61*.

Favara BE, Feller AC, Pauli M, Jaffe ES, Weiss LM, Arico M, Bucsky P, Egeler RM, Elinder G, Gadner H, Gresik M, Henter JI, Imashuku S, Janka-Schaub G, Jaffe R, Ladisch S, Nezelof C, Pritchard J. 1997. Contemporary classification of histiocytic disorders. The WHO Committee On Histiocytic/Reticulum Cell Proliferations. Reclassification Working Group of the Histiocyte Society. *Med Pediatr Oncol 29:157-66*.

Favara BE, Jaffe R, Egeler RM. 2002. Macrophage activation and hemophagocytic syndrome in langerhans cell histiocytosis: report of 30 cases. *Pediatr Dev Pathol 5:130-40*.

Gabrilovich D. 2004. Mechanisms and functional significance of tumour-induced dendritic-cell defects. *Nat Rev Immunol 4:941-52*.

Gabrilovich DI, Nagaraj S. 2009. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol 9:162-74*.

Gallina G, Dolcetti L, Serafini P, Santo CD, Marigo I, Colombo MP, Basso G, Brombacher F, Borrello I, Zanovello P, Bicciato S, Bronte V. 2006. Tumors induce a subset of inflammatory monocytes with immunosuppressive activity on CD8 T cells. *J Clin Invest 116:2777-90*.

Geissmann F, Lepelletier Y, Fraitag S, Valladeau J, Bodemer C, Debre M, Leborgne M, Saeland S, Brousse N. 2001. Differentiation of Langerhans cells in Langerhans cell histiocytosis. *Blood 97:1241-8*.

Ghassabeh GH, De Baetselier P, Brys L, Noel W, Van Ginderachter JA, Meerschaut S, Beschin A, Brombacher F, Raes G. 2006. Identification of a common gene signature for type II cytokine-associated myeloid cells elicited in vivo in different pathologic conditions. *Blood 108:575-83*.

Ginhoux F, Collin MP, Bogunovic M, Abel M, Leboeuf M, Helft J, Ochando J, Kissenpfennig A, Malissen B, Grisotto M, Snoeck H, Randolph G, Merad M. 2007. Blood-derived dermal langerin+ dendritic cells survey the skin in the steady state. *J Exp Med 204:3133-46*.

Grohmann U, Volpi C, Fallarino F, Bozza S, Bianchi R, Vacca C, Orabona C, Belladonna ML, Ayroldi E, Nocentini G, Boon L, Bistoni F, Fioretti MC, Romani L, Riccardi C, Puccetti P. 2007. Reverse signaling through GITR ligand enables dexamethasone to activate IDO in allergy. *Nat Med 13:579-86*.

Haniffa M, Ginhoux F, Wang XN, Bigley V, Abel M, Dimmick I, Bullock S, Grisotto M, Booth T, Taub P, Hilkens C, Merad M, Collin M. 2009. Differential rates of replacement of human dermal dendritic cells and macrophages during hematopoietic stem cell transplantation. *J Exp Med 206:371-85*.

Himoudi N, Nabarro S, Yan M, Gilmour K, Thrasher AJ, Anderson J. 2007. Development of anti-PAX3 immune responses; a target for cancer immunotherapy. *Cancer Immunol Immunother 56:1381-95*.

Himoudi N, Nabarro S, Buddle J, Eddaoudi A, Thrasher AJ, Anderson J. 2008a. Bone marrow-derived IFN-producing killer dendritic cells account for the tumoricidal activity of unpulsed dendritic cells. *J Immunol 181:6654-63*.

Himoudi N, Yan M, Papanastasiou A, Anderson J. 2008b. MYCN as a target for cancer immunotherapy. *Cancer Immunol Immunother 57:693-700*.

Hoeger PH, Diaz C, Malone M, Pritchard J, Harper JI. 2001. Juvenile xanthogranuloma as a sequel to Langerhans cell histiocytosis: a report of three cases. *Clin Exp Dermatol 26:391-4*.

Jansen PA, Kamsteeg M, Rodijk-Olthuis D, van Vlijmen-Willems IM, de Jongh GJ, Bergers M, Tjabringa GS, Zeeuwen PL, Schalkwijk J. 2009. Expression of the Vanin Gene Family in Normal and Inflamed Human Skin: Induction by Proinflammatory Cytokines. *J Invest Dermatol in press*.

Kissenpfennig A, Henri S, Dubois B, Laplace-Builhe C, Perrin P, Romani N, Tripp CH, Douillard P, Leserman L, Kaiserlian D, Saeland S, Davoust J, Malissen B. 2005. Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize

lymph node areas distinct from slower migrating Langerhans cells. *Immunity 22:643-54.*
Laman JD, Leenen PJ, Annels NE, Hogendoorn PC, Egeler RM. 2003. Langerhans-cell histiocytosis 'insight into DC biology'. *Trends Immunol 24:190-6.*
Lin EY, Li JF, Bricard G, Wang W, Deng Y, Sellers R, Porcelli SA, Pollard JW. 2007. VEGF restores delayed tumor progression in tumors depleted of macrophages. *Mol Oncol 1:288-302.*
Manitz MP, Horst B, Seeliger S, Strey A, Skryabin BV, Gunzer M, Frings W, Schonlau F, Roth J, Sorg C, Nacken W. 2003. Loss of S100A9 (MRP14) results in reduced interleukin-8-induced CD11b surface expression, a polarized microfilament system, and diminished responsiveness to chemoattractants in vitro. *Mol Cell Biol 23:1034-43.*
Meghari S, Berruyer C, Lepidi H, Galland F, Naquet P, Mege JL. 2007. Vanin-1 controls granuloma formation and macrophage polarization in Coxiella burnetii infection. *Eur J Immunol 37:24-32.*
Mellor AL, Munn DH. 2008. Creating immune privilege: active local suppression that benefits friends, but protects foes. *Nat Rev Immunol 8:74-80.*
Merad M, Manz MG, Karsunky H, Wagers A, Peters W, Charo I, Weissman IL, Cyster JG, Engleman EG. 2002. Langerhans cells renew in the skin throughout life under steady-state conditions. *Nat Immunol 3:1135-41.*
Merad M, Ginhoux F, Collin M. 2008. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. *Nat Rev Immunol 8:935-47.*
Movahedi K, Guilliams M, Van den Bossche J, Van den Bergh R, Gysemans C, Beschin A, De Baetselier P, Van Ginderachter JA. 2008. Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. *Blood 111:4233-44.*
Muller AJ, Sharma MD, Chandler PR, Duhadaway JB, Everhart ME, Johnson BA, 3rd, Kahler DJ, Pihkala J, Soler AP, Munn DH, Prendergast GC, Mellor AL. 2008. Chronic inflammation that facilitates tumor progression creates local immune suppression by inducing indoleamine 2,3 dioxygenase. *Proc Natl Acad Sci U S A 105:17073-8.*
Murakami I, Gogusev J, Fournet JC, Glorion C, Jaubert F. 2002. Detection of molecular cytogenetic aberrations in langerhans cell histiocytosis of bone. *Hum Pathol 33:555-60.*
Nabarro S, Himoudi N, Papanastasiou A, Gilmour K, Gibson S, Sebire N, Thrasher A, Blundell MP, Hubank M, Canderan G, Anderson J. 2005. Coordinated oncogenic transformation and inhibition of host immune responses by the PAX3-FKHR fusion oncoprotein. *J Exp Med 202:1399-410.*
Nagao K, Ginhoux F, Leitner WW, Motegi S, Bennett CL, Clausen BE, Merad M, Udey MC. 2009. Murine epidermal Langerhans cells and langerin-expressing dermal dendritic cells are unrelated and exhibit distinct functions. *Proc Natl Acad Sci U S A 106:3312-7.*
Pollard JW. 2008. Macrophages define the invasive microenvironment in breast cancer. *J Leukoc Biol 84:623-30.*
Pollard JW. 2009. Trophic macrophages in development and disease. *Nat Rev Immunol 9:259-70.*
Poulin LF, Henri S, de Bovis B, Devilard E, Kissenpfennig A, Malissen B. 2007. The dermis contains langerin+ dendritic cells that develop and function independently of epidermal Langerhans cells. *J Exp Med 204:3119-31.*
Puccetti P, Grohmann U. 2007. IDO and regulatory T cells: a role for reverse signalling and non-canonical NF-kappaB activation. *Nat Rev Immunol 7:817-23.*
Raes G, Beschin A, Ghassabeh GH, De Baetselier P. 2007. Alternatively activated macrophages in protozoan infections. *Curr Opin Immunol 19:454-9.*
Rolland A, Guyon L, Gill M, Cai YH, Banchereau J, McClain K, Palucka AK. 2005. Increased blood myeloid dendritic cells and dendritic cell-poietins in Langerhans cell histiocytosis. *J Immunol 174:3067-71.*
Romani L, Fallarino F, De Luca A, Montagnoli C, D'Angelo C, Zelante T, Vacca C, Bistoni F, Fioretti MC, Grohmann U, Segal BH, Puccetti P. 2008. Defective tryptophan catabolism underlies inflammation in mouse chronic granulomatous disease. *Nature 451:211-5.*
Schouten B, Egeler RM, Leenen PJ, Taminiau AH, Van Den Broek LJ, Hogendoorn PC. 2002. Expression of cell cycle-related gene products in langerhans cell histiocytosis. *J Pediatr Hematol Oncol 24:727-32.*
Senechal B, Elain G, Jeziorski E, Grondin V, Patey-Mariaud de Serre N, Jaubert F, Beldjord K, Lellouch A, Glorion C, Zerah M, Mary P, Barkaoui M, Emile JF, Boccon-Gibod L, Josset P, Debre M, Fischer A, Donadieu J, Geissmann F. 2007. Expansion of regulatory T cells in patients with langerhans cell histiocytosis. *PLoS Med 4:e253.*
Sharma MD, Baban B, Chandler P, Hou DY, Singh N, Yagita H, Azuma M, Blazar BR, Mellor AL, Munn DH. 2007. Plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate mature Tregs via indoleamine 2,3-dioxygenase. *J Clin Invest 117:2570-82.*
Sica A, Bronte V. 2007. Altered macrophage differentiation and immune dysfunction in tumor development. *J Clin Invest 117:1155-66.*
Steiner QG, Otten LA, Hicks MJ, Kaya G, Grosjean F, Saeuberli E, Lavanchy C, Beermann F, McClain KL, Acha-Orbea H. 2008. In vivo transformation of mouse conventional CD8alpha+ dendritic cells leads to progressive multisystem histiocytosis. *Blood 111:2073-82.*
Stoitzner P, Green LK, Jung JY, Price KM, Atarea H, Kivell B, Ronchese F. 2008a. Inefficient presentation of tumor-derived antigen by tumor-infiltrating dendritic cells. *Cancer Immunol Immunother 57:1665-73.*
Stoitzner P, Green LK, Jung JY, Price KM, Tripp CH, Malissen B, Kissenpfennig A, Hermans IF, Ronchese F. 2008b. Tumor immunotherapy by epicutaneous immunization requires langerhans cells. *J Immunol 180:1991-8.*
Sung SS, Fu SM, Rose CE, Jr., Gaskin F, Ju ST, Beaty SR. 2006. A Major Lung CD103 ({alpha}E)-beta7 Integrin-Positive Epithelial Dendritic Cell Population Expressing Langerin and Tight Junction Proteins. *J Immunol 176:2161-72.*
Van Ginderachter JA, Meerschaut S, Liu Y, Brys L, De Groeve K, Hassanzadeh Ghassabeh G, Raes G, De Baetselier P. 2006. Peroxisome proliferator-activated receptor {gamma} (PPAR{gamma}) ligands reverse CTL suppression by alternatively activated (M2) macrophages in cancer. *Blood 108:525-35.*
Willman CL, Busque L, Griffith BB, Favara BE, McClain KL, Duncan MH, Gilliland DG. 1994. Langerhans'-cell histiocytosis (histiocytosis X)--a clonal proliferative disease. *N Engl J Med 331:154-60.*
Yan M, Himoudi N, Pule M, Sebire N, Poon E, Blair A, Williams O, Anderson J. 2008. Development of cellular immune responses against PAX5, a novel target for cancer immunotherapy. *Cancer Res 68:8058-65.*
Yousem SA, Colby TV, Chen YY, Chen WG, Weiss LM. 2001. Pulmonary Langerhans' cell histiocytosis: molecular analysis of clonality. *Am J Surg Pathol 25:630-6.*

Yu RC, Chu C, Buluwela L, Chu AC. 1994. Clonal proliferation of Langerhans cells in Langerhans cell histiocytosis. *Lancet 343:767-8*.

Dr. Pieter J. M. Leenen
Dept. Immunology
Erasmus MC
Rotterdam
The Netherlands