7th International Workshop on Langerhans Cells

Stresa, Italy

September 7–9, 2001

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001

Langerin and the DC Asialoglycoprotein-Receptor: Two Closely Related Endocytic Type-II Lectins with Divergent Functions in Dendritic Cells

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Immature dendritic cells (DC) are highly specialized in antigen capture, notably via a number of endocytic receptors. These include type-I lectins, such as the mannose-receptor and DEC205, with multiple Ca^{++}-dependent (C-type) carbohydrate recognition domains (CRD). The important role of type-II lectins with a single CRD in DC endocytosis has been more recently realized. Langerin is a mannose-binding type-II lectin that we cloned from DC supplemented with TGFb. Langerin is expressed on Langerhans cells of the epidermis and epithelia. Strikingly, Langerin routes extracellular ligand into Birbeck granules (BG), the unique organelles of Langerhans-type cells. This process is paralelled by an active role of Langerin in the formation of BG by membrane superimposition and zippering when expressed in cell lines. Of note, the intracellular portion of Langerin contains a proline-rich (WPREPPP) domain but lacks the tyrosine-based internalization motif used by a number of other endocytic receptors. Mouse Langerin shares key features of its human counterpart, including a similar genomic organization. Langerin is most closely related to the rodent Kuppfer cell receptors, constituting a subfamily of C-type lectins characterized by an a-helical coil-coiled stalk between the transmembrane domain and the CRD. Studies with deleted and mutant forms of Langerin have shown that the CRD is essential for BG formation. Also, mouse Langerin in which a single conserved residue was modified within the CRD did not give rise to BG but to a different type of superimposed membranes similar to the cored tubules described in mouse Langerhans cells. We have also identified several isoforms of an asialoglycoprotein receptor (DC-ASGPR) on human DC. These represent splice-variants of the macrophage lectin HML, a galactose-specific type-II Ca^{++}-dependent lectin highly related to Langerin. However, in contrast to Langerin, the DC-ASGPR/HML is expressed by interstitial DC but not by Langerhans cells. The DC-ASGPR, which features an intracellular tyrosine-motif, internalizes extracellular ligand into DC with similar rapid kinetics as Langerin. However, the DC-ASGPR localizes to DC early endosomes indicative of a recycling receptor, and its expression in cell lines does not result in BG formation. Also, whereas Langerin does not intersect with the routing of MHC-II molecules, antigen targeted to the DC-ASGPR reaches the MHC-II pathway resulting in highly efficient presentation to T cells. Our data demonstrate that structurally closely-related endocytic receptors can have highly divergent functions in DC. Furthermore, as illustrated by Langerin, we speculate that a given receptor could have considerable plasticity in the routing of extracellular ligands, by generating distinct subcellular compartments upon endocytosis. Recent availability of mice with disrupted Langerin genes should be important to further understand the functional consequences of the diversity of type-II lectin endocytic receptors in DC.

002

Inflammatory Chemokine Transport and Presentation in HEV: A Remote Control Mechanism for Monocyte Recruitment to Lymph Nodes in Inflamed Tissues

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Interstitial fluid is constantly drained into lymph nodes (LN) via afferent lymph vessels. This conduit enables monocyte-derived macrophages and dendritic cells to access LN from peripheral tissues. We show that during inflammation in the skin, a second recruitment pathway is evoked that recruits large numbers of blood-borne monocytes to LN via high endothelial venules (HEV). Inhibition of monocyte chemoattractant protein-1 (MCP-1) blocked this inflammation-induced monocyte homing to LN. MCP-1 mRNA in inflamed skin was over 100-fold up-regulated and paralleled MCP-1 protein levels, whereas in draining LN MCP-1 mRNA induction was much weaker and occurred only after a pronounced rise in MCP-1 protein. Thus, MCP-1 in draining LN was primarily derived from inflamed skin. In MCP-1-/– mice, intracutaneously injected MCP-1 accumulated rapidly in the draining LN where it enhanced monocyte recruitment. Intravital microscopy showed that skin-derived MCP-1 was transported via the lymph to the luminal surface of HEV where it triggered integrin dependent arrest of rolling monocytes. These findings demonstrate that inflamed peripheral tissues project their local chemokine profile to HEV in draining LN and thereby exert ''remote control'' over the composition of leukocyte populations that home to these organs from the blood.

003

CCR7 Controls Defined Stages of Dendritic Cell Mobilization and Localization

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We recently identified the chemokine receptor CCR7 as an important organizer of the immune response. Due to the impaired migration of lymphocytes, CCR7-deficient mice reveal profound morphological and functional alterations in all secondary lymphoid organs. We now analyzed the role of CCR7 during the process of activation and mobilization of dendritic cells (DCs). We show that CCR7 is required at least at three different steps of the migratory process of dc. I) Langerhans cells need this receptor to migrate within the dermis towards the lymphatic vessels. II) Once these cells have been arrived within the marginal sinus of the draining lymph nodes they exploit CCR7 to make their way into the T cell-rich areas. III) Finally, both lymphoid- and myeloid-derived DCs require the interaction of CCR7 with its ligands to stay within their characteristic microenvironments within lymphoid organs. Within lymphoid organs CCR7-deficient DCs are dislocated to distinct areas where other homeostatic chemokines are highly expressed. Thus, our data also suggest that a hierarchy of chemotactic gradients within lymphoid organs controls the dynamic reorganization of lymphoid organs during an adoptive immune response.

004

Basic Research in Langerhans Cell Histiocytosis

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Until recently discoveries in the basic biology of Langerhans cell histiocytosis (LCH) have progressed at a slow pace as compared to the rapid advances in dendritic cell (DC) biology. My talk will summarize the work of several investigators in bringing new understanding to the pathobiology of LCH. The application of reagents used in DC research has provided better characterization of how the diseased Langerhans cells (LC) fit into the scheme of DC lineage and maturation. A major question has been whether the LC of LCH are mature or immature DC. Indeed the LC from LCH patient lesions are immature having no expression of CD83, CD86, or DC-Lamp and only intracellular expression of Class II antigens. These LC may be influenced by surrounding macrophages which that IL-10 and TGF-b could inhibit LC maturation. When LC from LCH lesions are put in culture they can be matured by cultivation with CD40. These important findings will be discussed in the context of previous understanding about the clonal proliferation of the LC in LCH that has perplexed investigators since no morphologic or cytogenetic markers of malignancy occur in this disease. Furthermore the etiology of elevated cytokine expression in LCH has not been elucidated although increased CD40L on surrounding T cells and CD40 on the LC in LCH has been identified along with TGF-b expression in macrophages. The multiple locations of LCH in patients suggests there are aberrations of the integrins or other cell surface proteins guiding LC migration. Low expression of E-cadherin has been associated with disseminated disease. Presence of CD2, CD11a and CD11b on LC has been hypothesized to show that they may contribute to migration of the LC. The complex interactions of lymphocytes and macrophages surrounding LC need to be dissected to determine the pivotal

005

Dendritic Cells and Peripheral Tolerance

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Dendritic cells (DCs) are best known for their immunogenic capacities. DCs have several known roles in the innate response and provide a direct link between innate and adaptive immunity. After initiating adaptive responses by CD4 and CD8 T cells, DCs also control T cell differentiation, such as the rapid polarization to Th1 type CD4 T cells and high affinity CD8 T cells. Much of the experimental work in the past has involved *ex vivo* derived DCs matured with different microbial and inflammatory stimuli. Together with the laboratories of Michel Nussenzweig and Kayo Inaba, we now find that a major function of DCs *in vivo*, in the unperturbed or steady state, is to induce peripheral T cell tolerance. DCs have been proposed to have tolerogenic capacities in the past, but we set out to assess this with DCs *in vivo*. We will first review evidence that DCs are continually capturing cells and soluble proteins in the steady state *in vivo*, and yet no autoimmunity or chronic inflammation is induced. When cellular and protein antigens are targeted selectively to DCs *in vivo*, the corresponding antigen-specific T cells undergo deletion, and the animals become tolerant or specifically unresponsive to antigenic challenge, even on mature immunogenic DCs. In contrast, if the DCs are simultaneously exposed to antigen and a maturation stimulus, immunity results, with an expansion of T cell numbers and a differentiation to effector T cells. We propose that the tolerogenic role of DCs is a vital part of their immunogenic function. When DCs are called upon to immunize an animal, they must capture antigen and respond to a maturation stimulus. Simultaneously however, e.g. during a microbial infection, the DCs must be capturing self antigens from dying infected cells as well as normal environmental antigens, such as proteins in the airway and intestine. If on the other hand in the steady state, i.e. prior to infection, DCs capture and induce tolerance to the peptides that can be extracted from cells dying during normal cell turnover or from nonpathogenic environmental proteins, then a subsequent exposure to an infection will lead preferentially to anti-microbial immunity and not autoimmunity or chronic inflammation. Therefore, the tolerizing role of DCs is not an occasional event. Rather it is a critical day-to-day role of DCs during their traffic through peripheral tissues and lymphoid organs in the steady state. The tolerizing and immunizing functions of DCs therefore seem critical for understanding the perplexing but essential capacity of the immune system to distinguish self from, as Janeway has stated it, ''infectious nonself''.

C1

$CD14^+$ $CD1a^-$ Dermal Migratory Cells Acquire the Characteristics of Langerhans After Short-Term Culture in GM-CSF, TGFβ-1 and IL-4

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It has been postulated that Langerhans cells (LC) precursors might be a skin-resident cell population; however, no data exist confirming this hypothesis. In the present work, we have characterized a population of human dermal-resident $CD14^+$ $CD1a^-$ cells that migrates "spontaneously" from human skin explants. After short-term culture in GM-CSF + TGFb-1 + IL-4 these cells acquire morphology, phenotype and function characteristic of epidermal LC. $CD14^+$ $CD1a^-$ cells did not adhere to plastic, expressed langerin, the myeloid markers CD13 and CD33, MHC-I and –II molecules, moderate levels of the costimulatory molecules CD80, CD86, and CD40 but they were negative for the maturation molecule CD83. $CD14^+$ $CD1a^-$ cells were positive for the intracytoplasmic markers protein S-100 and CD68, they showed a very weak expression of factor XIIIa and they lacked Birbeck granules (BG). In addition, $CD14^+$ $CD1a^-$ expressed CCR6 and migrated in a dose dependent manner in response to MIP-3a in chemotaxis assays. $CD14^+$ $CD1a^-$ cells were highly endocytic, and exhibited a weak stimulatory capacity for allogenic naive T cells. After short-term culture in GM-CSF + TGFb-1 + IL-4, $CD14^+$ $CD1a^-$ cells: i) expressed *de novo* CD1a and E-cadherin, ii) decreased langerin and CCR6 expression, iii) lost CD14 positivity, iv) acquired BG, and v) became a strong stimulators of allogeneic T cells. Our results show that skin migratory $CD14^+$ $CD1a^+$ cells might constitute a reserve pool of LC precursors resident in the dermis that are able to differentiate into epidermal LC in the appropriate cytokine environment.

C2

Neonatal Murine Dendritic Epidermal Cells Do Not Acquire the Phenotypic and Functional Characteristics of Adult Langerhans Cells Upon *In Vitro* Culture

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In epidermal sheets of adult mice, Langerhans cells (LC) exhibit a pronounced anti-MHC class II reactivity. Dendritic leukocytes of neonatal epidermis (NDL), in contrast, express only modest amounts of these proteins. To determine the differentiation and maturation potential of these cells, epidermal cell suspensions from newborn ($\leq$10 h old) and adult whole body skin were prepared and highly purified ($\geq$95%) for $CD45^+$/MHC class II^+ cells. Subsequently, the purified populations were cultured for three days in RPMI medium supplemented with FCS and GM-CSF. During culture, adult LC became highly dendritic and formed numerous homotypic clusters, while NDL remained round to polygonal and created only few small-sized clusters. After culture, both cell types were $CD3^-$ and exhibited similar intensity in surface expression of CD45, F4/80, and CD11b. NDL, however, expressed slightly more MHC class I, less CD40, considerably less MHC class II, CD54, CD80 and CD86, and were $CD25^-$/$CD205^-$. Functionally, cultured NDL were significantly weaker stimulators of naive, allogeneic $CD4^+$ and $CD8^+$ T cells than adult LC. Furthermore, freshly isolated NDL were twice as efficient as adult LC in the uptake of FITC-conjugated ovalbumin, but were not able to present ovalbumin to antigen-specific T cell hybridomas. In summary, we have shown that $CD45^+$/$CD3^-$ NDL do not acquire the morphology and phenotype typical of *in vitro* cultured adult LC, and are greatly impaired in their capacity to activate antigen-specific T cell proliferative responses. The finding that certain dendritic cells of neonatal mice do apparently not possess the molecular machinery needed for the initiation of productive T cell responses should facilitate the search for molecules involved and engaged in T cell activation.

C3

Anatomical Location and T Cell Stimulatory Functions of Mouse Dendritic Cell Subsets Defined by CD4 and CD8 Expression

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Mouse spleen contains three subsets of $CD4^+$, $CD8a^+$ and $CD4^-$/$CD8a^-$ dendritic cells (DC) in a 2 : 1 : 1 ratio. An analysis of over 60 surface and cytoplasmic antigens revealed several differences in adhesion molecule and costimulator expression between the three subsets. Immunohistochemical analysis revealed that both CD4 and CD8-expressing DC were present in the T cell areas, but large numbers of both subsets were also apparent in the splenic marginal zones. All three DC subsets showed similar migratory capacity in collagen lattices and were similar in their ability to stimulate unfractionated, allogeneic $CD4^+$ T cells. However, $CD8a^+$ DC were very weak stimulators of highly purified allogeneic resting or CTL $CD8^+$ T cells, even at high stimulator to responder ratios. Neither LPS nor CD40 treatment significantly improved the stimulatory capacity of the $CD8^+$ DC subset. In contrast $CD8a^+$ DC did efficiently stimulate NP68 peptide–specific $CD8^+$ F5 transgenic T cells, but this required higher DC: T cell ratios and peptide concentrations. The turnover rate of class I/peptide complexes $CD8a^+$ DC was similar to the $CD8a^-$ subsets, suggesting that a rapid loss of antigenic complexes from $CD8a^+$ DC was not responsible for their poor $CD8^+$ T cell stimulation. Mixing experiments showed that $CD8a^+$ DC could not down-regulate the ability of the other DC subsets to stimulate resting $CD8^+$ T cell proliferation. Therefore $CD8a^+$ DC are poor stimulators of resting $CD8^+$ T cells, and do not appear to tolerise, induce apoptosis, or otherwise interfere with the proliferative ability of $CD8^+$ T cells.

C4

The Migration of Langerhans Cells is Closely Associated with the Expression of the Actin Bundling Protein Fascin

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We have demonstrated that the DC actin bundling protein fascin is involved in the development of dendrites and plays a critical role in activating T cells. In this study we evaluated the regulation of fascin expression in migrating LC *in vivo*.

Normal skin and lymph node were obtained from mice and human and double stained for fascin and MHC Class II. Fascin expression was quantitated using Matlab software. Isolated human epidermal cell suspensions were further evaluated for fascin and MHC Class II staining by flow cytometry. Human lymph cells were examined for fascin and MHC Class II expression. Suprabasal LC in the epidermis did not stain for fascin. However, basal LC were fascin positive and had a more rounded appearance. Strong staining for fascin of a dermal DC population was seen. The fascin positive DC were frequently clustered around lymphatics suggesting that these are migrating DC. Human lymphatic DC also expressed fascin. Numerous DCs were noted in the marginal sinuses and paracortical zones of the lymph nodes. Fascin expression quantitatively increased from the dermis into the T cell areas of the lymph node. Fascin expression was confined to migrating LC. Similar results were seen in mouse and human indicating an important conserved function. This distribution pattern of fascin expression suggests an important role for its expression in the migration of LC. An increase in fascin expression in lymph node DC suggests a second function of fascin, possibly in the interaction of DC and T cells.

C5

Selective and Transient Accumulation of Polyubiquinated Proteins During Dendritic Cell Maturation

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Dendritic cells (DCs) are antigen presenting cells (APCs) with the unique capacity to initiate primary and secondary immune responses. DCs acquire antigens in peripheral tissues and migrate to lymphoid organs where they present processed peptides to T-cells. DCs have a remarkable pattern of differentiation (maturation) that is accompanied by striking changes in morphology, organization and function. In particular, DCs exhibit unique mechanisms to control antigen presentation by regulating the transport and the loading of MHC class I and II molecules. MHC class I molecules function to present peptides eight to 10 residues long to CD8 + cytotoxic T cells. These peptides are mostly derived from cytosolic proteins which in turn have to be ubiquinated and degraded by the proteasome, the major cytosolic protease complex. Here we show, that upon inflammatory stimulation, Langerhans cells accumulate ubiquitinated proteins in large cytosolic structures reminiscent of aggresomes or inclusion bodies observed in many amyloid or neurogenerative diseases. Interestingly, these Dendritic cell Aggresome Like Induced Structures (DALIS) are transient and require continuous protein synthesis for their existence. Our observations suggest the existence of an organized prioritization of protein degradation in maturing DCs, likely to be important for maturation and regulating MHC class I presentation during this necessary functional switch.

C6

Crosspresentation of Antigens on MHC Class I Molecules by Dendritic Cells after Endocytosis via DEC-205 *In Vivo*

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Dendritic cells (DCs), including Langerhans cells, express a distinct receptor for adsorptive endocytosis, DEC-205. The receptor contains 10 contiguous C-type lectin domains (it is a DECalectin), but its ligands are not yet known. Therefore, we are using anti-DEC-205 antibodies as surrogate ligands for this receptor. Previously, we found that DEC-205 greatly enhances the efficiency of ligand presentation to $CD4^+$ T cells in culture. We now are asking if DEC-205 mediated uptake can be exploited for the targeting of antigens to DCs in mice. In fact, anti DEC-205 antibodies efficiently target to DCs in the T cell areas of lymph node. To begin to establish that this targeting gives rise to MHC-peptide complexes, we coupled the model antigen ovalbumin (OVA) to anti-DEC-205 and control Abs. After s.c. injection, CD11c + ve lymph node DCs were isolated and tested for presentation to MHC class I restricted (OT-1) and MHC class II restricted (OT-II) T cell receptor transgenic cells. The CD11c + ve DCs induced strong proliferative responses from both CD8 + and CD4 + T cells, with the presentation on MHC class I ("exogenous pathway" or "cross presentation") being TAP dependent. The presentation of OVA following injection of DEC-205 OVA conjugate was at least 100-fold more potent than control antibody OVA conjugates. Also the CD11c + ve DCs were at least 30 fold more potent than the CD11c –ve nonDCs. These results show that the DEC-205 receptor efficiently delivers antigens to DCs *in vivo* for presentation on MHC class I and II products.

C7

A Dendritic Cell-Restricted Mechanism for the Phagocytosis of Apoptotic Cells

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The typical fate for apoptotic cells is rapid engulfment and degradation by phagocytes. Previously, we described a novel pathway in which dendritic cells (DCs) phagocytose apoptotic material via the a_vb_5 integrin receptor and generate MHC I peptide epitopes from the internalized material, thus allowing for the activation of antigen-specific CTLs. Notably, the a_vb_5 integrin is not found on macrophages and may account in part for the unique nature by which DCs handle internalized apoptotic material. Despite the significance of the cellular events associated with phagocytosis of apoptotic cells via this receptor, little is known about the molecular events involved. We now show that apoptotic cells are recognized and internalized via the a_vb_5 integrin receptor, with an absolute requirement for the cytoplasmic tail of the b_5 integrin. Notably, this constitutes the first demonstration that integrin receptors play a direct role in phagosome formation, and do not simply serve to ''stick'' the apoptotic material to the phagocyte as was originally predicted. We have also demonstrated that activation of a_vb_5 results in phosphorylation of $p130^{cas}$ and recruitment of both CrkII and Dock180. Upon phagocytosis, Dock180 activates Rac1 and which is the proposed trigger for cytoskeletal rearrangement. Interestingly, Dock180 expression is restricted to DCs as compared to macrophages, which employ the a_vb_3 integrin receptor and the Dock-M protein (a family member of Dock180). Additionally, we find that Dock180 is markedly down-regulated during DC maturation, consistent with its role in antigen capture. Present studies are focused on identifying additional molecules that distinguish the mechanism by which DCs capture and traffic apoptotic material. By uncovering the molecular events involved in the phagocytosis of apoptotic cells, we hope to better understand the mechanism of antigen trafficking and cross-presentation by DCs.

C8

Heat Shock Proteins 70 and 60 Share Common Receptors which are Expressed on Human Monocyte-Derived But Not Epidermal Dendritic Cells

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Priming of CTLs by means of heat shock proteins (hsps) is dependent on APCs, which present the hsp-associated peptides, via their cell surface MHC class I molecules, to $CD8^+$ T cells. It has not yet been established how human (hu)hsp70 interacts with the major (hu)APCs, the dendritic cells (DCs). Here we show that (hu)hsp70 is specifically internalized into $CD14^-$ Toll-like receptor 4-monocyte-derived (hu)DCs by receptor-mediated endocytosis. We further demonstrate that (hu)hsp70 and (hu)hsp60 share the same putative receptors. Both molecules as well as MHC class I molecules are spontaneously internalized and reach the MHC class II enriched compartments. Finally, freshly isolated Langerhans cells (LCs), as well as $CD34^+$-derived LCs, do not bind hsp60 or hsp70. Given the immunological importance of the internalization of hsp70 by APCs, in the induction of the immune responses, the finding that hsp60 and hsp70 are internalized through the same receptor(s) may explain why microbial hsp60 represents a major T cell antigen. This may rationalize the use of microbial hsp60 to prime immune responses against microbes. Finally, the lack of hsp60/70 receptors on LCs raises the crucial question as to whether absence of priming of the skin and mucosal immune systems by hsp-polypeptide complexes could account for some tissue specific diseases.

C9

Functional Analysis of Dendritic Cells Generated *In Vitro* from Blood Monocytes and $CD34^+$ Progenitors and *In Vivo* with FLT3 Ligand

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The successful use of DC as cellular vaccine adjuvants in immunotherapy of cancer is dependent on clearly understanding DC subsets and their functional diversity. DC can be generated from either monocytes (McDC) or from $CD34^+$ progenitors ($CD34^+$DC) or expanded *in vivo* with Flt3 ligand (FL). We evaluated the ability of these DC subsets to respond to 3 classes of stimuli. (1) pro-inflammatory mediators; (2) T cell-derived signals (CD40L) and (3) pathogen-derived signals. All 3 types of DC responded to pro-inflammatory mediators, CD40L or bacterial signals and DC stimulated with any one class of stimuli were phenotypically indistinguishable. However, these phenotypically similar DC were found to be functionally distinct. A putative dichotomy in DC functions was identified. Immature DC that encounter pro-inflammatory cytokines acquire migratory capacity towards chemokines and secrete low levels of cytokines. Conversely, immature DC that encounter pathogens or CD40L in the absence of pro-inflammatory mediators (and presence of IL-10) are activated into pro-inflammatory, cytokine secreting cells which are poor migratory cells. Finally, CD40L-mediated cytokine secretion requires the presence of both T cell-derived cytokines (e.g. IL-4 and IFN-g) as well as cytokines produced by non-T cells (IL-1b and IFN-a) suggesting dual roles for CD40L function in the lymph nodes and periphery. It appears therefore that not all mature DC nor all DC subsets are destined to migrate to lymphoid organs and that the sequence in which stimuli are encountered significantly affects which functions are expressed. This highlights that immature DC may have multiple developmental options once recruited from the resting precursor pool in the periphery.

C10

Identification of a Clinical Grade Maturation Factor for Dendritic Cells

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Dendritic cells are essential for the generation of primary adaptive immune responses, but their full immuno-stimulatory capacities are reached only upon maturation. Despite a growing interest for the use of antigen-loaded DC in immunotherapy, few effective activating agents compatible with clinical use have been identified so far. In this study, several bacteria-derived adjuvants were compared for their ability to induce phenotypic maturation and cytokine secretion (IL-12, IL-10) by monocyte-derived dendritic cells (Dendritophages™, DF) differentiated with GM-CSF and IL-13 in single-use cell processors (VacCell®). Efficiency in the induction of MelanA-specific T cell responses by mature DF was evaluated using ^{51}Cr-release assay, ELISPOT, and tetramers. Although MonophosphorylLipidA, BCG, and Ribomunyl, all appeared to be able to provide the signal necessary for initiation of DF maturation, Ribomunyl (composed by membrane and ribosomal bacterial fractions) was more efficient than MonophosphorylLipidA and did not imply an active cellular infection as does BCG. Addition of IFN-g at the onset of maturation modulated both quantitatively and qualitatively cytokine secretion, allowing higher levels of IL-12 concomitant with lower levels of IL-10. Moreover, contact of DF with Ribomunyl (± IFN-g) for 6 h was sufficient to trigger a maturation process that would proceed and complete spontaneously. MelanA-specific CTL responses could be efficiently induced *in vitro* by Ribomunyl + IFN-g-treated DF even in the absence of exogenous cytokines. Thus, Ribomunyl in association with IFN-g represents a suitable agent for the *ex vivo* production of mature monocyte-derived DC for use as cellular vaccines. DF matured with Ribomunyl and IFN-g will be tested in a melanoma clinical trial.

C11

A Costimulatory Role of Hyaluronan in Dendritic Cell–T Cell Interaction

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Hyaluronan (HA), a high molecular weight glycosaminoglycan expressed abundantly in extracellular and pericellular matrices, is thought to be involved in a variety of cellular functions including leukocyte migration and activation. Most studies on HA function have employed inhibitors of CD44, a cell surface glycoprotein that binds HA. Because CD44 binds many ligands, these studies failed to examine the function of HA directly. To better define physiological roles of HA, we developed an HA-binding peptide (GAHWQFNALTVR), termed Pep-1, that specifically binds to HA and inhibits leukocyte adhesion to HA (J Exp Med 192:769, 2000). Based on the reported costimulatory role of CD44 in mitogen-induced T cell activation, we investigated the contribution of HA in dendritic cell (DC)–T cell interaction using Pep-1. First, the XS52 DC line was cultured with a KLH-reactive $CD4^+$ Th1 clone (HDK-1) with antigen (KLH). Pep-1 inhibited in a dose-dependent manner the secretion of TNFa (by XS52 and HDK-1 cells), IL-6 (by both cell types) and IFN (by HDK-1 cells). To study the impact of HA on primary T cell activation, we employed a second system. Splenic DC freshly isolated from BALB/c mice pulsed with $OVA_{323-339}$ peptide and cocultured with $CD4^+$ T cells isolated from DO11.10 transgenic mice expressing transgenic TCR a and b chains specific for the OVA peptide. Pep-1, but not a control peptide, inhibited DC-dependent and antigen-specific activation of naive T cells. Based on these results, we propose a previously unrecognized function of HA in antigen presentation.

C12

The Expression of Serotonin Receptors by Murine Langerhans Cells

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Serotonin has been known to show various physiological and pharmacological activities through its specific receptors in the nervous, vascular, and gastrointestinal systems. Moreover, it has been reported to regulate the cytokine production and the expression of surface molecules including MHC class II by the immunocompetent cells such as macrophages and monocytes, suggesting that it also plays an role in the immune system. Seven families of serotonin receptors (5-HT1~5-HT7), some of which include several subtypes, have been identified so far. The expression of serotonin receptors and the effect of serotonin on Langerhans cells (LC) were investigated. Murine LC were enriched from BALB/c epidermal cell suspension by panning method using anti $I\text{-}A^d$ antibody and the high purity (~95%) achieved by this procedure was confirmed by flow cytometry. Reverse transcriptase-polymerase chain reaction revealed the expression of mRNA for 5-HT1A, 5-HT2A, and 5-HT2B by LC. The expression of 5-HT2A was confirmed by Western blot analysis. In order to study the function of the serotonin receptors on LC, the effect of serotonin on the expression of $I\text{-}A^d$, B7-1 and B7-2 was further examined. Although the expression of B7-1 and B7-2 on LC was unchanged, $I\text{-}A^d$ expression was suppressed by 1 mg/ml serotonin, suggesting the LC function was, at least in part, regulated by serotonin through its specific receptors.

C13

Dendritic Cells are Induced to Proliferate *In Vivo* by Interaction with Microparticles of Specific Size

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Microparticles are readily taken up by phagocytic antigen presenting cells (APC) and have been used to target antigen to these cells for vaccine development. A new phenomenon is presented by which stimulation with microparticles *in vivo* induces proliferation of cells expressing dendritic cell (DC) and Langerhans cell markers. Our results suggest that microparticles may provide a danger signal to the immune system manifested by amplification of cellular responses including expansion in the number of potent APC types. The optimal microparticle size and composition capable of stimulating these proliferative responses was determined and the mechanism by which DC were induced to expand was explored by testing this phenomenon in a series of lymphokine deficient knockout mice.

C14

CD1a Positive Dendritic Cells Intracellularly Transport Antigen from the Skin to the Regional Lymph Nodes in the Induction Phase of Allergic Contact Dermatitis

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Dendritic cells are potent stimulators of T cell-mediated immune responses. In contact hypersensitivity reactions in animals dendritic cells have been reported to pick up antigens in the skin and migrate to the regional lymph node. In this study we analysed whether skin-derived dendritic cells transport contact antigens via the afferent lymph in humans. By means of microsurgical technique lymph cells were collected after painting a defined skin region on the leg of 10 volunteers with the sensitizing agent 2,4 dinitrochlorobenzene 2%. Interestingly, in the collected skin lymph specifically draining this painted area there was no significant change in flow, output or composition of cells after antigen painting. Using flow cytometric analysis we were able to detect the antigen in CD1a+ dendritic cells of the afferent lymph 15-25 h after antigen application. However, the antigen could only be detected after permeabilizing the dendritic cells, indicating that the main part of the antigen is transported intracellularly and not on the surface of these cells. Further analysis of cell surface antigens such as CD80, CD86, HLA-DR, CD11a, CD14, CD23, CD25 and CD54 revealed that in the course of cutaneous sensitization the phenotype of the dendritic cells does not seem to be altered in the afferent lymph. These results provide direct evidence that during the induction phase of an allergic contact dermatitis in humans antigen-bearing dendritic cells internalize the antigen and migrate from the skin via the afferent lymph vessels to the lymph node.

C15

Osteopontin is Involved in the Initiation of Cutaneous Contact Hypersensitivity by Inducing Langerhans and Dendritic Cell Migration to Lymph Nodes

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Osteopontin (OPN) is a chemotactic protein that attracts inflammatory cells. The sensitization phase of cutaneous CHS is dependent upon the migration of antigen-carrying Langerhans cells/dendritic cells (LC/DC) from the skin to draining lymph nodes (LN). We investigated the expression of OPN in the sensitization phase of CHS and found that OPN mRNA is up-regulated both in the TNCB hapten sensitized skin and in LN draining such skin after hapten application. Within skin especially endothelial cells expressed OPN. Speculating that OPN expression could be a crucial factor in attracting DC to lymphatic organs, we investigated the role of OPN in DC trafficking. Migration-assays revealed that OPN-induced DC migration in a chemotactic manner. *In vivo* s.c. injected OPN initiated LC emigration from the epidermis and OPN injected in close proximity to LN draining the skin attracted DC into these nodes. The OPN receptors CD44 and $\alpha v\beta 3$ integrin are known mediators of OPN induced cell migration. We found both receptors on DC upon their maturation in bone-marrow cultures. Antibodies against αv and CD44 partially blocked OPN mediated LC/DC migration *in vitro* and *in vivo*. When ultimately investigating OPN function *in vivo* we found that OPN deficient mice had a significantly reduced CHS response to TNCB. Furthermore, when wild type DC were injected into OPN deficient mice, migration of DC to regional LN was reduced dramatically compared to wild type mice. In conclusion we demonstrate that OPN is a crucial factor in the initiation of CHS by guiding DC from skin into lymphatic organs.

C16

Interleukin-18 is a Key Cytokine in the Initiation of Langerhans Cell Migration and Contact Hypersensitivity Response in Mice

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Following skin sensitisation, Langerhans cells (LC) are stimulated to migrate from the epidermis via afferent lymphatics to draining lymph nodes where they accumulate as immunostimulatory dendritic cells. It has been demonstrated previously that interleukin (IL)-1β and tumour necrosis factor-a (TNF-α) provide important signals for the initiation of LC migration. In the present study, the ability of IL-18 to regulate LC migration and contact hypersensitivity (CHS) was examined using IL-18 deficient mice. Thus, IL-18−/− and control (wild type; WT) mice were sensitised epicutaneously on abdominal skin with 1% oxazolone (OX) and challenged on the dorsum of the ear with 0.5% OX 5 days later. Although WT mice responded with vigorous ear swelling, significant suppression (36%, $n = 3$; $p < 0.05$) of this response was observed in IL-18−/− mice. Local pre-treatment of mice by intradermal injection of IL-18 (50 ng) prior to sensitisation with OX, restored completely the CHS response in IL-18−/− mice ($n = 3$ experiments). To investigate the influence of IL-18 in allergen-induced LC migration, epidermal LC numbers were determined following exposure of IL-18−/− and WT mice to 1% OX. Topical application of the contact allergen resulted in a significant decrease in the frequency of epidermal MHC class II^+ LC 4 h after exposure (26%, $n = 3$; $p < 0.05$) in control mice, as expected. However, this decrease was absent in IL-18−/− mice. We next examined the response of LC in WT and IL-18−/− mice to intradermal injection of IL-1β, TNF-α and IL-18. All three cytokines caused a significant decline in epidermal LC numbers 4 h (IL-1β and IL-18) or 30 min (TNF-α) after exposure in both WT and IL-18−/− mice ($n = 3$ experiments) indicating that IL-18−/− LC are capable of migration when provided with the appropriate cytokine stimuli. Taken together these data demonstrate that IL-18 is a critical proximal mediator of contact allergen-induced LC migration and CHS and suggest that this cytokine may be a key mediator of cutaneous immune responses.

C17

Differential Kinetics of Langerhans Cell Migration Stimulated in Mice by Chemical Contact and Respiratory Allergens

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We have demonstrated previously, that repeated topical exposure of BALB/c strain mice to chemical contact and respiratory allergens results in the selective activation of T helper 1 (Th1)- and Th2-type cells, respectively. Thus, under conditions of exposure that induce equivalent levels of immunogenicity with respect to IgG antibody production and draining lymph node cell (LNC) proliferation, only treatment of mice with respiratory allergens elicits IgE antibody production and a Th2-type cytokine secretion profile by LNC. We have now investigated the migration of Langerhans cells (LC) from the epidermis and the accumulation of dendritic cells (DC) in draining lymph nodes (DLN) induced following topical exposure of mice to the contact allergens dinitrochlorobenzene (DNCB; 1%) and oxazolone (Ox; 0.5%). These responses were compared with those provoked by trimellitic anhydride (TMA; 25%) and fluorescein isothiocyanate (FITC; 1%); sensitizers that stimulate a Th2 type cytokine profile. We demonstrate that whereas both Ox and DNCB provoke significant reductions in epidermal MHC class II^+ LC frequencies within 4 hr of exposure, the response to TMA reached significance only after 17 hr. DC accumulation was also more vigorous, with DNCB and Ox inducing approximately 5-fold increases in the accumulation of DC in DLN compared with 2- to 2.5-fold increases for TMA and FITC. These data demonstrate that under conditions of similar immunogenicity, chemical contact and respiratory allergens display differential kinetics of LC migration. Such differences may ultimately influence the selective activation of Th cell subpopulations by these classes of chemical allergen.

C18

Poly I:C-Exposed Human Keratinocytes Acquire the Ability to Induce Type-1 Polarized Phenotype in Dendritic Cells (DC1)

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Epidermal epithelial cells, keratinocytes (KC), represent the major constituent of the skin and participate actively in the skin immune system by producing, constitutively or upon stimulation, various soluble mediators. Immature dendritic cells (iDC) reside in the epithelia in close contact with KC. Upon activation iDC undergo final maturation and migrate toward secondary lymphoid tissues, where they prime naive Th cells. Amongst others, IL-1β and TNF-α, which are produced by KC, can induce iDC to mature. Therefore, we questioned whether the products of differentially activated KC could induce DC polarization. To this aim, KC were pulsed with poly I:C and or with IL-1β/TNF-α and KC-derived supernatants (50%, v/v) were added to iDC. Exposure of maturing DC to poly I:C-activated keratinocyte supernatants enhanced their expression of CD83. Neutralization of TNF-α reduced the phenotypic maturation of monocyte-derived DC induced by poly I:C-exposed KC supernatants suggesting that TNF-α produced by poly I:C-activated KC induced DC maturation. DC matured in the presence of poly I:C-activated KC were able to induce the differentiation of polarized Th1 cells, although IL-12p70 production was not affected. In contrast, DC mature in the presence of IL-1β/TNF-α-activated KC induce the differentiation of a mixed population of Th1 and Th2 cells. Our results indicate that activated KC may initiate specific immune responses by direct activation and polarization of DC.

C19

Psoriatic Lesional Keratinocytes Promote the Maturation of Human Monocyte-Derived Langerhans Cells

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Langerhans cells (LCs) are a kind of important antigen-presenting cells in epidermis and may play a key role in the pathogenesis of psoriasis. It has been proved that LCs isolated from psoriasis are abnormal. However, the reason of the abnormality of LCs from psoriasis has not been reported so far. In the present study, we investigated the effect of psoriatic lesional keratinocytes on the maturation of LCs. At first, the monocytes isolated from the healthy peripheral blood could differentiate into LCs in the presence of GM-CSF, IL-4 and TGF-β1 for 5 days. Then the human monocyte-derived LCs were cultured with the supernatants from psoriatic lesional keratinocytes for another 2 days. Their phenotypes and phagocytic capacity were analyzed by flow cytometry. IL-12 secreted by the LCs was determined by ELISA. Their immunostimulatory capacity was also detected by mixed lymphocyte reaction (MLR). The results showed that the supernatants from psoriatic lesional keratinocytes could up-regulate the expression of HLA-DR and CD86 on LCs more significantly than the supernatants from healthy keratinocytes, but less powerfully than LPS. Their immunostimulatory capacity was enhanced markedly and the levels of IL-12 secreted from LCs also increased. But the expression of CD1a on LCs and their phagocytic capacity were reduced. The results demonstrated that human monocyte-derived LCs cultured with the supernatants from psoriatic lesional keratinocytes displayed the characteristics of maturation. It suggests that psoriatic lesional keratinocytes might secrete some factors that could promote the maturation of LCs, which may play important roles on immune reaction correlated to psoriasis.

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C20

"Tracking & Tracing" of Migrating Langerhans Cells

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In response to inflammatory stimuli, Langerhans cells migrate from the epidermis to draining lymph nodes and, if charged with antigen, generate T cell immunity there. Dermal dendritic cells behave alike. In order to learn about the relative contributions of these two types of cutaneous dendritic cells we studied migration in a murine skin explant model and *in vivo* using a novel antibody recognizing the mouse homologue of Langerin. This allowed us to distinguish Langerhans cells from dermal dendritic cells. Migrating dendritic cells in lymphatic vessels ("cords") consisted of both Langerhans cells and dermal dendritic cells in roughly equal proportions. This was also true for dendritic cells that emigrated into the culture medium. Numbers of Langerin-expressing cells in the lymph nodes increased upon epicutaneous application of a contact sensitizer (FITC). These Langerin-positive cells in the nodes were also FITC-positive indicating that they carried the antigen. A substantial proportion of Langerin-negative but FITC-containing cells was also detected. This would emphasize an important role for dermal dendritic cells in antigen carriage from the skin and, specifically, in contact hypersensitivity. Our data also suggest that Langerin is a reliable Langerhans cell marker *in vivo*, as opposed to epidermal cell cultures where it disappears during culture.

C21

Regulation of CCR6 Expression in Langerhans Cell Precursors: Suppression by IL-4 and IFN-γ and Induction by IL-10

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Immune responses are initiated by Dendritic Cells (DC) that form a network comprising different populations. In particular, Langerhans cells (LC) appear as a unique population of cells colonizing epithelial surfaces. We have recently shown that MIP-3α/CCL20, a chemokine secreted by epithelial cells, induces the selective migration of LC among DC populations. Here we studied the effects of cytokines on the expression of the MIP-3α receptor, CCR6, during differentiation of LC. We found that both IFNγ and IL-4 blocked the expression of CCR6 and MIP-3α responsiveness when added during LC development. In addition, when added on $CCR6^+$ differentiated LC precursors, both cytokines down-regulated CCR6 expression and antagonized their migration in response to MIP-3α. The IFNγ induced down-regulation of CCR6 was irreversible, in contrast, when IL-4 was withdrawn from the culture both CCR6 expression and MIP-3α responsiveness were recovered. When other cytokines involved in DC and T cell differentiation were tested, we found that IL-10, unlike IL-4 and IFNγ, maintained CCR6 expression. The effect of IL-10 was reversible and upon IL-10 withdrawn, CCR6 was lost concomitantly to final LC differentiation. In addition, IL-10 induced the expression of CCR6 and responsiveness to MIP-3α in differentiated monocytes. Finally TGF-β which induces LC differentiation, did not alter early CCR6 expression but triggered its irreversible down-regulation, in parallel to terminal LC differentiation. Taken together these results suggest that the recruitment of LC at epithelial surface might be suppressed during Th-1 and Th-2 immune responses, and amplified during regulatory immune responses involving IL-10 and TGF-β.

C22

Inflammatory Stimuli Promote Differentiation of Langerin+ Immature LC Type Cells *In Vitro*, and the Recruitment of Immature LC within the Draining Lymph Node *In Vivo*

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Maturation of dendritic cells (DC), i.e. switching from low to high expression of MHCII antigen complexes and costimulatory molecules, is a critical event in the induction of immunity. DC maturation in the periphery and DC migration to draining lymph nodes are thought to be coordinated, but little is known regarding these events for Langerhans cells (LC). Using LC-type cells cultured in the presence of TGFβ1, we found that inflammatory stimuli such as TNFα and bacterial products induce expression of Langerin-a LC specific C-type lectin- in up to 60% of immature DCs. TNFa also induces LC migration towards lymph node chemokines. Although these inflammatory stimuli induce the maturation of interstitial type DC they did not induce the maturation of LC type cells *in vitro*. As an *in vivo* counterpart, we examined a series of inflamed-skin human draining lymph nodes. Strikingly, these lymph nodes selectively exhibited a massive recruitment of immature LCs in T cell areas. Our results show that Langerhans cells can migrate to secondary lymphoid organs in an immature stage in response to inflammation. Therefore stimuli that induce maturation of LC appear to be distinct from those leading to their accumulation in lymph nodes. The role of LC in the regulation of immune responses should thus be re-evaluated, since antigen bearing LCs can either transmit antigen to bystander DCs within lymph nodes, undergo maturation, or induce tolerance in absence of maturation.

C23

Dendritic Cells Exposed to Extracellular ATP Acquire the Migratory Properties of Mature Dendritic Cells and Reduce the Capacity to Attract Type 1 T Lymphocytes

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We recently reported that chronic stimulation with low, noncytotoxic doses of extracellular ATP, induced a distorted maturation of dendritic cells (DCs) with reduced production of TNF-α and IL-12 but unchanged release of IL-10. As a result, DCs matured in the presence of ATP showed an impaired capacity to initiate Th1 responses *in vitro*. Here we examined the effects of ATP on the chemokine receptor asset of DCs. As shown by FACS analysis and RNase protection assay, ATP strongly induced CXCR4 expression both in immature and in LPS-stimulated DCs. Moreover, CCR7 was slightly up-regulated mainly in mature DCs. In contrast, ATP reduced CCR5 expression on immature DCs. ATP increased migration and intracellular calcium transients of immature and mature DCs to SDF-1 and MIP-3β, while responses to MIP-1β were reduced. Mature DCs are an important source of chemokines influencing the recruitment of distinct T lymphocyte subsets. ATP significantly reduced LPS-induced production of IP-10 and RANTES, but did not affect the release of MDC and TARC. Consistent with this finding, supernatants from mature DCs exposed to ATP attracted less efficiently Th1 and Tc1 cells, whereas migration of Th2 and Tc2 cells was not affected. In aggregate our data suggest that ATP provides a signal for lymph node localization of DCs and impairs their capacity to attract type 1 T lymphocytes.

C24

The CD1a+ CD11c+ Langerhans Cell Precursors are the Most Susceptible Blood Leucocytes for HIV Infection

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Using skin explants we have previously shown HIV-1 entry into Langerhans cells and preferential transmission of macrophage tropic HIV-1 after virus exposure. Primate studies have mostly supported this role for DCs however, recent studies of SIV in macaques have shown CD4 T cells are the first to express HIV-1 at the site of entry. Models based on initial infection of T cells or on lectin binding and carriage by DC do not allow for HIV-1 selection. Since CCR5 is involved in HIV-1 selection and viral entry whatever the route of infection we have examined directly isolated blood leucocytes to determine if initial entry is via the different DC subpopulations. DCs, identified as lin- [CD3-, 14-, 16-, 19-] HLA-DR+ cells were sorted into subpopulations using the additional markers CD123, CD11c and CD1a. After exposure of sorted uncultured blood leucocytes for 2 h HIV-1 entry was determined using quantitative realtime PCR at 24 h. The highest frequency of infection was found in the CD1a+CD11c+ Langerhans cell precursors. The CD11c+CD1a- and CD11c- DCs were infected at a lower frequency and the CD14+ and CD16+ monocytes and lymphocytes were infrequently infected. A similar preferential entry of HIV-1 into the CD11c+ DCs was found if the whole PBMCs were infected and cultured for 0, 12, 36 h before determining entry into the sorted sub-populations. Efficient virus production occurred after DC infection and coculture with T cells but only low levels of virus production occured after infection of the monocytes or T cells.

C25

Correlation Between CD123+ (Lymphoid) Dendritic Cell Recovery and Better Infection Control in Primary HIV Infection

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Successful immunological control of HIV infection is only achieved in rare individuals. Dendritic Cells (DCs) are required for specific antigen presentation to naive T lymphocytes and for antiviral, type I Interferon secretion. We have previously found a decreased proportion of blood $CD11c^+$ (myeloid) DCs in chronic HIV^+ patients. In this study, we have used rare event analysis of three-colour flow cytometry data. We have studied longitudinally 13 patients during primary infection, before and after highly active antiretroviral therapy. Twenty six to 57 days after contamination, $CD123^+$ (lymphoid) and $CD11c^+$ DC numbers were dramatically reduced in the 13 HIV^+ patients compared to 13 controls ($p = 0.0002$ and 0.001, respectively). After 6-12 months of highly active antiretroviral therapy, DC subpopulation average numbers remained low, but $CD123^+$ DC numbers increased again in 5/13 patients. A strong correlation was found between this increase and CD4 T cell count increase ($p = 0.0009$) and plasmatic viral load decrease ($p = 0.009$). DC number restoration may be predictive of immune restoration and may be a goal for immunotherapy to enhance viral control in a larger proportion of patients.

C26

Comparative Inhibition of R5 HIV Infection in Epidermal Langerhans Cells by Novel Chemically Modified RANTES Analogues

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Initial biologic events that underlie sexual transmission of HIV are poorly understood, although it is believed that mucosal Langerhans cells (LC) are initial targets for virus. It is also known that the majority of transmitted viruses utilize CCR5 as a co-receptor for entry into cells (i.e. R5 viruses). To model these events, we recently reported that LC within epithelial tissue explants could be preferentially infected with R5 HIV isolates (Kawamura et al, J Exp Med, 2000). Here, to help elucidate a potent inhibitor of HIV infection in LC, we directly compared the HIV blocking ability of four novel chemically modified chemokines that bind CCR5 (AOP-RANTES, NNY-RANTES, PSC-RANTES, and UCB-RANTES) using our explant model. As quantified by intracellular HIV p24 staining of LC and flow cytometry, all analogues could block R5 HIV in a dose-dependent manner (1-100 nM) when pre-incubated with explant tissue for 20 min prior to HIV exposure. Furthermore, all analogues could inhibit LC-mediated HIV infection of CD4+ T cells that were co-cultured with LC. Interestingly, the newer analogues, especially PSC-RANTES and UCB-RANTES, performed significantly better than AOP-RANTES at a dose of 10 nM ($p < 0.01$). In summary, we document R5 HIV infection in single LC following exposure to virus within epithelial tissue and show that this infection can be blocked by pre-incubation of tissue with drugs that compete with virus binding and entry into cells. The fact that RANTES analogues could also block transmission of virus to T cells suggests that LC infection, and not virus bound to LC via a C-type lectin, is the most important mechanism by which LC transmit HIV to T cells. Importantly, these results suggest that a topical microbicide containing a chemically modified RANTES analogue may be useful in blocking sexual transmission of HIV.

C27

CD40-Ligation *In Vivo* Promotes Activation and Migration of Dendritic Cells and Enhances Resistance to Infection with Mycobacterium tuberculosis

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Adaptive immune responses begin after antigen bearing dendritic cells (DCs) traffic from peripheral tissues to lymph nodes where they play a key role in the initiation of specific T-cell responses. Ligation of CD40 is known to be an important signal in the activation and migration of Langerhans cells (LC). Furthermore the importance of CD40 ligation in the generation of protective immune responses against infectious agents (*Leishmania major, Trypansoma cruzi*) and tumors has been demonstrated in a number of studies. *M.tuberculosis* has been shown to use a number of evasion strategies to avoid an effective immune response including impairment of antigen presentation and down-regulation of CD40 ligand. We investigated whether there is an impairment of Langerhans cell migration in *M.tuberculosis* infected mice and whether ligation of CD40 using an anti CD40 mAb could overcome any antigen processing/presentation defect in *M.tuberculosis* infected mice and result in increased immunity against *M. tuberculosis* infection. We have demonstrated that CD40 ligation *in vivo* results in Langerhans cell migration from the epidermis with virtual clearing of LC by day 7 following anti-CD40 mAb. Epidermal LC have a more mature phenotype and dendritic cells accumulate in the draining nodes of anti-CD40 treated mice. *M.tuberculosis* infected mice were treated with either anti-CD40 or control antibody. The colony counts of *M.tuberculosis* in the lungs and spleens showed a 10-100 fold reduction in viable *M.tuberculosis*. This effect is greater than any other immunological therapy to date (including dendritic cell immunotherapy and DNA vaccination) and suggests potential new approaches in multidrug resistant *M.tuberculosis* and vaccine design.

C28

DermaVir: A Langerhans Cell-Mediated DNA Immunization Technology for the Induction of Cellular Immune Responses

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A practical, non-invasive transcutaneous gene transfer technology has been developed to genetically engineer large numbers of lymph node dendritic cells in order to induce potent T cell-mediated immune responses. Plasmid DNA was formulated with mannosylated polyethylenimine (PEIm) in a glucose-water solution to transduce Langerhans cells in the epidermis. DNA-expressing Langerhans cells migrated to the T cells area of the draining lymph node, interdigitated as dendritic cells and presented DNA-derived antigens to T cells. The unprecedented high efficacy of this approach was demonstrated in a non-human primate model where 0.025 mg DNA applied on the surface of the skin resulted in ca. 20 000 gene-expressing dendritic cells in one draining lymph node. Large number of SIV-specific T cells were detected in monkeys immunized on the surface of the skin with a SHIV expression plasmid ($DermaVir_{SHIV}$). This novel technology is expected to provide a new, general scheme for developing therapeutic and preventive vaccines against viral and neoplastic diseases.

C29

Active Migration of Human Monocyte-Derived Dendritic Cells from Skin to Regional Lymph Nodes Depends on their Stage of Maturation

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The feasibility to produce high numbers of dendritic cells (DC) from human monocytes (moDC) has stimulated increasing interest in DC based immunotherapy of tumor patients. Whereas a high T cell stimulatory property of moDC has clearly been shown *in vitro*, reliable data on their *in vivo* migratory capacities are lacking. To determine the *in vivo* migratory capacity of moDC, 2 Mio moDC indium-111 labeled cells were injected intra- and subcutaneously in a voluntary patient, who as a physician was himself interested in these data. Imaging over the injection sites and draining lymph nodes was obtained at 4, 24, 48 and 96 h and the percentage of clearance for each area of interest calculated. Immature DC, obtained from monocytes by a 7d culture with GM-CSF and IL-4 were bad migrators: only following i.c. injection a small amount of the injected activity was found in the draining inguinal and iliacal lymph nodes. In contrast, ic. and sc. injections of moDC that were further matured over 3d with a cocktail of inflammatory cytokines (IL-1, IL-6, TNF, PGE2) yielded 8-12% activity within regional nodes. No nodal activity was detected when matured DC were ethanol fixed prior injection. This is the first *in vivo* proof that human monocyte derived DC, if properly matured, do actively and efficiently migrate from peripheral injection sites to draining lymph organs. Thus there is no need for difficult intranodal injections of DC in patients, which even bear the risk of damaging the lymph node architecture.

C30

Generation of Optimal Monocyte-Derived Dendritic Cells for Immunotherapy: Influence of Maturation Conditions on Antigen Presentation

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Monocyte derived dendritic cells (MODC) are increasingly used for immunotherapy of cancer. For human clinical trials, U.S. regulatory agencies dictate that the cells must be generated under Good Manufacturing Practice (GMP) conditions, using no animal-derived products. Consequently, we have established serum-free culture conditions for human MODC that result in a highly purified preparation. These cells efficiently take up different antigenic preparations, including recombinant proteins, inactivated virus, and whole tumor cells, and can stimulate specific responses to these antigens *in vitro*. To induce maturation, we use heat-killed Baccille Calmette-Guerin (BCG), or BCG + gamma interferon (IFNγ). Inclusion of IFN. during maturation induces the production of high levels of interleukin 12 and tumor necrosis factor (TNFα), and down-regulation of interleukin 10. Both immature MODC, as well as cells matured with BCG alone or with BCG + IFNγ are able to stimulate peptide-specific T cell lines, whereas especially MODC matured with BCG + IFNγ effectively stimulate CD4+ and CD8+ primary T cell responses. For immunotherapy of hormone refractory prostate cancer patients, we prepared MODC loaded with prostate specific membrane antigen (PSMA). Upon injection into the patients, these cells generated significant humoral and cellular immune responses. A current update of clinical data from this trial, including CT scans, bone scans and PSA values, will be presented and discussed.

C31

IL-12-Secreting Dendritic Cells are Required for Optimal Activation of Human Secondary Lymphoid Tissue T-Cells: Implication for Anti-Tumoral Vaccination Protocols

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Successful antitumor immunization requires that mature dendritic cells (mDC) prime T cells in secondary lymphoid tissue (LT). However, in contrast to peripheral blood T-cells, multiple costimulatory signals are required for triggering LT T-cell activation. This study was designed to optimize mDC immunogenicity in accordance with lymphoid tissue T-cell requirements. Monocyte-derived DC differentiated in GM-CSF/IL-13 were matured for 24 h with soluble CD40Ligand (sCD40L-mDC) or IFN-γ combined with sCD40L (sCD40L/IFNγ-mDC). T cells from secondary lymphoid tissues were purified from spleen, tonsil and lymph node samples by immunomagnetic selection. We report here that 1) Although mDC expressed similar membrane phenotype including CD83 expression, only sCD40L/IFNγ signaling induce high levels of IL-12p70 secretion by mDC. 2) sCD40L/IFNγ-mDC increased allogeneic LT T-cell proliferative responses as compared with sCD40L-mDC. IL-12 neutralizing antibody prevented the effect of IFN-γ addition. 3) IL-12-secreting DC promote IFN-γ production from LT CD4+ cells. LT T-cells IFN-γ release was partially inhibited by neutralizing IL-12 antibody. 4) sCD40L/IFNγ-mDC enhanced the cytotoxic CD8+ generation from lymphoid tissue T-cells. In conclusion, IL-12-secreting DC are required for optimal proliferation, IFN-γ secretion and cytotoxic responses of LT T-cells. DC to be used for immunization protocols should be characterized by their IL-12 secretion as an additional costimulatory signal potentially critical for the triggering of an antitumor immune response by LT T-cells.

C32

Autologous MUC-1 Pulsed Dendritic Cells are a Safe, Feasible Treatment Approach in Patients with Cancer and are Associated with Cellular Immune Responses

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Dendritic cells (DC) are professional antigen presenting cells capable of inducing MHC restricted antigen specific CD4 and CD8 T cells *in vitro* when pulsed with tumor antigens. DC function in patients with malignancy is deficient and re-administration of *ex-vivo* cultured DC may reverse this. MUC-1 is a *trans*-membrane glycoprotein aberrantly glycosylated in some human cancers soan occult epitope is exposed, providing an immuno-therapeutic target. A phase 1 clinical trial of MUC-1 pulsed DC began in July 1999. 12 patients with various primary cancers have been treated. DC were generated from adherent peripheral blood mononuclear cells using GM-CSF and IL4, and pulsed with a liposomal preparation of a 25-mer peptide from MUC-1 (BLP-25, Biomira Inc) before re-administration by subcutaneous injection. Patients received between 0.075×10^6 and 1.0×10^6 dc per kg body weight in one or two doses. Followed up for toxicity, immune response and clinical effects at days 1, 7, 14, 28 and 90. 11 patients are assessable, more than 28 days from treatment. Minor, grade 1 toxicitites reported by 8 patients. Grade 2 fatigue seen in 2, myalgia in 1 and anaemia in 1. No grade 3 or 4 toxicities. 4 patients had stable disease radiologically and clinically at 28 days, but all progressed by 3 months. Immunologically a small but significant increase occurred in proliferative response to the muc-1 antigen in patients over time compared with controls ($n = 6$). Increased responses seen to repeated skin testing with ppd, a recall antigen, following the dc therapy ($n = 9$). Patients mean pre-treatment value was 3.2mm, post treatment 13mm ($p = 0.03$, students t test), controls ($n = 5$), showed no response to re-testing over the same time period (initial test mean 17.6mm, repeated test mean 23.6mm, $p = 0.13$, students t test). This phase 1 trial demonstrates a safe, feasible treatment approach with biological effects in patients with a range of malignancies.

C33

Accumulation of Langerhans Cells in the Skin of Epithelium-Specific PTEN Knockout Mice

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The *PTEN* gene is a highly mutated tumor suppressor gene. Somatic deletions or mutations of this gene have been identified in a wide range of tumors such as gliomas, endothelial, prostate and breast cancers. The PTEN tumor suppressor plays a critical role in cell programming apoptosis and cell cycle arrest. In addition, *in vitro* studies have demonstrated that PTEN may participate in the regulation of T cell survival and TCR signaling, implicating a potential role of PTEN in immunity. However, *in vivo* studies have been impeded by the embryonic lethality of the homozygous PTEN knockout mice. To directly address the role of PTEN in skin immunity, we have recently developed epithelium-specific PTEN knockout mice (PTEN WAP–/–). These mutant mice are viable and demonstrate a specific PTEN deficiency in the epidermis. Intriguingly, PTEN WAP–/– mice spontaneously developed chronic cutaneous inflammation in the trunk, head and ears. Clinically, the dermatitis was characterized by the thickened and reddened skin, as well as abnormal hairs. Histologically, lesional skin demonstrated hyperkeratosis, acanthosis, dermal thickening and mixed cellular infiltration. Most interestingly, Langerhans cells were dramatically increased in the epidermis and follicular epithelium, suggesting a potential role of PTEN in differentiation and/or activation programs of cutaneous dendritic cells. Collectively, our data demonstrate a significant role of PTEN in the cutaneous inflammatory and immune response.

C34

High Numbers of Infiltrating Langerhans Cells in the Absence of MHC II^{high} Macrophages is Associated with Skin Tumour Regression

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Dendritic cells (DC) such as Langerhans cells (LC) are important in the induction of anti-tumour immunity and are currently being used in clinical trials. To address their interaction with tumour cells, UV-radiation induced skin cancers which spontaneously regress upon transplantation into syngeneic mice because they activate the immune system were compared to progressing skin tumours which evade immunological destruction. Flow cytometric analysis revealed that regressing tumours were infiltrated by greater numbers of mature DC ($CD11c^+CD45^+$) compared to progressing skin tumours. DC derived from both tumour groups had an "activated" phenotype compared to *in vitro* matured epidermal LC, with high surface expression of MHC II, CD80, CD86 and Fas. However in contrast to both fresh and matured epidermally-derived LC which expressed moderate levels of the chemokine receptors CCR5 and CXCR4, skin tumour derived DC had no surface expression of these receptors. Thus, it is possible that there is a dysregulation in the ability of DC from both tumour groups to respond to chemokines. DC derived from both tumours were "myeloid-related" with high levels of Mac-1 and F4/80 but no CD4. No "lymphoid-related" $CD8\alpha^+$ DC could be detected in either tumour type. Progressing but not regressing skin tumours were heavily infiltrated by MHC II^{high} macrophages. Thus, it is possible that the larger number of DC in regressor tumours stimulates immunity, although they may not be able to migrate from the tumour as they failed to express chemokine receptors. In contrast the macrophages present in progressor tumours may inhibit the induction of immunity.

C35

Blood-Derived Myeloid and Plasmacytoid Dendritic Cells Differ in Responsiveness to Pathogenic Compounds and Expression of Toll-Like Receptors

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Specific immunity to pathogens requires activation of sentinel dendritic cells (DC). Distinct subsets from different lineages, e.g. myeloid DC and plasmacytoid DC, have been described. Using newly described antibodies (BDCA1-4) we have isolated three distinct DC-subsets from peripheral blood and analysed these subsets for their responsiveness to different pathogenic compounds and their Toll-like receptor (TLR) expression. Myeloid BDCA1+ or BDCA3+ DC produced IL-12p70, IL-6 and TNF-α but no IFN-α in response to LPS or dsRNA, whereas plasmacytoid BDCA2+ DC produced high levels of IFN-α, TNF-α and IL-6 but no IL-12p70 in response to bacterial CpG-motifs but not to LPS or dsRNA. To our surprise also myeloid BDCA3+ DC responded to CpG motifs with the production of IFN-α, TNF-α and IL-6 but no IL-12p70. Analysing the mRNA expression of TLR1-9 we found that TLR2 and 4, the receptors for LPS, were expressed by all 3 subsets, which is not in line with their LPS-responsiveness. However, as expected from the responsiveness to CpG motifs, the receptor for CpG, e.g. TLR9 was found in BDCA2+ and BDCA3+ DC but not in BDCA1+ DC. This study suggest that reactivity to pathogenic compounds is mediated by distinct subsets of DC with different cytokines profiles and expression of TLR.

C36

BDCA-2, a Novel Plasmacytoid Dendritic Cell-Specific C-Type Lectin Type II Transmembrane Protein: Molecular Cloning and Functional Characterization

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Plasmacytoid dendritic cells (PDCs, also known as interferon (IFN)-α/β-producing cells or type-2 pre-dendritic cells) are present in lymphoid and non-lymphoid tissue and contribute substantially to both innate and adaptive immunity. Recently, we have described several mAbs that recognize a PDC-specific antigen which we have termed BDCA-2. Molecular cloning of BDCA-2 revealed that BDCA-2 is a novel C-type lectin type II transmembrane protein with 50% identity and 69% homology at the amino acid level to human DCIR. Anti BDCA-2 mAbs are rapidly internalized and efficiently presented to T-cells indicating that BDCA-2 can function in ligand internalization and presentation. Furthermore, ligation of BDCA-2 suppresses both influenza virus-induced and DNA/anti dsDNA mAb complex-induced IFN-α/β production by PDCs, presumably by a mechanism dependent on calcium mobilization and protein-tyrosine phosphorylation by Src-family protein-tyrosine kinases. Inasmuch as production of IFN-α/β by PDCs is believed to be a key pathophysiological factor in systemic lupus erythematosus (SLE), BDCA-2 represents an attractive target for blocking production of IFN-α/β in SLE patients.

C37

Rapid Chemokine-Mediated Binding of T Cells to Dendritic Cells (DC) Under Dynamic Conditions

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The binding of a T cell to an antigen-laden DC is a critical step of the acquired immune response. Activated DC synthesize a variety of chemokines including CCL22 (a CCR4 ligand) that can attract T cells. Herein, we address whether DC-produced CCL22 can induce the arrest of T cells to DC. Maturing bone marrow-derived DC were allowed to adhere to anti-CD40 pre-coated plastic tissue culture plates at high density. Within a parallel plate flow chamber, shear-dependent arrest of B 9.1 T cell hybridoma cells was observed with maximal arrest at or below 0.2 dynes/cm^2. Arrest of B 9.1 cells was inhibited by > 85% with anti-CCL22 or anti-CD18 (but not anti-CXCL13) antibodies (Ab), pretreatment of B 9.1 cells with CCL22 (but not CCL5 or CXCL10) or pertussis toxin (PTX) ($p < 0.01$). At 0.2 dynes/cm^2, ovalbumin-immunized (memory) T cells (mTC) from DO11.10 mice showed 3-fold more efficient binding to DC than did naïve T cells (nTC) although nTC demonstrated consistent binding to DC. mTC (but not nTC) binding to DC was inhibited (> 80%, $p < 0.01$) by anti-CCL22 antibodies, PTX, and CCR4 desensitization with CCL22. mTC to DC arrest occurred in less that 0.5 s. Our results demonstrate that DC-produced CCL22 induces rapid binding of T cells under dynamic conditions and that mTC and nTC fundamentally differ with respect to chemokine-dependent binding to DC.

C38

Regulation of Immune Responses by Interleukin-10 Treated Dendritic Cells *In Vivo*

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Interleukin-10 (IL-10) is an immuno-modulatory cytokine expressed by various immune cells as well as tumor cells. IL-10 treatment of dendritic cells (DC) affects the expression of MHC and costimulatory molecules such as CD80 and CD86. It was shown *in vitro* that IL-10 treated human blood-derived DC induce anergy in CD4+ and CD8+ clonal or naive T cells. In this study we analysed the effect of IL-10 treated DC *in vivo* using the OVA-TCR transgenic and the murine experimental autoimmune encephalopathy (EAE) system, a model system for multiple sklerosis. In the OVA-TCR transgenic system dendritic cells not only prevented OVA-specific immune responses in naive mice but also inhibited immunity in previously sensitized mice. This inhibition was detected by antigen specific proliferation *ex vivo* and by DTH reactions *in vivo*. The effect was long lasting and could not be reversed by subsequent antigen stimulation. In the EAE model mice injected with IL-10 treated DC prior to EAE induction developed no clinical symptoms. DC injection 5 days past EAE induction also prevented disease-onset. In the EAE system the inhibitory effect of IL-10 treated DC was not antigen-specific. As IL-10 treated DC represent a population of immature DC which have been shown to induce regulatory T cells we are currently studying whether induction of regulatory cells might account for the effects seen in both systems.

C39

Immature Langerhans Cells: Central Role in Tolerance Induction in Carcinogen-Treated and Neonatal Skin

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Antigen presented across dimethylbenzanthracene (DMBA)-treated BALB/c skin results in the induction of antigen specific tolerance rather than immunity. DMBA treatment of skin triggers the enhanced migration of Langerhans cells (LC) to draining lymph nodes and epidermal replacement by immature LC. Morphologically, these new arrivals are small rounded cells with poorly formed dendrites. These immature cells carry less antigen to lymph nodes, have a reduced costimulatory molecule expression and impaired capacity to induce T cell proliferation. Comparative analysis in 4 day old neonatal skin revealed that the LC were small and rounded with poor dendrite formation. Likewise they have an impaired ability to transport antigen to the draining lymph nodes. They have reduced costimulatory molecule expression, fail to express DEC205 and are unable to induce T cell proliferation. Antigen applied across such skin also results in antigen specific tolerance. By day 14 the LC of neonatal skin have a typical dendritic morphology and antigen applied across this skin results in a positive immune response. We are now using immature LC as an approach to down-regulate the development of autoimmune gastritis. In short, the active antigenic peptide associated with this disease, when presented across neonatal skin, curtails the development of the autoantibody against parietal cells and the pathological lesions. It is concluded that pulsing immature LC with autoantigens offers a strategy to prevent the development of autoimmune disease.

C40

Inhibition of Epidermal Langerhans Cells in Early Events of Murine Schistosomiasis

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Epidermal Langerhans cells (LCs) play a key role in immune defence mechanisms and in numerous immunological disorders. We show that percutaneous infection of C57BL/6 mice with the helminth parasite *Schistosoma mansoni* leads to the activation of LCs but, surprisingly, to their retention in the epidermis. Moreover, using an experimental model of LC migration induced by TNFα, we show that parasites transiently impair the departure of LCs from the epidermis and their subsequent accumulation as dendritic cells in the draining lymph nodes. The inhibitory effect is mediated by soluble lipophilic factors released by the parasites and not by host-derived anti-inflammatory cytokines. We find that prostaglandin (PG)D_2, but not the other major eicosanoids produced by the parasites, specifically impedes the TNFα-triggered migration of LCs through the adenylate cyclase-coupled PGD_2 receptor (DP receptor). Moreover, the potent DP receptor antagonist BW A868C restores LC migration in infected mice. Finally, in a model of contact allergen-induced LC migration, we show that activation of the DP receptor not only inhibits LC emigration but also dramatically reduces the contact hypersensitivity responses after challenge. Taken together, we propose that the inhibition of LC migration could represent an additional stratagem for the schistosomes to escape the host immune system and that PGD_2 may play a key role in the control of cutaneous immune responses. The putative efficiency of PGD_2 analogues in diseases where reduction of immune cutaneous response is sought (dermatitis) or, conversely, in diseases where stimulation of LC migration would be beneficial (carcinomas, infectious pathologies) will be discussed.

P1

Ontogeny of Langerin Expression in Mouse Skin

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As Langerhans cells populate the epidermis in fetal and neonatal life, they acquire surface molecules in a stepwise fashion. In the mouse, ATPase-expressing and CD45-positive cells of dendritic morphology appear in late fetal life. These cells do not yet express MHC class II molecules, neither on the suface nor intracellularly. MHC class II expressing cells show up only after birth, they increase in numbers and by the 5th postnatal day virtually all Langerhans cells express MHC class II. It has not been systematically studied when in ontogeny Birbeck granules arise. Therefore, we made use of a newly developed mAb recognizing the murine homologue of Langerin. Epidermal sheets were prepared from the skin of late fetal (i.e. fetal days 9-19) and postnatal (i.e. day of birth, days 1, 5, 18, and adult) BALB/c mice. They were labelled with anti-Langerin mAb and visualized with FITC-conjugated second-step antibodies.

We found that Langerin expression was very low or absent on Langerhans cells from mice 5 days after birth. In contrast, Langerhans cells from mice on day 18 after birth were like Langerhans cells from adult mice: There was an almost 100% overlap of HC class II and Langerin. We conclude that Birbeck granules appear relatively late in the development of Langerhans cells. It appears that *in vivo* Langerhans cell precursor cells do not yet possess Birbeck granules.

P2

The Concept of Lymphoid *Versus* Myeloid Dendritic Cell Lineages Revisited: Both CD8α⁻ and CD8α⁺ Dendritic Cells are Generated from CD4^{low} Lymphoid-Committed Precursors

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Two dendritic cell (DC) subsets have been identified in the murine system on the basis of their differential CD8α expression. CD8α⁺ DCs and CD8α⁻ DCs are considered as lymphoid- and myeloid-derived, respectively, because CD8α⁺ but not CD8α⁻ splenic DCs were generated from lymphoid CD4^{low} precursors, devoid of myeloid reconstitution potential. Although CD8α⁻ DCs were first described as negative for CD4, our results demonstrate that around 70% of them are CD4⁺. Besides CD4⁻ CD8α⁻ and CD4⁺ CD8α⁻ DCs displayed a similar phenotype and T cell stimulatory potential in MLR, although among CD8α⁻ DCs, the CD4⁺ subset appear to have a higher endocytic capacity. Finally, experiments of DC reconstitution after irradiation in which, in contrast to previous studies, donor-type DCs were analyzed without depleting CD4⁺ cells, revealed that both CD8α⁺ DCs and CD8α⁻ DCs were generated after transfer of CD4^{low} precursors. These data suggest that both CD8α⁺ and CD8α⁻ DCs derive from a common precursor, and hence do not support the concept of the CD8α⁺ lymphoid-derived and CD8α⁻ myeloid-derived DC lineages. However, since this has to be confirmed at the clonal level, it remains possible that CD8α⁻ DCs arise from a myeloid precursor within the CD4^{low} precursor population, or alternatively that both CD8α⁺ and CD8α⁻ DCs derive from an independent non-lymphoid, non-myeloid, DC precursor. In conclusion, although we favor the hypothesis that both CD8α⁺ and CD8α⁻ DCs derive from a lymphoid-committed precursor, a precise study of the differentiation process of CD8α⁺ and CD8α⁻ DCs is required to define conclusively their origin.

P3

Langerhans Cells Develop from a Lymphoid-Committed Precursor

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Langerhans cells (LCs) are specialized dendritic cells (DCs) strategically located in stratified epithelia, such as those of the skin, oral cavity, pharynx, oesophagus, upper airways, urethra and female reproductive tract, which are exposed to a wide variety of microbial pathogens. LCs play an essential role in the induction of T lymphocyte responses against viruses, bacteria and parasites which gain access to those epithelial surfaces, due to their high antigen capture and processing potential and their capacity to present antigen peptides to T cells upon migration to the lymph nodes. Although LCs have been classically considered of myeloid origin, recent reports demonstrating the existence of lymphoid DCs derived from multipotent lymphoid precursors devoid of myeloid differentiation potential, raise the question of the lymphoid or myeloid origin of LCs. Here we show that mouse lymphoid-committed CD4^{low} precursors, with capacity to generate T cells, B cells, CD8⁺ lymphoid DCs and NK cells, also generate epidermal LCs upon intravenous transfer, supporting the view that LCs belong to the lymphoid lineage.

P4

Pro-Inflammatory Skin-Derived Cytokines Play a Critical Role in the Regulation and/or Maintenance of Langerin Expression Induced on Monocytes in Synergy with IL-13/TGFβ

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Keratinocytes (KC) are activated by environmental stimuli such as UV to produce a variety of cytokines (TNFα, GM-CSF, TGFβ,…) that can affect the immune responses as well as the cell growth and differentiation of skin cells and mononuclear cells which have recently colonized the epidermis. Furthermore, other cells present in the dermis such as IL-13-secreting mast cells, IL-15-secreting endothelial cells or IL-13-/IL-4-secreting activated T cells may also contribute to the differentiation of the mononuclear cells while they enter the skin. The epidermal dendritic cells (DC) termed Langerhans cells specifically express the type II lectin, Langerin. In this study we showed that the addition of TNFα to peripheral CD14⁺ monocytes cultured for 6 days in presence of IL-13 in combination with GM-CSF, TGFβ optimized the generation of Langerin⁺ DC which were similar to the Lag⁺ Langerhans-like cells generated in presence of IL-4 (Geissmann et al, J. Exp. Med, 1998). We further demonstrated that a short incubation with TNFα (< 10-18h) up-regulated the membrane expression of Langerin on the monocytes after 6 days of culture whereas a longer incubation with TNFα (> 24h-48h) led to: (i) the phenotype of activated mature DC correlated to the up-regulation of characteristic markers such as CD86, CD83, DC-LAMP and CCR7; (ii) the translocation of MHC-class II onto the membrane of Langerin⁺ DC; (iii) the internalization of membrane Langerin. It is noteworthy that in absence of TNFα, the up-regulation of the Langerin was dramatically reduced on the plasma membrane. Taken together, these results suggest that the generation of a Langerhans cell phenotypic profile from peripheral monocytes which colonize epidermis is dependent on a large amount of pro-inflammatory cytokines, i.e. IL-13, TGFβ and TNFα.

P5

Peripheral Homeostasis of Immature Dendritic Cells Mediated through TRANCE–RANK Interaction

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TRANCE (tumor necrosis factor-related activation-induced cytokine) and its receptor RANK (receptor activator of NF-kB) are required for bone homeostasis and lymph-node organogenesis and are involved in cell survival. Factor XIIIa⁺ CD14⁺ CD1a⁻ interstitial dermal DC are present in the skin, where they capture antigen and thereafter, migrate to draining lymph nodes where they present it to T cells. We have generated immature dermal DC *in vitro* using M-CSF and show that they mature in response to LPS and CD40 ligand and become capable of 6Ckine/MIP3β-mediated chemotaxis and naive T cell activation. Immature dermal DC carry both TRANCE and RANK on the cell surface, and the constitutive TRANCE-RANK ligation is responsible for high Bcl-2 levels and long-term cell survival. Dermal DC also promote the survival of RANK⁺ CD1a⁺-derived Langerhans DC. These findings provide evidence that, in addition to bone homeostasis, TRANCE and RANK play a key role in the homeostasis of immature peripheral DC.

P6

The Role of Maturation and Caspase-Activation in UVB, Steroid, CD95 (FAS/APO-1) and MHC-Class II Induced Cell Death in Murine Dendritic Cells

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We have investigated potential pathways of dendritic cell (DC) death through a comparison of the relative role of steroids, ultraviolet light, CD95 and class II in inducing DC apoptosis. Bone marrow derived, mature DC were relatively resistant to a low level UVB treatment (50 mJ per cm²) and only became susceptible as the dose was increased up to 100-250 mJ per cm². In contrast, immature DC showed complete sensitivity to low level (50 mJ per cm²) UVB doses. Though much less effective than UVB, both hydrocortisone and dexamethasone induced apoptosis in sub-populations of both immature and mature DC. All immature DC were highly susceptible to CD95 induced apoptosis but upon maturation became completely resistant. Crosslinking of class II with the N22 or 2G9 anti-IA/IE monoclonal antibodies resulted in rapid onset of DC death, but only in mature DC. Steroid, UVB and CD95 induced cell death was only partially (5-50%) inhibited by caspase-inhibition with zVAD-fmk whilst class II-induced cell death was completely caspase-independent. In spite of the low requirement for caspase activation in DC seen during CD95 induced apoptosis, we were able to detect strong activation of both caspase 8 and caspase 3 within 6h of CD95 crosslinking. In summary, although UVB and CD95 are highly effective agents for induction of DC apoptosis, their efficacy is reduced upon DC maturation. Steroids are less effective than UVB, but appear to act independently of DC maturation while class II crosslinking may be an important signal for the removal of only mature DC *in vivo*.

P7

Localization of CD1e in Dendritic Cells

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CD1e is expressed in monocytes derived dendritic cells (DCs) and Langerhans cells (LCs). In constrast to other CD1, which are first expressed on the surface of DCs and may then internalize into early (EE) or late (LE) endosomal compartments, CD1e is mainly detected in the Golgi of immature DCs or freshly isolated LCs, and never reachs the surface. Upon maturation CD1e is mainly found in LE, cleaved into a soluble form. DCs were shortly incubated with LPS and/or with bafilomycin, known to block the transport between EE and LE/lysosomes, the acidification of LE and, consequently the cleavage of CD1e in a soluble form. Relocalization of CD1e after these treatments was investigated by confocal microscopy using mAbs against the luminal or cytoplasmic (VIIC7) domains of CD1e and Abs staining differents cellular compartments. After one hour of LPS treatment, while HLA-DR compartments are not yet redistributed, $CD1e^+$ vesicules docking on $MHCII^+$ compartments are observed, demonstrating a relocalization to compartments implicated in processing and loading of antigens on CD1b, -c or HLA-DR. Absence of colocalization with EE markers after treatment with both bafilomycin and LPS suggests that CD1e move directly from the Golgi to LE. Surprisingly, under these conditions, VIIC7 does not stained endosomal compartments, supporting the idea that a post-traductional modification or the association with a partner, masking the VIIC7 epitope, is implicated in the transport of CD1e to LE.

P8

Differentially Expressed Proteins in Immature and Mature Dendritic Cells

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Upon activation, tissue residing immature dendritic cells (DC), such as Langerhans cells (LC), start to migrate towards the draining lymph node and mature into efficient antigen presenting cells. During maturation DC loose their capacity to endocytose antigens, change their surface expression of adhesion molecules, chemokine receptors and costimulatory molecules, and their morphology. We have now employed proteomics to identify additional differentially expressed proteins in immature and mature DC. DC were obtained by culturing monocytes with IL-4 and GM-CSF for 6 days. LC were isolated and enriched from normal human skin. Maturation was induced by the addition of LPS. Proteins were separated on 2-dimensional polyacrylamide gels and individual protein spots were analysed by mass-spectrometry. Two of the proteins found to be differentially expressed in immature and mature DC are involved in modulating actin dynamics: Cofilin, which increases the rate of actin-depolymerisation, is highly expressed in immature DC and low in mature DC, whereas profilin, which increases elongation of actin filaments, is up-regulated in mature DC compared to immature DC. Regulation of actin-filament dynamics allows the cell to respond to extracellular signals by moving, changing shape and translocating intracellular organelles. The role of cofilin and profilin in migration of DC, formation of the typical dentritic shape and/or transport of antigens towards the MHC class II rich compartment and of MHC class II-peptide complexes towards the cell surface is currently under investigation.

P9

The Dendritic Cell Cytoskeleton is Critical for the Formation of the Immunological Synapse

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Activation of T cells requires the formation of an immunological synapse between the interface of the T cell and an APC. The T cell cytoskeleton plays an active role in this process; however, based on studies using B cells, it has been widely believed that the APC cytoskeleton is not involved. Using an allogeneic model, we evaluated whether the DC cytoskeleton is important for the ability of DC to cluster and activate resting $CD4^+$ T cells. DC were allowed to cluster with either resting allogeneic or syngeneic $CD4^+$ T cells. Confocal microscopy was used to evaluate the redistribution of the cytoskeletal proteins F-actin and fascin. During clustering with allogeneic T cells, 87% of DC polarized their F-actin and fascin toward the interface with the T cell compared with only 23% of DC clustered with syngeneic T cells, indicating that these changes are antigen-dependent. The cytoskeletal changes in DC are an active process since inhibition of DC actin polymerization using Cytochalasin D (CytD) reduced their ability to cluster resting but not activated allogeneic $CD4^+$ T cells. In addition, pretreatment of DC with CytD prior to mixing with resting $CD4^+$ T cells reduced the MLR in a dose dependent manner. This novel finding provides strong evidence of the DC cytoskeleton's dynamic involvement in the establishment of the immunological synapse with resting T cells. This may partially explain why DC are unique at clustering and activating resting T cells as compared to other APC.

P10

Dendritic Cells Form the Immunological Synapse at a Wide Range of MHC-Peptide Concentrations

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We have demonstrated the active involvement of the DC cytoskeleton in the formation of the immunological synapse with resting $CD4^+$ T cells using an allogeneic system. In this study, we examined these changes in a model that allows evaluation of DC cytoskeleton participation over a wide range of MHC-peptide concentrations. We used the DO11.10 TCR transgenic system, where the transgenic $CD4^+$ T cells recognize an OVA specific peptide in the context of MHC class II (IA^d). Resting $CD4^+$ T cells mixed with DC in the presence of agonist peptide ($OVA_{323-339}$) proliferate in a peptide dose dependent manner with an optimal proliferation at the dose range 300-3000 nM. In contrast, no proliferation was seen with no peptides or antagonist peptide ($OVA_{324-334}$). We used confocal microscopy to evaluate the redistribution of the cytoskeletal protein, F-actin. In the presence of agonist peptide (300 nM), 96% of the DC clustering resting $CD4^+$ T cells polarize their F–actin at the interface with the T cell. However, only 39% and 13% F-actin polarization is seen in DC clustering resting $CD4^+$ T cells in the presence of antagonist (300 nM) or no peptide, respectively. Pretreatment of DC with Cytochalasin A, an inhibitor of the cytoskeletal rearrangement, prior to their mixing with resting $CD4^+$ T cells and agonist peptide significantly reduced the MLR indicating that these cytoskeletal changes are a dynamic process. This study demonstrates that the DC cytoskeleton plays a critical role in the formation of the immunological synapse at a wide range of peptide doses.

P11

Erythrocytes Efficiently Deliver Tat Antigen to Human Dendritic Cells for Initiation of Specific Type 1 Immune Responses *In Vitro*

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Dendritic cells (DC) are an important target for vaccine development against viral infections. The capacity of DC to induce $CD4^+$ and $CD8^+$ T cell responses is enhanced when antigens are provided as particulates. Here, we analyzed human red blood cells (RBC) as a delivery system for Tat protein, and studied whether IFN-γ could improve the antigen-presenting functions of DC. DC were generated from peripheral blood monocytes in human serum, and matured with monocyte-conditioned medium (MCM) in the presence or not of IFN-γ. Tat was conjugated to RBC (RBC-Tat) through avidin-biotin links. DC efficiently and rapidly internalized RBC-Tat. Compared to DC treated with soluble Tat, DC pulsed with RBC-Tat were much more efficient at inducing specific $CD4^+$ and $CD8^+$ T cell responses. DC matured in the presence of MCM were more effective than immature DC in inducing $CD4^+$ and $CD8^+$ T cell proliferation and IFN-γ release. Stimulation of DC with IFN-γ inhibited the production of IL-10 but increased the release of IL-12 and TNF-α. Moreover, IFN-γ-treated DC markedly up-regulated the release of CXCL10 (IP-10) and significantly reduced the secretion of CCL17 (TARC) chemokines, attracting preferentially Th1 and Th2 cells, respectively. Finally, immature or mature DC exposed to IFN-γ were better stimulators of allogeneic T cells, and induced a higher IFN-γ production from Tat-specific $CD4^+$ and $CD8^+$ T lymphocytes. In conclusion, RBC appear an effective tool for Tat delivery into DC, and IFN-γ could be advantageously used for augmenting the ability of DC to induce Th1 responses.

P12

A Comparison of CTL Induction by Different Methods of Antigen Loading of Monocyte-Derived Dendritic Cells

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During this study we aimed to compare the efficacy of three different methods of antigen loading of dendritic cells at inducing MHC-class I restricted antigen specific responses, using allogeneic leukemic cells as a source of antigens for processing and presentation by the dendritic cells to autologous T cells. Dendritic cells were pulsed with either whole cell tumor lysates prepared by three freeze/thaw cycles or with apoptotic leukemic cell fragments, or were fused directly to the leukemic cells to generate hybrid cells. Dendritic cell/leukemic hybrids and dendritic cells pulsed with apoptotic leukemic cell fragments were both efficient at generating CTL responses when tested against antigen-pulsed dendritic cells used as targets. However, the hybrid cells induced a more potent CTL response as judged by a significantly higher percentage of targets being lysed. We also report that the CTL responses were MHC class I restricted and antigen-specific, as shown by the inability of the CTL to lyse antigen-pulsed allogeneic dendritic cells or autologous dendritic cells pulsed with different antigens, respectively. On the other hand, dendritic cells pulsed with freeze/thaw lysates prepared from the leukemic cells induced very weak CTL responses. We thus conclude that the method used for loading antigens into the dendritic cells is critical to the outcome of the CTL response. Our data suggests that dendritic cells that express endogenously synthesised antigens as is the case with the dendritic cell hybrids are better stimulators of MHC class I restricted CTL responses to dendritic cells which acquire exogenous antigens, suggesting further that the classical pathway of antigen presentation is more efficient than cross-presentation for the induction of CTLs.

P13

UV-A Irradiation Inhibits the T-Cell Stimulatory Function of Mature Murine Langerhans Cells

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UV irradiation of Langerhans cells (LC) leads to different effects on their viability and their capacity to stimulate T cells. This depends critically on the wavelength (i.e. UV-A vs. UV-B). UV-A has been shown to inhibit LC function because CD54 and co-stimulatory molecules (CD80, CD86) are not up-regulated any longer during subsequent culture. We tested the susceptibility of mature LC, i.e. LC that already express high levels of CD54, 80, and 86, to UV-A. A pure UV-A source (''Sellmair lamp''), which emits exclusively above 320 nm, was used to irradiate 2-day-cultured mature LC. A clearcut dose-dependent reduction of T cell proliferation occurred, starting at 20 kJ per m^2 and leading to almost ''T cell only'' background at 60 kJ per m^2. However, after two further days in culture, about 60% of these LC had survived both in irradiated (60 kJ per m^2) and unirradiated control cultures. LC, that had been irradiated with 60 kJ per m^2 on day 2, re-gained their full ability to stimulate T cells by day 4. This is in sharp contrast to UV-B irradiation which is not reversible and where LC eventually die. A similiar inhibitory effect could be seen with ''Sylvania F20 T12'' and ''Osram-Vitalux'' lamps that are commonly used as sources for UV-B. These lamps also have a small emission window in the UV-A area which is energetically sufficient to cause an effect as shown in experiments where all the UV-B (< 320 nm) was filtered out, yet the inhibitory effect still remained. We conclude that UV-A may not be as ''innocent'' as often assumed, and further investigations seem warranted.

P14

Cross-Presentation of Vaccinal Lipopeptides Derived from HIV-1 Reverse Transcriptase by Human Dendritic Cells

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An efficient vaccine against HIV must induce good cellular immune responses and primary stimulation of T $CD8^+$ lymphocytes requires dendritic cells (DC) as presenting cells. Lipopeptides are currently used in vaccine trials. We have already shown that a model lipopeptide containing a short epitopic peptide from HIV-1 was endocytosed and presented in association with MHC class I by human DC. To determine more precisely this cross-presentation pathway, we have used a longer lipopeptide ($Pol_{461-484}$), which requires processing to be presented. As the shorter model, this lipopeptide was rapidly endocytosed into immature DC, as shown by co-localization of Rhodamine-labeled analog and FITC-Dextran (by confocal microscopy). The lipopeptide induced functional stimulation of HLA-A*0201-restricted $Pol_{476-484}$ epitope-specific $CD8^+$ T lymphocytes, as detected by intracellular IFN-γ flow cytometric analysis. The presentation was not inhibited by monensine, which inhibits endosomal acidification and therefore enzymatic degradation. Conversely, it was inhibited by a proteasome specific inhibitor and by brefeldin A. Therefore, the cross-presentation pathway followed by this long vaccinal lipopeptides in human DC was endocytosis, then degradation in the cytoplasm before association with MHC I molecules. These well-defined molecules are a good model for studying antigen processing in the cross-presentation pathway, and the results will be useful for vaccine design and to address vaccines into this particularly efficient pathway for protective $CD8^+$ T lymphocyte induction.

P15

Cross Presentation of Cell-Associated Antigens by Dendritic Cells in the Steady State Leads to Peripheral Tolerance *In Vivo*

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Dendritic cells (DCs) serve as powerful adjuvants for the initiation of cell-mediated immunity, if the DCs are charged with antigens *ex vivo* and then injected into mice or to humans. To probe the function of DCs that are found *in vivo* in the steady state, i.e. in the absence of any *ex vivo* manipulation, we injected mouse splenocytes charged with ovalbumin (OVA) according to the method of Carbone et al (J Exp Med 171: 377-8, 1990). It is known that the OVA in the injected splenocytes is efficiently cross-presented on the MHC class I products of the recipient DCs (J Exp Med 192: 1685-96, 2000; J Immunol 166: 6099-103, 2001). We have obtained similar findings when we inject OVA-charged splenocytes from mice genetically depleted of the TAP transporters (TAP–/–) into wild type C57BL/6 (TAP+/+) recipients. Specifically, when we isolate CD11c positive (DC-enriched) and CD11c negative (DC-depleted) fractions from the recipients of OVA charged splenocytes, only the DCs cross-present antigen to OT-I TCR transgenic, OVA-specific, CD8+ T cells. When the OT-I T cells are injected into mice, followed by an intravenous infusion of TAP–/– OVA splenocytes in the steady state, the T cells proliferate rapidly for 2 days first in the draining lymphoid tissue (spleen) and later reach distal lymph nodes. However the T cells are ultimately deleted, because few can be found in spleen, lymph node or blood 10 days later. Likewise, the OT-1 injected mice became unresponsive systemically to antigen rechallenge *in situ* if the splenocytes are captured by DCs in the steady state, but immune if the OVA-splenocytes are captured by DCs maturing in response to an agonistic anti-CD40 antibody. The tolerance to OVA that is induced by DCs in the steady state is seen even with powerful adjuvants, i.e. rechallenge with OVA in complete Freund's adjuvant or pulsed onto mature *ex vivo* derived DCs. The OT-I cells from control mice given splenocytes charged with an irrelevant protein, Hen Egg Lysozyme (HEL), remain strongly responsive to OVA, being able to proliferate and produce IFN gamma in ELISPOT assays. We conclude that the capture of cell-associated antigens by DCs *in vivo* in the steady state leads to peripheral tolerance. It is proposed that this is an essential role for DCs *in vivo*, i.e. to induce tolerance to self-peptides so that subsequent immune responses to dying cells will focus on microbial rather than self antigens. Without the establishment of peripheral tolerance to those cell-associated self-peptides that can be presented by DCs, one would be at great risk of developing autoimmunity whenever DCs are called upon to present infected self tissues.

P16

Effect of Dinitro-Fluorobencene in Amphibian Langerhans Cells

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Recently, we demonstrated two subsets of amphibian epidermal dendritic cells (DC) similar to mammalian Langerhans cells. One was located suprabasally and was ATPase+/class II– whereas the other had a basal location and was ATPase+/class II+. Both subsets had ultrastructural pattern of Langerhans cells except for the Birbeck granule. The objective of these work was to search the modifications in the amphibian DC with an hapten. Ten Rana pipiens were painted with 50 ml of DNFB at 0.1% 0.5, 1, 1.5 and 2 hrs. Epidermal sheets were obtained and treated to demonstrate ATPase and class II molecules. Other samples were processed for electron microscopy. We observed a diminution of ATPase+/class II– and an increase in the ATPase+/class II+ subset densities. We appreciated too an increased number of class II+ DC in the underlying connective tissue. At ultrastructural level we observed that DC had numerous vesicles with some myelinic bodies, but no evidence of Birbeck granules was shown. Results suggest that ATPase+/class II– dc diminished as a result of depletion of the enzyme caused by the continuous endocytosis process, whereas ATPase+/class II+ dc increased by the amplified synthesis and membrane expression of class II molecules for antigen presentation. We considered that both subsets are the same type but in a different functional state. On the other hand, the lack of Birbeck granules is not an evidence that these cells do not represent the homology of langerhans cells in amphibians, because they have all the other morphological characteristics of mammalian Langerhans cells.

P17

Cholera Toxin and its B Subunit Differentially Modulate Antigen Presentation by Mucosal Dendritic Cell Subsets

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Mucosal administration of antigens linked to cholera toxin B subunit (CTB) can induce peripheral T cell tolerance whereas co-administration of cholera toxin (CT) breaks it. The mechanisms that govern induction or abrogation of tolerance in that system remain unknown. We examined the phenotypical and functional properties of DC subsets in the gut-associated lymphoid tissues from mice fed either CT or CTB. Two CD11c+ DC subsets were identified in both Peyer's patches (PP) and mesenteric lymph nodes (MLN), based on expression or not of CD8a. A third and predominant subset with intermediate levels of expression of CD8 was exclusively disclosed in MLN. Further, a discrete subset of CD11c+ but CD8– DCs was identified in the intestinal wall and could constitute the muco-epithelial equivalent of LCs. Feeding mice with CT led to the selective increase of the CD8int DCs in MLN and of intestinal wall DCs whereas CTB had no apparent effect. Thus, this MLN DC subset may play a major role in the induction of mucosal immune responses to gut luminal antigens adjuvantized by CT. Adoptive transfer studies are now underway to examine the potential role of these APCs in the induction or abrogation of oral tolerance (EC Biotech IV).

P18

Effective Treatment of Established Melanoma Tumors with Antigen Loaded Dendritic Cells Generated Under FCS-Free Conditions Requires IL-2

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Melanoma, despite its aggressive growth characteristics, is an immunogenic tumor expressing several characterized neo- and differentiation antigens. Dendritic cells (DC) when pulsed with defined peptides induce tumor specific T cell responses and protect animals from challenge with melanoma. However, DC treatment of metastatic disease so far has failed to induce significant tumor regressions. We therefore set up a therapeutic model using C57BL/6J mice and the immunogenic melanoma cell line B78-D14. To produce skin and lung tumors groups of mice received s.c. or i.v. injections of 2×10^6 tumor cells. After 1 and 2 wks mice were vaccinated with 20.000 DC, either bone marrow derived DC or epidermal Langerhans cells (LC), loaded with the a TRP2 peptide. Generally, DC cultured with FCS proved to induce a dominant unspecific response. This was not seen using LC cultured in 1% mouse serum, however, vaccination with TRP2 loaded FCS-free LC alone failed to influence the growth of established B16 tumors. A reproducible reduction of tumors in size and weight was only obtained, if LC vaccinations with TRP2 were followed by a 5 d treatment of mice with IL-2, 200.000 IU i.p. twice/d. Omitting the TRP2 peptide abolished the efficacy of this combined treatment, demonstrating the crucial role of priming a melanoma specific T cell response. Collectively our data suggest that a DC based T cell priming needs an IL-2 mediated expansion of T cells to become effective and that this combined treatment might be helpful in melanoma patients with metastatic disease.

P19

Long Lasting Immunity in DNA-Vaccinated Mouse is Maintained by Antigen-Pulsed Spleen Dendritic Cells

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The mechanism underlying the long-lasting humoral and cellular immunity due to a single injection of DNA vaccine was studied. Plasmid pCAGGS(S) constructed by inserting the genes of hepatitis B surface antigen (HBsAg) into the multiple cloning site of the parental plasmid, pCAGGS, was injected (100 μg per mouse) once into the regenerating anterior tibialis muscle of C57BL/6 mouse. HBsAg-specific memory T cells were isolated from C57BL/6 mouse injected twice with 10 μg of HBsAg. Spleen dendritic cell (DC) were isolated by density centrifugation. Infiltration of DC and expression of HBsAg were studied at the site of injection by immunohistochemistry. A single injection of pCAGGS(S) induced antibody to HBsAg and HBsAg-specific T cells in C57BL/6 mouse, which persisted for as long as 48 weeks. Infiltrating cells including DC were detected at the site of DNA injection and some injection of pCAGGS(S) induced proliferation of HBsAg-specific memory lymphocytes without addition of HBsAg, indicating that the spleen DC from these mouse were pulsed with HBsAg *in situ*. Existence of HBsAg-pulsed DC in the spleen may have a critical role in the persistence of long-lasting immunity due to a single injection of DNA vaccine.

P20

p38 MAPK is Activated by the Chemical Sensitizer DNFB in a Fetal Skin Dendritic Cell Line

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Dendritic cells (DC) play a pivotal role in the induction of cutaneous immune response, namely in the sensitization phase of allergic contact dermatitis (ACD). However, the intracellular mechanisms involved in the activation of these antigen presenting cells (APCs) remain unclear. We investigated the effect of a strong skin sensitizer, 2,4-dinitrofluorobenzene (DNFB), and its inactive analogue, 2,3-dicloronitrobenzene (DNCB), on the activity of mitogen activated protein kinases (MAPKs) in a dendritic cell (DC) line generated from fetal mouse skin (FSDC), precursors of Langherhans cells. DNFB (5 μg per ml) increased significantly the phosphorylation (activity) of p38 high osmolarity glycerol protein kinase (p38/HOG), but not the phosphorylation of extracellular regulated kinase (ERK), or stress-activated protein kinase/c- jun NH_2- terminal kinase (SAPK/JNK). Maximal phosphorylation of p38 was observed after 30-45 min incubation with DNFB, as determined by Western blot, using phospho-specific antibodies. The results indicating that stimulation with the strong sensitizer DNFB, at low concentration, activates p38 MAPK suggest that this kinase may be involved in the activation of APCs during the sensitization process of ACD.

P21

Characterisation of CD11c/CD1a Positive Dendritic Cells for Identification of Contact Sensitisers

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In vitro alternatives for the identification of contact sensitisers have examined the use of human blood derived dendritic cell (DC) cultures. Cultures exposed to the allergen 2,4-dinitrochlorobenzene (DNCB) have shown up-regulation of certain markers, although with variability and not always with evidence of specificity. We aimed to derive a purified DC population (CD11c+ cells), a cell type more relevant for identification of skin allergens. Pure populations of DC were isolated from human peripheral blood using BCDA-1 magnetic bead separation. The cells were cultured for 5 days in a combination of granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-4 (IL-4) and transforming growth factor–beta (TGF-β), which generates ''Langerhans cell like'' cells. This approach produced a population which was 95% CD11c/CD1a positive as determined by flow cytometry. The cells expressed HLA-DR but not CD86 or CD83, demonstrated endocytic ability (FITC-dextran uptake), were weak stimulators of the mixed lymphocyte reaction and thus could be regarded as an ''immature'' phenotype. Exposure of these cells to subtoxic doses of DNCB resulted in elevated expression of HLA-DR (6-7 fold increase in mean fluorescence intensity [MFI]) and CD86 (15-20 fold increase in MFI) compared with control cells. Concurrent treatment with subtoxic doses of the irritant sodium lauryl sulphate or 0.1% DMSO (vehicle control) did not induce up-regulation of HLA-DR or CD86. Culture of blood derived CD11c+ dendritic cells may provide a population of Langerhans like cells for evaluation of potential future *in vitro* approaches to the identification of skin sensitisers.

P22

Reactivity of Langerhans Cells in Reconstructed Human Epidermis to Exposure to Allergens and UV Radiation

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To study the behavior of Langerhans cells in reconstructed human epidermis, we have analyzed their density, dendricity, and the expression of IL-1β and CD86 mRNA after exposure to known allergens or Solar Simulated Radiation (SSR). $CD34^+$ cord blood progenitors were cultured for 7 days in StemBio A medium in the presence of SCF (replaced by TGF-β at day 4), GM-CSF and TNF-α to generate a differentiated dendritic cell population. These cells together with human keratinocytes and melanocytes were seeded onto human de-epidermized dermis or the Episkin® support. After 2 weeks of culture, the resulting reconstructed epidermis containing the Langerhans cells was exposed to topically applied allergens or SSR. The reactivity of the Langerhans cells was analyzed 24 h later. The number of Langerhans cells and their morphology were analyzed after Langerin immunostaining. Changes in IL-1β and CD86 mRNA expressions were evaluated by RT-PCR. Exposure of the reconstructed epidermis to allergens resulted in a dose-dependent decrease in the number of Langerhans cells and an over-expression of IL-1β and CD86 mRNA. Exposure of the reconstructed epidermis to SSR caused a decrease in the number of the Langerhans cells and a reduction in dendricity of the remaining Langerhans cells. The SSR-induced changes, which resemble very much *in vivo* observations attributed to the phenomena of immunosuppression, were prevented by the topical application of a sunscreen before irradiation. We propose this model to predict the sensitizing potential of new chemicals and to evaluate the protective effect of sunscreens against UV-induced immunodamages.

P23

Reduced Susceptibility of Human Immature Dendritic Cells (iDC) to NK Cell Lysis After Interaction with the Opportunistic Yeast Malassezia furfur (Mf)

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The yeast *Mf*, normally present in the human skin microflora, is nevertheless a factor contributing to the severity of the chronic inflammatory skin disease atopic dermatitis (AD). The aim of this study was to assess whether co-culture of *Mf* and human iDCs would affect subsequent interactions of DCs with autologous NK cells. iDCs pre-incubated with *Mf* showed a reduced susceptibility to NK cell killing when compared to untreated iDCs. Moreover, also when iDCs were treated with *Mf*-iDC co-culture supernatant, a reduced susceptible to killing was observed, indicative of the presence of soluble factors. The numbers of CD40, CD80, CD83 and CD86+ DCs increased and HLA-DR expression was up-regulated after supernatant treatment. NK ($CD56^+/CD3^-$) cells were found in the dermis of skin biopsy specimens from healthy individuals and AD patients. However, in the AD patients $CD56^+$ cells were also found in the epidermis. These data suggest that NK cells and iDCs can interact, that *Mf* affects this interaction and that NK cells may play a role in regulating DCs. Furthermore, the data imply that DCs and NK cells can meet in the skin.

P24

Study of the Phenotype and Function of Langerhans Cell Histiocytosis Cells

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Langerhans cell histiocytosis (LCH) consists of lesions composed of cells with characteristics of Langerhans cells (LC). The clinical course of LCH ranges from spontaneous resolution to a chronic and sometimes lethal disease. We studied 25 patients with various clinical forms of the disease. In bone and chronic lesions, LCH cells had immature phenotype and function. They coexpressed LC antigens CD1a and Langerin together with monocyte antigens CD68 and CD14. Class II antigens were intracellular and LCH cells almost never expressed CD83, CD86 or DC-Lamp despite their CD40 expression. Consistently, LCH cells sorted from bone lesions (eosinophilic granuloma) poorly stimulated allogeneic T-cell proliferation *in vitro*. Strikingly, however, *in vitro* treatment with CD40L induced the expression of membrane class II and CD86 and strongly increased LCH cell allostimulatory activity, to a level similar to that of mature DC. Numerous $IL\text{-}10^+$, $Langerin^-$, $CD68^+$ macrophages were found within bone and lymph node lesions. In patients with self-healing and/or isolated cutaneous disease, LCH cells had a more mature phenotype. LCH cells were frequently $CD14^-/CD86^+$ and macrophages were rare or absent, as were IL-10 expressing cells. We conclude that LCH cells in the bone/chronic forms of the disease accumulate within tissues in an immature state, that most probably results from extrinsic signals, and may be induced to differentiate towards mature DC after CD40 triggering. Drugs that enhance the *in vivo* maturation of these immature DC, or that induce their death may be of therapeutic benefit.

P25

Cholera Toxin Induces Activation of Langerhans Cells *In Vivo*

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The development of non-invasive methods for the delivery of proteins through the permeability barriers, such as the intact skin, will greatly facilitate the administration of human and veterinary vaccines. Our studies have demonstrated that topical application of antigens formulated in lipid-based delivery systems induces antigen-specific immune responses. These responses can be significantly enhanced by coadministration of antigens with cholera toxin (CT). The mechanism by which CT exerts its immunoadjuvant effect is not known but it is likely related to its ability to activate dendritic cells (DC). In this study we sought to evaluate the effect of CT on epidermal dendritic cells (Langerhans cells; LC) following topical application to intact skin. Our results indicate that within 8 h of application of CT, epidermal LC cells become round and exhibit enhanced expression of MHC class II and costimulatory molecules. These changes are accompanied by increased ICAM-1 expression in the skin and increased migration of fluorescent labeled cells from the skin to the draining lymph nodes. Taken together, these findings suggest that CT induces activation of LC and stimulates the migration of cells to the lymph nodes. These results provide important information for the development of effective and safe transcutaneous vaccines.

P26

Factors Affecting the Differentiated State of Langerhans Cells in Epidermis

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Cytokines, in particular GM-CSF and TNF-α, together with serum factors, are known to influence the differentiation of precursors into Langerhans cells (LC) and of the latter into "mature" dendritic cells. This research has addressed the role of serum separately from that of cytokines in maintaining the differentiated state of LC within epidermis. Epidermal sheets isolated from human skin biopsies were incubated without supplements or with either 10% fetal calf serum or cytokines (TNF-α, 0.25-25 ng per ml; GM-CSF, 0.05-0.5 ng per ml; or a combination of the two at highest doses). Upon labeling for CD1a, the mean cell surface area (an estimate of cell volume) and perimeter length (an estimate of dendriticity) decreased significantly ($p < 0.01$, anova) within 48 h culture without supplements. These decreases were almost entirely prevented by addition of serum ($p < 0.01$ vs. without serum), but not by any cytokine or their combination, at variance with previous results with serum. Upon labeling for major histocompatibility complex class II molecules, all parameters analyzed exhibited inconsistent, not significant variations among experiments and among experimental conditions. The results suggest that CD1a varies in distribution upon culture, without changes in cell shape and size, and that the two antigens analyzed are differentially regulated in LC within epidermis. The distribution of CD1a seems to be in part dependent on serum factors, therefore the previously recognized effects of cytokines in the presence of serum may depend on concomitant factors acting on LC, or be mediated by keratinocytes whose response to cytokines possibly depends on trophic stimuli given by serum.

P27

Expression and Function of Histamine Receptors in Human Dendritic Cells

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Dendritic cells (DC) are antigen-presenting cells specialized in the activation of naive T lymphocytes and the initiation of immune responses. Immature DC resides in most tissues, where they are adapted to capture antigens and alert for danger signals such as microorganisms, inflammatory cytokines and cell necrosis. Histamine is a well-known mediator eliciting different responses in immune and non immune cells. Here we show that immature and mature dendritic cells (DCs) express the mRNA for H_1, H_2 and H_3 histamine receptors. Histamine induced intracellular Ca^{2+} transients, actin polymerization and chemotaxis in immature human DC. Maturation of DC resulted in the loss of histamine-induced chemotaxis, actin polymerization and Ca^{2+} responses. However, in maturing DC histamine dose-dependently enhanced intracellular cAMP levels and stimulated interleukin (IL)-10 secretion, while it inhibited production of IL-12. As a consequence, the ability of mature DC to generate allogeneic type 1 responses was impaired by histamine. The use of specific histamine receptor agonists or antagonists revealed that Ca^{2+} transients, actin polymerization and chemotaxis of immature DC were prevalently due to stimulation of H_1 and H_3 receptors receptor subtypes. Modulation of IL-12 and IL-10 secretion by histamine involved exclusively the H_2 and H_3 receptors. Our study suggests that histamine has important and complex biological effects on DC activities, opening the possibility that histamine released during inflammatory or immune responses could regulate DC functions and ultimately favor type 2 lymphocytes-dominated immunity.

P28

Neuropeptide Y and Cutaneous Immunity

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Epidermal Langerhans cells (LC) reportedly can be stained with antibodies to neuropeptide Y (NPY) in the setting of atopic dermatitis. Therefore, our laboratory examined expression of NPY and the related factor peptide YY (PYY) by the epidermis-derived dendritic cell line XS52 (BALB/c) and highly-enriched populations of BALB/c LC. While both populations of cells were found to contain mRNA for NPY and PYY by RT-PCR, radioimmunoassay of supernatants conditioned by these populations of cells revealed the presence of detectable levels of PYY secretion only from XS52 cells. The effects of NPY are mediated by at least 6 G protein-coupled receptors termed Y1, Y2, Y3, Y4, Y5 and Y6. By RT-PCR, Y1, Y2, Y4, Y5 and Y6 receptors could not be detected on XS52 cells or on highly-enriched populations of murine LC. In order to examine whether production of these factors by cells in the epidermis may have immunologic consequences, naïve mice were injected intradermally with 500 pmol of NPY, PYY or diluent alone. Fifteen minutes later 5 mice from each group were immunized by topical application of 5 μL of 1% dinitrofluorobenzene (DNFB) at the injected site. One week later all mice as well as non-immunized negative control animals were challenged on the ears by application of 5 μL of 0.2% DNFB to each side of earch ear and 24 h ear swelling was assessed. Mice immunized at NPY injected sites exhibited a significantly decreased contact hypersensitivity response compared to the diluent and PYY groups. The locus of action of NPY in this activity remains unknown. NPY may have a regulatory role for immune reactions within the skin.

P29

The Adenosine Receptors A2a and A3 are Differentially Expressed During Dendritic Cell Maturation

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In an attempt to characterize dendritic cell (DC) functional stages we performed a gene array of immature vs. mature monocyte derived DC. One group of differentially regulated molecules were the adenosine receptors A2a and A3. The mRNA levels were ~4 fold up-regulated and ~10 fold down-regulated during maturation, respectively. We found little expression of mRNA coding for the other known adenosine receptors, A1 and A2b in immature DC only. To collect information on the possible involvement of adenosine and it's receptors in DC function we added the stable adenosine-agonist 5¢-N-ethylcarboxyamidoadenosine (NECA) to maturing DC. Like adenosine, NECA binds to all adenosine receptors. Using untreated and treated DC we compared T-cell activating capacity (mixed leukocyte reaction), surface marker expression (FACS analysis), T-cell cytokine profile (ELISA) and transcription of genes coding for a pannel of DC function related molecules (quantitative PCR analysis). Preliminary results show an induction of mRNA transcription coding for proinflammatory cytokine receptors (CXCR4, CCR7) in immature DC. These receptors are known to be up-regulated during maturation. To find out whether these changes are mediated by the A2 or the A3 receptors we are currently using specific receptor antagonists in similar experiments.

P30

Are Extracellular Nucleotides Danger Signals for Dendritic Cell Activation?

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Factors released at sites of tissue damage, or during the early phases of inflammation can have a role in shaping the immune response. Thus, it has been postulated that cells undergoing stress or necrotic cell death release factors that trigger immune cells, in particular dendritic cell (DC) activation, and initiate primary immune response. Identity of such factors is largely unknown, but for a few examples such as CD40L, TNFα, IL-1β. Substances already present in the cell cytoplasm (constitutive danger signals) may alert the immune system when released into the extracellular environment. We provide evidence for a role of intracellular nucleotides (in particular ATP) as endogenous danger signals. ATP is normally present at a 5–10 mM concentration in the cytosol of all cells. Its concentration in the extracellular milieu is negligible. Release of ATP occurs following cell membrane stretching or injury, or upon stimulation with inflammatory agents such as LPS. Once in the extracellular medium, ATP stimulates in a autocrine/paracrine fashion specific plasma membrane receptors, named purinergic P2, and triggers an increase in the intracellular Ca^{2+} concentration, MAPK activation, cytoskeletal rearrangement, chemotaxis, IL-1β and TNFα secretion. In DC, low ATP concentrations may also drive maturation. Sustained exposure to high ATP concentrations on the contrary causes DC apoptosis. We conclude that extracellular ATP is a powerful activator of DC and other immune cells and suggest that this nucleotide may have an important role as a trigger and modulator of the early phases of the immune response.

P31

Dehydroepiandrosterone Sulfate Regulates Cortisol-Induced Suppression of Epidermal Langerhans Cells

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Dehydroepiandrosterone (DHEA) which decreases as people are aged is considered as an antagonizing factor to aging and stress. Effects of DHEA sulfate and cortisol on Langerhans cells were examined. Epidermal cells were prepared from Balb/c mouse skin. Langerhans cells were enriched by I-A antibody-bound magnetic beads. Langerhans cell population was determined by flow cytometry. Mixed epidermal cell lymphocyte reaction (MELR) was performed with T cells prepared from C57BL mice by nylon wool. Langerhans cell population decreased after incubation with cortisol for 24 h. The down-regulation was blocked by DHEA sulfate. DHEA sulfate alone did not affect the population. MELR was suppressed by pretreatment of epidermal cells with cortisol. The suppression was not observed in the presence of DHEA sulfate. Addition of cortisol to the mixed culture caused more suppression and the suppression was blocked by DHEA sulfate little. DHEA was suggested to antagonize the suppressive effect of cortisol on Langerhans cells.

P32

Identification of Genes Specifically Modulated in Dendritic Cells During Maturation

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The application of high density DNA array technology to monitor gene expression transcription has been responsible of a paradigm shift in biology and we have now the ability to measure the expression of a significant proportion of the genome in single experiment. In the field of immunology the analysis of gene expression profiles has revealed that cells respond to an activation signal with waves of coordinated gene expression profiles. DCs represent the professional antigen presenting cells which uptake, process and present antigens to naive T cells inducing their priming. In order to acquire this ability DC have to undergo a process of maturation that involve the activation of hundreds of genes. Global expression profiles of DCs stimulated with Gram-negative bacteria at different time points has been performed using microarrays representing 11000 known genes and ESTs. Gene expression data have been organized using two different type of clustering, the hierarchical clustering and the self-organizing maps. Approximately 30% of the genes displayed on the chips were called present at each time point after bacteria stimulation. Using stringent conditions, we identified about 1500 gene differentially expressed during DC maturation: 800 ESTs and 700 known genes. These genes mostly encoded enzymes and proteins involved in the control of cell cycle, apoptosis, cytoskeleton rearrangements and activation and control of inflammation.

P33

Crosslinking of Leukosialin (CD43) with Monoclonal Antibody 6F5 Induces Apoptosis in Dendritic Cells

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Dendritic cells (DC) play a central role in the induction and regulation of immune responses. This capacity has to be controlled in order to avoid overwhelming immune reactions. One of these potential control mechanisms is programmed cell death of DC. Here we report that a monoclonal antibody (mAb) against CD43 generated in our laboratory induces apoptosis in DC. CD43 is broadly expressed on peripheral blood leukocytes. Yet, we observed that CD43 triggered apoptosis is specific for DC, since engagement of CD43 on monocytes, lymphocytes, granulocytes or cell lines did not result in apoptosis. CD43 induced DC death is rapid and efficient and occurs in immature as well as mature monocyte-derived DC, which is in contrast to apoptosis observed after HLA-DR triggering. Interestingly, also peripheral blood DC (LIN-, HLA-DR+) were driven into apoptosis upon CD43 triggering with 6F5mAb. Cross-linking of the CD43 epitope was found to be mandatory, since Fab-fragments of 6F5 failed to induce apoptosis. DC apoptosis appears to be specific for the epitope recognized by the 6F5 mAb, since an antibody against a different epitope showed no such effects. We conclude from these data that the mAb 6F5 recognizes an epitope of CD43 which represents a potential target structure via which DC can be selectively eliminated.

P34

Stimulation of Blood-Derived Dendritic Cells by Ultraviolet B Radiation During Apoptosis

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UVB is considered to suppress skin immune system by killing or altering LC function. However, we have previously demonstrated that some LC subpopulations showed enhanced expression of costimulatory molecules and increased proinflammatory cytokine production after UVB, proposing the possibility that UVB partly potentiates skin immune reactions. In this study, we examined the mechanism of the stimulation or apoptosis of LC induced by UVB using blood-derived dendritic cells (BDC). BDC, which were obtained from CD14-selected PBMC by culturing them with GM-CSF and IL-4 for 6 days, were irradiated with various doses of UVB (25-200 J per m^2). Flow cytometry was used to detect cell surface molecules on BDC. RT-PCR and ELISA were utilized for the analysis of cytokines. UVB caused apoptotic cell death on BDC in a dose-dependent manner, while simultaneously stimulated BDC to up-regulate CD86 surface expression, and mRNA or protein synthesis of proinflammatory cytokines such as IL-1β, IL-6, IL-8, and TNF-α. Inhibition experiments using p38 MAPK inhibitor, SB203580 showed that p38 MAP kinase cascade was largely involved only in the former process. These results suggest that UVB exerts contradictory effects on BDC which involve the complex intracellular pathways.

P35

A Clinically Approved Oral Vaccine Against Urinary Tract Infections Induces the Terminal Maturation of CD83$^+$ Immunostimulatory Dendritic Cells

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Dendritic cells (DCs) are important antigen-presenting cells of the immune system, which have attracted interest as cellular adjuvants to induce immunity in clinical settings. We have investigated the effects of Uro-Vaxom®, an oral vaccine composed of different E. coli strains on human monocyte-derived dendritic cells (moDCs). Uro-Vaxom® induced the terminal maturation of CD83$^+$ moDCs. MoDCs stimulated with Uro-Vaxom® displayed a phenotype of activated DCs with high levels of major histocompatibility complex (MHC) molecules and increased levels of adhesion and co-stimulatory molecules. In addition, moDCs activated with Uro-Vaxom® exhibited enhanced T cell-stimulatory capacity in the allogeneic mixed leukocyte reaction. Uro-Vaxom® at 100 µg per ml was as potent as TNF-α at 1000 U per ml in activating human moDCs. In DC-based immunotherapy, Uro-Vaxom® could be used as a stimulus of DC maturation, which meets the standards of good manufacturing practice (GMP). In addition, vaccination with Uro-Vaxom®-loaded moDCs may be an attractive treatment option in preventing recurrent urinary tract infections in predisposed individuals.

P36

Direct Transfection and Activation of Human Langerhans Cells *In Situ* Using Biolistic Delivery of Naked DNA

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Delivery of antigens to Langerhans cells (LC) in order to induce specific immune responses against tumors represents a challenge for vaccine development and for this purpose genetic immunization strategies have emerged as an attractive approach. In the present work, to address the possibility of "*in situ*" transfection of human Langerhans cells (LC), we used the gene gun to transduce human skin organ cultures with plasmid DNA encoding the reporter green fluorescent protein (pEGFP) or firefly luciferase (pLuc). To analyze the function of transfected LC we used a plasmid DNA encoding melanoma antigen MART-1 under the control of the hIE-CMV promoter. Gold delivery to LC and the expression EGFP was analyzed *in situ* in the population of CD1a$^+$ cells in epidermal sheets by confocal microscopy and in cytospin preparations of epidermal cell suspensions. The quantification of transgenic proteins was determined in epidermal or dermal cell suspensions as well as in purified CD1a$^+$ cells, from skin samples transfected with pLuc. The viability of CD1a$^+$ LC and skin migratory dendritic cells was analyzed after gene delivery in cytospin preparations and by transmission electron microscopy. Activation and migration of LC from transfected epidermis was determined 1, 3, 6, 12, 24 and 48 h after transfection of skin samples with pEGFP and pLuc, or after delivery of empty plasmid backbones or unloaded gold particles. The possibility of direct transfection of skin dendritc cells (DC) was analyzed by RT-PCR in the population of migratory DC from skin samples transfected with genes under the control of the hIE-CMV or the keratinocyte specific promoter K14. The ability of transfected LC to process and present transgenic peptides in MHC-I restricted manner was analyzed in co-cultures of LC transfected with a plasmid DNA encoding the human melanoma antigen MART-1 with a specific CTL clone HLA-A2 restricted. We conclude that: i) LC can be directly transfected using a gene gun, ii) the viability of LC is not compromised by the transfection method employed iii) biolistic gene delivery is sufficient to stimulate the activation and migration of epidermal LC and iv) LC can efficiently process transgenic proteins and present the resulting peptides to a MART-1 specific CTL.

P37

Entrapment and Antigen Loading of Langerhans Cells In Situ by an Artificial Chemokine Gradient

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Many dendritic cell (DC)-based vaccine protocols have been developed for the treatment of cancer patients. However, time- and cost-consuming "customizing" processes are required in the current format, e.g. isolation, expansion, and antigen-pulsing of DC. Here we report a novel format that requires no *ex vivo* DC manipulation. We sought to entrap migratory Langerhans cells (LC) by creating an artificial chemokine gradient in their homing path. We formulated ethylene-vinyl-acetate (EVA) polymer rods releasing MIP-3β (LC-attracting chemokine) in a controlled fashion. When MIP-3β rods were implanted subcutaneously into mouse abdomen and DNFB was applied over the implantation sites, marked LC accumulation was observed around the rods. FITC-triggered LC homing to draining lymph nodes (LN) (assessed by counting IA+/FITC+ LN cells) was markedly inhibited by MIP-3β rod implantation at 24 hr. Recovery of IA+/FITC+ LN cells increased thereafter to the normal level at 72 hr, indicating that LC can be entrapped transiently without affecting their homing potential. As an attempt to load migratory LC with tumor-associated antigens (TAA) at the entrapment sites, we co-implanted MIP-3β rods together with the second EVA rods releasing ovalbumin (OVA) and applied DNFB 24 hr later. These mice developed potent CTL activities to lyse the OVA-transduced E.G7-OVA tumor target, and exhibited almost full protection against subsequent challenge with the same tumor cells. Thus, tumor-specific protective immunity is readily inducible by combining MIP-3β-mediated LC entrapment and EVA polymer-based LC loading with relevant TAA. Our *in situ* LC vaccine strategy represents a major breakthrough moving the DC-based vaccine concept toward practical medicine.

P38

Role of Matrix Metalloproteinases in Migration of *In Vitro* Generated Langerhans Cells and Epidermal Langerhans Cells, Implication of TNFα

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Langerhans cells (LC) are potent dendritic cells for induction of primary T cell-mediated immune responses. The implication of matrix metalloproteinase-9 (MMP-9) in the migration was suggested by Kobayashi et al in mouse model and by Lebre et al in human skin explants. In our *in vitro* model using Langerhans like dendritic cells derived from $CD34^+$ cord blood cells (LLDC), we investigated the role of MMP-9 and other MMPs on human cell migration.

Immature LLDC, as epidermal LC, are activated with Bandrowski' Base (BB) a chemical allergen or with recombinant Birch pollen allergen 1 (rBet v 1). Non-specific activation is obtained by addition of TNFα. Contact of LLDC or LC with BB or rBet v 1 triggers migration in 10 experiments over 10, migration is maximum at 24 h of contact with allergens. Migration is preceded by a production of MMP-2 and –9, part of the molecules is recovered as pro-MMPs in cell culture supernatant and part is associated to cell membrane proteins. MMP-1, –3, –7 and –13 as MT1- and MT3-MMP are also identified at the cellular level. Addition of TNFα in the upper compartment of migration chambers with non-activated LLDC or freshly isolated LC initiates MMP-2 and MMP-9 production then cell migration with a dose dependent effect. These data imply that TNFα is a key molecule for MMPs production and cell migration. Furthermore, activation of LLDC with BB induces a synthesis of TNFα and expression of TNF-R2 on the cell membrane suggesting an autocrine loop. In conclusion, membrane associated MMP-2 and –9 rather than soluble ones are implicated in the cell migration, however, the system is probably more complex, other MMPs being implicated either in the migration or in MM-2 or –9 activation.

P39

Adhesion of Dendritic Cells Derived from $CD34^+$ Progenitors to Resting Human Dermal Microvascular Endothelial Cells is Down-Regulated Upon Maturation and Depends on CD11a, CD11b and CD36

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Dendritic cells (DC) function as sentinels of the immune system. In order to reach their target tissue, bone marrow-derived DC precursors need to bind and migrate specifically through *microvascular* endothelial cells. Binding of DC to primary endothelial cells of the skin has not been investigated. We therefore determined adhesion of DC at different stages of development to human dermal microvascular endothelial cells (HDMEC). DC were generated from human cord blood-derived $CD34^+$ hematopoietic progenitors by culture in the presence of GM-CSF, TNF-α and hSCF. To enhance DC maturation, cells were additionally exposed to a defined cocktail of IL-1β/IL-6/TNF-α/PGE2 ("cocktail DC"). Adhesion was quantified by phase contrast microscopy and by a fluorimetric adhesion assay. Significantly more DC progenitors on day 5 ($37.8 \pm 9.7\%$) than mature DC on day 13 ($13.8 \pm 6.3\%$ for conventionally matured DC and $2.5 \pm 0.87\%$ for "cocktail DC") bound to unstimulated HDMEC. Pretreatment of HDMEC with TNF-α and IFN-γ resulted in an enhanced attachment of both DC progenitors and mature DC by 50–160%. Mature DC lacked expression of CD31, CD36, CD45RA and CLA and expressed lower levels of CD11a, CD11b and CD49d as compared to day 5 progenitors. MAb's against CD11a, CD11b, and CD36 markedly inhibited DC binding (mean inhibition 65-70%), whereas anti-CLA, anti-DC-SIGN and anti-CD49 Ab's did not. Our data support the hypothesis of immunosurveillance with selective recruitment of blood DC progenitors (including langerhans cell progenitors) to resting, and, more so, to inflamed skin. They have potential relevance for anti-cancer immunotherapy strategies favouring the intracutaneous application of mature DC.

P40

A Three-Dimensional View on Langerhans Cell Migration – A Scanning Electronmicroscopic Study

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Langerhans cells and dermal dendritic cells initiate primary immune responses after migrating from sites of antigen uptake to lymphoid organs. The skin is a feasible model to study the morphology and regulation of dendritic cell migration. Both features are incompletely known and understood. We used murine skin explant cultures for tracking the pathways of dendritic cell migration by scanning electronmicroscopy and noted the following ultrastructural features. (1) In 48-h cultures of epidermal sheets we observed numerous Langerhans cells migrating out between keratinocytes in a time-dependent manner. These cells appeared mature as judged by their expression of thin cytoplasmic processes ("veils"). They also possessed pseudopodia that appeared to be actively involved in migration. (2) The basement membrane represented a tightly packed and dense network of fibrils which seemed to leave no space for emigrating dendritic cells unless they actively digested the meshwork by proteinases. (3) Inspection of the dermal compartments in 48-h cultures of whole skin revealed dendritic cells "struggling" through the collagen lattice and extending their cytoplasmic processes, apparently to pull themselves along the collagen fibrils until they reach a lymph vessel. These observations visualize the cumbersome pathway that dendritic cells have to take when they generate immunity (or tolerance). They thereby emphasize the need to better understand the cellular and molecular events that regulate this migration in order to be able to improve it in vaccination approaches.

P41

How Migrating Langerhans Cells Cross Physical Borders – A Transmission Electron Micrososcopic Study

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On their way from the epidermis to the lymph node, Langerhans cells must cross some solid physical barriers, first the basement membrane and then the endothelial cells of the lymphatic vessels. We were interested in the morphology of these processes. A murine skin explant culture system was used. Explants were cultured on medium for 48 h, fixed and further processed for transmission electronmicroscopy. On rare occasions could we observe Langerhans cells that were just leaving the epidermis. They seem to first extend a pseudo-pod. Then they penetrate the basement membrane, presumably with the help of matrix metallo-proteinases. Disruption of the basement membrane (lamina densa) was only seen where a Langerhans cell was actively pushing out of the epidermis, emphasizing the spatially focussed action of these enzymes. Entry into dermal lymph vessels appeared to occur through (sometimes wide) gaps in the thin endothelial lining of the vessels. This very moment was indeed caught by EM, although very rarely. Transmigration between adjacent endothelial cells is also conceivable, even more so since cell-cell contacts in the lymph endothelial lining appeared loose; cell endings often overlapped to some degree. The clear physical separation of the electron-dense ground substance of the connective tissue and the electron-lucent lumen of the vessel in the gaps suggests that there is still a molecular border (e.g. barely visible lamina lucida materials) that needs to be surmounted by migrating Langerhans cells with the help of known (e.g. matrix metalloproteinases) and still unknown molecular tools.

P42

Verapamil Inhibits Murine Epidermal Langerhans Cell Migration *In Vitro* and *In Vivo*

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Verapamil (VPM) has been found previously to compromise the migration of Langerhans cells (LC) from human skin explants through a mechanism believed to be dependent upon inhibition of multi-drug resistance protein (MDR1) activity. More recently, it was suggested that murine dendritic cells (DC) do not express substantial levels of MDR1 but that both human and murine DC express the multidrug resistance associated protein 1 (MRP1), a transporter of leukotriene C_4 (LTC_4). It is known that VPM inhibits both MDR1 and MRP1 activity and also resultant LTC_4 release. We have now examined the influence of VPM on LC migration in mice, both in skin explants and *in vivo*. Early (4 hr) migration of MHC class II^+ LC from murine skin explants was inhibited completely by addition of VPM to culture medium. Subsequently, mice were exposed topically on the dorsum of both ears to VPM 2 hr prior to application of the contact allergen oxazolone (Ox). VPM inhibited the decrease in epidermal LC densities observed 4 hr following exposure to Ox and compromised also the accumulation of DC in draining lymph nodes 18 hr later. Identical pre-treatment of mice with VPM attenuated LC migration and DC accumulation provoked by intradermal injection of tumour necrosis factor α (TNF-α), but was without effect on either response stimulated by exogenous interleukin 1β (IL-1β). It is proposed that one function of VPM is to regulate the secretion by LC of IL-1β either directly through inhibition of MDR1/MRP1 activity or through a mechanism involving LTC_4 transport.

P43

Macrophage Inflammatory Protein (MIP)-3α Expression is Increased in Human Inflammatory Bowel Disease: Role in Crohn's Disease

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MIP-3α, the ligand of CC chemokine receptor 6 (CCR6), has recently been identified the key chemokine attracting Langerin$^+$ Langerhans cell-like dendritic cells (DCs) to inflamed epithelial cell surfaces. Inflammatory bowel disease (IBD), constituting Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory disorders characterized by infiltration of Th1 and Th2 lymphocytes, respectively, in the lamina propria of the gut. The role of DCs in IBD is barely defined. In the current study, we asked whether MIP-3α expression is altered in IBD. Colonic biopsies were obtained from IBD patients. Control biopsies were obtained from patients undergoing routine colonoscopy for tumor screening, which were found normal. MIP-3α mRNA levels in colonic biopsies were determined by real-time PCR analysis. Furthermore, whole tissue cultures of colonic biopsies were performed and MIP-3α secreted into supernatant determined by specific enzyme-linked immunosorbent assay. We demonstrate that MIP-3α mRNA levels are significantly elevated in both, lesional and non-lesional colonic biopsies in CD compared to controls. MIP-3α mRNA levels varied widely in UC patients, and were not statistically different from controls. MIP-3α levels secreted from colonic biopsies cultured for 24 h were elevated in both, lesional and non-lesional CD compared to controls. In UC, non-lesional cultured biopsies secreted MIP-3α comparable to controls, while MIP-3α secretion was significantly elevated in lesional UC. MIP-3α has a predominant role in the migration of LC-like DC precursors to inflamed epithelial surfaces, thus in directing DCs to sites of antigen-uptake. Enhanced expression of MIP-3α in human CD might therefore attract DCs to epithelial surfaces, and thus could play a role in the initiation of the Th1 immune response finally resulting in bowel inflammation. Another important function of enhanced MIP-3α expression in CD might be the direct attraction of T lymphocytes, since CCR6 expression has also been reported on gut-dedicated a_4a_7-expressing memory T lymphocytes.

P44

Inhibition by Lactoferrin of Mechanical Trauma-Induced Langerhans Cell Migration in Humans

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Topical exposure of volunteers to the sensitising chemical diphencyprone, or intradermal administration of homologous recombinant tumour necrosis factor α (TNF-α) or interleukin (IL)-1β, each provokes significant reductions (approximately 30%) in CD1a$^+$/HLA-DR$^+$ Langerhans cells (LC) in epidermal sheets prepared from punch biopsies. Furthermore, lactoferrin (LF), a protein believed to inhibit TNF-α production, compromises both allergen- and IL-1β-induced LC migration but is without effect on responses provoked by TNF-α. We have now investigated the utility of epidermal LC measurements in blister roofs taken following suction blister formation; a technique that permits the concomitant measurement of locally produced cytokines. We demonstrate that CD1a$^+$ LC numbers in blister roofs taken from suction blisters (12mm) formed on photoprotected buttock skin were reduced by 31 ± 3% (6 volunteers) compared with those derived from concurrent punch biopsy (6 mm) material. Treatment of sites with LF prior to induction of suction blisters inhibited almost completely the loss in LC numbers observed as a result of this manipulation (3 volunteers). Injection of IL-1β (50U) prior to formation of suction blisters, conditions shown previously to induce significant LC migration in humans, failed to provoke further decreases in LC densities (4 volunteers). Taken together these data suggest that although the suction blister technique may represent a potentially useful tool for analysis of soluble cutaneous mediators, this process is itself traumatic. The suggestion is that this procedure induces local cytokine release, including THF-α, with concomitant loss of responsive LC from the epidermis; a response that may be prevented by pre-treatment with LF.

P45

HIV Capture by Langerhans Cells

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DC-SIGN is a C-type lectin expressed on the surface of dendritic cells (DCs) that efficiently binds and transmits human immunodeficiency viruses (HIV) to T cells in *trans*. It was postulated that DC-SIGN allows HIV to divert mucosal DCs from their immunological function and to use them as transporters for the virus to reach lymphoid organs, where DCs transmit a vigorous infection to T cells. The recent discovery of DC-SIGN as a specific and efficient HIV receptor raises the possibility that other attachment factors expressed on relevant cell types may exist and could have profound consequences on the transmission of HIV infection. By their localization in mucosal epithelium, Langerhans cells (LCs) are likely to be the initial HIV targets and are thought to play an important role in the early steps of viral dissemination. In this work, we found that LCs do not express DC-SIGN but can still bind envelope glycoproteins of different HIV strains. This interaction is inhibited by mannan (a mannose polymer), suggesting the participation of a lectin receptor in the capture of viral envelopes by LCs. We hypothesize that LCs may express specific HIV attachment receptor(s), allowing these cells to capture virus in mucosa and transport HIV virions to lymph nodes, the major site of HIV replication *in vivo*.

P46

Expression of Functional HIV CXCR4, CCR5, and CD4 Receptors on Fresh and Cultured Human Langerhans Cells

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HIV can cross the intact epithelium of genital mucosae via Langerhans cells. Fresh Langerhans cells are known to express CD4 and CCR5. The presence of CXCR4 has been described on the surface of cultured but not freshly isolated Langerhans cells. In the present study, we demonstrate that CXCR4 was expressed by fresh Langerhans cells isolated and purified from epidermis. However, the percentage of Langerhans cells expressing CXCR4 or CCR5 increased during maturation of the cells in culture, especially in the presence of exogenous GM-CSF. To determine if CXCR4 was functional, freshly isolated Langerhans cells were infected with HIV-LAI, a T-cell tropic strain and P24 production was measured in culture supernatants. A P24 production was observed when infected Langerhans cells were cocultured with SupT1 cells. However, the presence of HIV provirus DNA was evidenced within the infected Langerhans cells by nested PCR. Ultrastructural studies confirmed that there was the formation of syncytia when Langerhans cells were cocultured with SupT1 cells. Preincubation of Langerhans cells with AZT or SDF1-a, a natural ligand for CXCR4, prevented infection. These data demonstrate that CXCR4 is present on the surface of Langerhans cells, freshly isolated from human skin epidermis, and that this expression is functional.

P47

Extracellular-Matrix Environment Favors HIV-1 Transmission by Setting Dendritic Cells as Replicative Reservoirs

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In primary HIV-1 infection, dendritic cells (DC) are used by the virus to traverse the epithelial barrier of the female genital tract. *In vitro*, the replication of monocyte (M)-tropic strains is hypothesized to be efficient in immature DC but not in cultured mature DC although infectious virus can be efficiently transmitted to CD4+ T cells. We focused our interest on the susceptibility of human interstitial DC (IntDC) to be infected after M- and T lymphocyte (T)-tropic strains exposure when they were integrated in a reconstructed *lamina propria*. For this purpose, CD34 precursors were cultured in presence of GM-CSF+ TNFα and generated more than 80% of IntDC (Langerine$^-$ or CD207$^-$) and less than 5% of Langerhans cells (LC) (Langerine$^+$ or CD207$^+$). The mixed DC subpopulations were either cultured in fibroblasts-retracted collagen lattices or in suspension. Infection of both culture models was performed after 7 or 13 days of culture of CD34$^+$ cells by a T- (Laï) or a M- (BaL) tropic strain. Virus replication was controled by measuring p24 in supernatants. Detection of provirus was carried out by *in situ* PCR. Although DC were infected by both virus strains when they were integrated or not in lattices, only the M-tropic strain was efficiently replicated by immature DC (infected on day 7 of the CD34 culture). Integrated in lattices, DC efficiently replicated virus when they were infected on day 13 of the culture whereas their counterparts lost this capacity in suspension. These results suggest that sustained capacity of DC to replicate M-tropic strains is correlated to the delay of DC maturation induced by their integration into a 3D-reconstructed *lamina propria*. In this 3D model, the productive infection of IntDC may contrast with the ability of DC-SIGN to capture the virus and not to mediate its entry, unless migrating LC act as viral vectors by enhancing the infection of IntDC in *trans*.

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Inhibition of HIV Infection in a Reconstructed Mucosa Integrating Langerhans Cells

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We have performed a reconstructed *in vitro* mucosa integrating Langerhans cells (LC), and we have investigated the infection of this model by HIV-1. Our epithelium consists of vaginal keratinocytes cultured on de-epidermized dermis in submerged medium for two weeks. LC precursors, obtained by differentiation of CD34$^+$ cord blood progenitor cells in presence of GM-CSF, TNFα, TGFβ and Flt3L, are added in the reconstituted epithelium after 4–6 days of culture. Immuno-histochemical and ultrastructural studies showed that LC were detected in a physiological location in the well-stratified, differentiated epithelium. T-tropic (LAI, NL4-3) and M-tropic (BaL) HIV-1 strains were added 1° on a epithelium of vaginal cells, 2° on isolated fresh LC, and 3° on the model of the reconstructed mucosa. Nested PCR revealed the presence of proviral DNA of the three HIV-1 strains in cultured LC as in LC integrated in vaginal mucosa. In contrast, we could not detect any HIV-1 proviral DNA in epithelial cells. NL4-3 infection of LC included in our model could be totally inhibited by 100 ng per ml of SDF-1 (the natural ligand of CXCR4) or by AZT. LAI infection was also inhibited by these drugs (SDF-1 and AZT) but less efficiently. Then, in our experiments, LC could be infected by both M and T-tropic HIV isolates in this *in vitro* vaginal model. Moreover, T-tropic HIV infection could be specifically inhibited by SDF-1 confirming the presence of a functional CXCR4 on LC. Such model seems to be a useful tool for the study of the mechanisms involved in heterosexual transmission of HIV.

P49

Langerhans Cells in Human Periodontal Diseases

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Gingival Langerhans cells (LC) are strongly implicated in the initiation and maintenance of inflammatory periodontal diseases. In order to initiate the immune response LC migrate towards lymph nodes and present pathogenic antigens to T lymphocytes. Their migration and the crossing of the epithelial basement membrane probably need the release of metalloproteinases (MMPs) leading to the local degradation of matrix macromolecules. Our works were focused on quantitative and morphological studies as well as MMPs expression by gingival LC in periodontal diseases. Fixed or frozen gingival samples of control, gingivitis or periodontitis patients were stained by single or double immunohistochemical method using antibodies directed against CD1a (LC), CD45, CD3, CD8, CD20, TIA-1, GrB, MMP-1, MMP-2 and MMP-9. Quantitative and morphological studies were performed by morphometric and automated image analysis. MMPs expression of gingival LC was studied with a confocal microscope on sections stained by double immunofluorescence method. Results of these studies showed (1) a significant decrease in the CD1a + LC number correlated with the severity of the inflammatory phenomenon evaluated by the quantitative study of cellular and collagen fibres components; (2) a significant decrease of the surface, perimeter and equivalent diameter of CD1a + LC in periodontitis when compared with controls and within the basal epithelial layer when compared with the upper layers; (3) an expression of the collagenase MMP-1 and the gelatinases MMP-2 and MMP-9 by LC in healthy and diseased gingival tissues. In conclusion, during periodontal diseases LC contribute to the extracellular matrix degradation.

P50

Different Behavior of Mycobacterium Tuberculosis in Human Macrophages and Dendritic Cells

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The interaction of *Mycobacterium tuberculosis* (*Mtb*) with dendritic cells (DCs) is likely to have a strong impact on the initiation of the immune response, the host's capacity to control primo-infection, and possible further reactivation of latent infection. Here, we compared the interaction of virulent *Mtb* H37Rv with human monocyte-derived DCs and macrophages (MΦs) generated *in vitro* from the same donors. As reported by others, *Mtb* infection induced DC activation and maturation, with up-regulation of CD83, HLA-class II and costimulatory molecules, whereas it resulted in only limited activation of MΦs. Unexpectedly, the ability of *Mtb* to multiply in DCs over a 7-day period was markedly reduced relative to its growth in MΦs. Confocal microscopy analysis revealed differential trafficking of *Mtb* in the two cell types: although the mycobacterial phagosome did not acidify nor fuse with host cell lysosomes in both DCs and MΦs, in DCs the bacterial vacuoles appeared to be disconnected from endocytic and recycling pathways, as demonstrated by the absence of Rab5a and Rab11 markers on the vacuole membrane and the inability of this compartment to accumulate and recycle exogeneously administered transferrin. Such a segregation may be responsible for impaired access of the bacteria to essential nutrients. Altogether, our findings suggest that human DCs may have evolved special mechanisms to control the growth of intracellular mycobacteria, a property that could allow safe and efficient priming of naive T cells in secondary lymphoid organs without contributing to spreading the pathogen.

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The Effects of Treponema Pallidum on Human Dendritic Cells

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Cell mediated immune responses play a prominent role in the syphilis, which is caused by *Treponema pallidum* (TP). TP enters human body through skin or mucous membrane, where dendritic cells (DC) may be important antigen presenting cells to present treponemal antigens to the lymphocytes. The role of DC in the syphilitic infection is not well understood in human. In the present study, we studied interaction of TP with DC, generated from human peripheral blood mononuclear cells with GM-CSF and IL-4. After adding TP to DC culture at day 7 for 16 h, the change of surface antigens on DC was checked with flow cytometry and the amount of IL-12 in culture supernatant of DC was checked by ELISA. We have observed an efficient phagocytosis of TP by electron microscopy as early as 2 h after addition of TP to DC. Interaction of DC with TP resulted in increased surface expression of CD83 which was proportionally increased according to the number of TP. Addition of normal rabbit serum, normal rabbit testicular tissue, and media, which served as negative controls, did not induce any change of CD83 on DC. CD80, CD86 expressions on DC were slightly increased and HLA-DR was not changed. The amount of IL-12 in the culture supernatant of DC was increased (1099 pg per ml) after the addition of TP which was similar (1191 pg per ml) in the supernatant of mature DC. However, there was no secretion of IL-12 from DC after adding normal rabbit testicular tissue and serum. From the above results, we found that TP can stimulate DC to mature by showing increased expression of surface molecules and production of IL-12.

P52

Human Melanoma Cells with Different Invasive Properties Inhibit the Earliest Steps of Differentiation of Langerhans Cell Precursors, but Fail to Affect the Functional Maturation of Epidermal Langerhans Cells

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This study addressed the question of whether invasive and non invasive human melanoma cells may affect the generation and/or functional maturation of human epidermal Langerhans cells (LC). LC were generated *in vitro* from $CD34^+$ cord blood progenitors under GM-CSF/TNF-α/TGF-β1. $CD34^+$ cells were co-cultured with or without melanoma cells from a metastatic and invasive clone (T1C3) or a non-metastatic and non-invasive clone (IC8) using transwell dishes. After 7 or 11 days of co-culture, $CD34^+$-derived cells displayed a non-adherent undifferentiated morphology, high level of monocytic CD14 marker, low level of LC markers (CD1a, E-cadherin) and DC markers (CD40, CD80, CD54, CD58, CD83, CD86, HLA-DR, HLA-class I), and were less potent than control LC in inducing allogeneic T cell proliferation. The generation of a $CD14^+$ population was correlated with a decrease in $CD1a^+$ population, without any statistical differences between the two clones. Interestingly, melanoma cells affect differentiation of $CD34^+$ cells only during their proliferation stage (day 0–day 6), and the earlier the co-culture was started, the more the $CD14^+$ population was enhanced. This effect was not the result of factors including IL-10, TGF-β1 and VEGF, as assessed by using blocking antibodies. By contrast, a 2-day coculture of melanoma cells from both clones with fresh LC isolated from healthy epidermis did not affect their phenotype and function. Collectively, our data demonstrate that melanoma cells produce factor(s) that block(s) the earliest steps of LC differentiation, but fail(s) to affect the functional maturation of LC, thus highlighting the differential sensitivity of LC and their precursors to melanoma-derived factor(s). We suggest that melanoma cells might participate in their own escape from immunosurveillance by preventing LC generation in the local cutaneous microenvironment.

P53

Breast Carcinoma Cell Lines Favor the Differentiation of CD34+ Cells into Two Different Subpopulations ($CD1a^+Lang^-CD86^-$, $CD1a^+Lang^+CD86^+$)

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It has been reported that breast carcinoma (BCC) tumors are infiltrated by immature dendritic cells (DC) ($CD1a^+CD83^-CCR6^+$). The aim of this study was to investigate the effect of BCC conditioned media (BCC CM) as well as irradiated BCC cell lines on the differentiation of $CD34^+$ progenitors into DC in presence of GM-CSF^+ TNFα. In contrast with renal cell carcinoma, previously described to block DC differentiation through IL-6 and M-CSF production, BCC CM as well as irradiated cell lines favored DC differentiation towards a specific phenotype in this model: BCC CM promoted the differentiation of $CD34^+$ into $CD1a^+$ DC as early as day 6, in particular strongly enhanced $CD34^+$ cell differentiation into Langerhans cells at day 6; at day 12, cells cultured in the presence of BCC CM showed a long fine shape with long dendrites and were loosely adherent. In addition, a specific subpopulation of cells expressing high levels of the CD1a Ag among those $Langerin^+$ and $CD86^-$ subpopulations were obtained in the presence of BCC CM. Similar results were observed in presence of rhTGFβ, known to favor the differentiation of $CD34^+$ progenitors into Langerhans cells. Pan TGFβ polyclonal antibody decreased the MFI of $CD1a^+$ cells, blocked the appearance of the $CD1a^+Lang^+CD86^+$ population and diminished by 50% the $CD86^-CD1a^+$ population. These results strongly suggest the involvement of TGFβ in the effects of BCC CM. The functional consequences of this shift of DC phenotype induced by BCC CM will be discussed.

P54

Generating the Optimal Dendritic Cell for Immunotherapy

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Dendritic cells (DCs) have the unique ability to initiate an immune response *in vivo*. Therefore DCs became a major tool in cancer immunotherapy. Recently, it has been shown that mature DCs are more potent in eliciting an immune response than immature DCs. Nonetheless, not all mature DCs induce optimal T cell responses. The secretion of IL-12p70 by DCs seems to play a central role in the stimulation of Th1 cells providing essential help for the activation of cytotoxic T cells. In this study we are aiming to optimize the maturation conditions for human DCs with respect to clinical applicability. For this reason we investigate the effect of different stimuli, like soluble CD40L, polyI:C or a cytokine mixture of TNF-α, IL-1β, IL-6 and PGE2 on DC maturation, IL-12p70 production and T cell stimulatory capacity of the DCs. "Clinical grade" DCs where generated under serumfree conditions and treated with different maturation stimuli. Mature DCs were analyzed by FACScan or used as stimulators in mixed lyphocyte reactions. We found that all of the stimuli mentioned above induce maturation but do not elicit IL-12p70 production of DCs when given alone. However the combination of the cytokine mixture with soluble CD40L or polyI:C leads to production of considerable amounts of IL-12p70. Furthermore this IL12p70 production could be enhanced by the addition of IFN-γ. DCs which were matured with the cytokine mixture showed the best T cell stimulatory capacity which could not be enhanced by the addition of soluble CD40L, polyI:C or IFN-γ. So far our experiments show that proinflammatory cytokines are necessary that DCs stimulate Th cells efficiently, but are not sufficient to induce IL-12p70 production of DCs. Currently we are investigating the cytokine profile of Th cells activated by these DCs.

P55

Alteration of Peripheral Blood Dendritic Cells in Connective Tissue Disease

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The pathogenesis of connective tissue diseases has remained unclear. Dendritic cells (DCs) are specialized APC and play central roles in eliciting primary immune responses. Thus, it is possible that DCs are involved in breakdown of self tolerance, resulting in the genesis of connective tissue diseases. We recently identified 3 fractions of human peripheral blood DCs including the monocyte-associated fraction 1 and 2 ($CD1a^+/CD11c^+$ and $CD1a^-/CD11c^+$) and the lymphoid-associated fraction 3 ($CD1a^-/CD11c^-$). Here, we attempted to determine whether these fractions were altered in connective tissue disease including primary Sjogren's syndrome (pSS), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and polymyositis/dermatomyositis (PM/DM). We analyzed peripheral blood DCs from 23 pSS, 10 SLE, 11 SSc, 5 PM/DM untreated with immunosuppressant, and 22 normal volunteers as control. Total amount (/total PBMC) of fraction 1 DC was decreased in pSS, SLE and PM/DM. Fraction 3 DC were also decreased in only SLE patients' peripheral blood. In SSc patients, neither fraction of circulating DC was altered. Furthermore, immunohistochemical staining of the small salivary gland from pSS patients indicated that $CD11c^+$ myeloid DCs scattered among the infiltration of $CD4^+$ T cells. These findings suggest the selective trafficking of DCs into the focal inflammatory sites in pSS. Amount of circulating DCs and their fractions were reduced in some connective tissue disease in different patterns, suggesting that DCs participate in the pathogenesis of these diseases in different ways.

P56

Gangliosides Purified from Human Melanoma Tumors Alters the Phenotypic and Functional Differentiation of Monocyte-Derived Dendritic Cells

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Gangliosides are membrane-associated sialic acid-containing glycosphingolipids which may be involved in the suppression of the antitumor immune response. Indeed, tumor cells synthetize and shed large amounts of gangliosides into their microenvironment and several studies have unravelled the immunosuppressive properties of these compounds. In the present study, we analyzed the effects of total or purified ganglioside fractions derived from human melanoma tumors on the differentiation of dendritic cells (DC). To this aim, monocytes were purified from normal peripheral blood and cultured for 6 days with GM-CSF and IL-4 in the presence or not of gangliosides at different concentrations, before phenotypic and functional studies were carried out. Gangliosides were prepared from human melanoma tumors and, as described earlier, the total ganglioside fraction mostly contained GM3 and GD3 gangliosides. At the concentration of 25 µg per ml, the total ganglioside fraction did not alter the cell yield but substantially altered the phenotype of monocyte-derived cells. HLA-DR, CD1a, CD54, CD80 and CD40 antigens were strongly down-regulated while the percentage and mean fluorescence intensity of CD86 was dramatically increased. CD14 and CD83 expression remained negative. At 50 µg per ml, the viable cell yield was decreased by about 30-40% and the phenotypic alterations were even more pronounced. Furthermore, the allostimulatory capacity of viable monocyte-derived cells was significantly decreased. GM3 and GD3 were purified by high pressure liquid chromatography in order to analyze their respective effects on DC differentiation. Both fractions were found to induce the above mentioned phenotypic and functional changes. In conclusion, the results demonstrate an inhibitory effect of gangliosides on phenotypic and functional DC differentiation, which may be an additional mechanism of human melanoma escape.

P57

Increased Tumor Necrosis Factor Family Signaling Elements in Peripheral Blood Lymphocytes of patients with Langerhans Cell Histiocytosis

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Langerhans Cell Histiocytosis (LCH) is a reactive/proliferative disease characterized by increased numbers of CD1a+ Langerhans cells (LCs), lymphocytes (lym), eosinophils and CD1a– macrophages infiltrate in single or multiple organs. Cytokines up-regulated in LC include tumor necrosis factor alpha (TNF-α) and CD40L by an unknown mechanism. RANK and its ligand RANKL influence T cells and dendritic cells interactions. OPG (osteoprotegerin) a decoy receptor of RANKL may be involved. We have studied peripheral blood lymphocytes from 7 children with active LCH and compared them with normal controls to determine if changes in CD40/CD40L, RANK/RANKL or OPG relate to systemic aberrations of the immune system accompany localized or diffuse disease. Results: by flow cytometric analysis CD19+ peripheral blood B cells had a marked increase in CD40, but no increase in CD80, CD86, CD40L or rank expression. We did not find any increase in myeloid dendritic cells (CD11c+/11b+) or lymphoid dendritic cells (CD11c+/llb-) or their expression of CD40. RT-PCR analysis of mRNA content in lym showed increased amounts for RANKL and OPG. Conclusions: unlike the classical T-cell–antigen presenting cell interaction it appears that in LCH patients the CD40 is up-regulated in B cells and that mRNA for RANKL and its decoy receptor opg are increased. The CD40/CD40L interaction between T and B cells may be preferentially used instead of CD28/CD80 in LCH lymphocytes.

P58

Langerhans Cell-Related Dendritic Cells Expand in a Virally Induced Mouse Model of Histiocytosis

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The malignant histiocytosis sarcoma virus (MHSV) induces a proliferative disease in mice involving cells characterized initially as malignant macrophages. Therefore, this disease was suggested to represent a mouse model for malignant histiocytosis (MH). Upon infection, virally affected cells first appear in spleen and bone marrow and later metastasize to visceral organs. To investigate the specificity of the virus in affecting distinct subsets of mononuclear phagocytes, we further characterized the virally transformed cells in this model. Immunohistochemical and flow cytometric analysis showed that distinct MHSV-infected subpopulations expand simultaneously. These populations include CD11c/CD13-positive DC, MOMA-1-positive metallophilic Mφ and ER-MP58-positive myeloid precursor cells. DC represent the major subset of transformed cells in both primarily and secondarily affected organs. Early in viral infection, all affected splenic DC express CD8α, whereas affected DC in other peripheral organs virtually lack CD8α expression. In later stages, also transformed DC in spleen show heterogeneous CD8 expression. In the mouse, CD8α is expressed by lymphoid-related DC as well as by Langerhans cells which have been activated immunologically *in vivo* or *in vitro*. Similarly activated myeloid DC, however, do not express CD8α. Since MHSV only affects myeloid cells and CD8α is expressed by MHSV-transformed DC in a non-constitutive manner, we conclude that affected DC in this mouse model are most likely related to Langerhans cells.

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Methotrexate and 6-Mercaptopurine in the Treatment of Langerhans Cell Histiocytosis (LCH)

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We present results and experiences of treating children with LCH with methotrexate (MTX) and 6-mercaptopurine (6MP) after unsuccessful previously administered therapy. Eleven children (7_ and 4_) was diagnosed with LCH in the period of 1983-2000 and had a median age at onset of 1.8 ys. (range 0.4-12.0). In nine cases the diagnosis was definite (CD1-pos./Birbecks granules), and in other two cases presumptive. At onset seven patients had multisystem, and four had single system disease. Ten children had bone involvement among those seven had multiosseous LCH, and one child only skin involvement. Three children received MTX and 6MP together with prednisone and/or vinblastine, while the other eight received the two drugs after recurrent disease (1-7 recurrences) with a median period of 0.9 yr. (range 0.6-8.3) from diagnosis of LCH. In all cases, previous therapy included, i.e. prednisone, cytostatics and/or immunomodulating agents. Six patients also got other therapy, but in all cases tapered off before MTX and 6MP were stopped. The dose of MTX was 20 mg per m^2 per week and 6MP 50 mg per m^2 per day. In all cases improvement occurred along the treatment as judged by resolvement of clinical symptoms, e.g. pain, limp, fever, regression of skin elements, imaging studies showing healing of lytic lesions. Side-effects were limited to transient myelosuppression, reversible liver affection, and to a mild degree loss of appetite. All patients seem to benefit from the treatment with MTX and 6MP,which were given in all but one case after recurrence of LCH, and in all cases led to remission.

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Fascin and Langerhans Cells: Function, Regulation and Langerhans Cell Manipulation

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Maturation of Langerhans cells (LC) into fully mature dendritic cells (DC), capable of primary stimulation of naive T cells, can be mimicked by *in vitro* culture of epidermal cell suspensions for three days. Applying a differential screening approach we recently discovered that the actin-bundling protein fascin is up-regulated during this maturation process. Furthermore, we showed that fascin expression is induced during maturation of human monocyte-derived dendritic cells as well. Inhibition of fascin expression by antisense oligonucleotides inhibits formation of numerous dendrites, indicating that fascin is pivotal for dendrite formation in DC. Here we report on the isolation of the human und the murine fascin genes and characterization of the corresponding promoters. Promoter fragments were subcloned to drive the expression of the reporter genes enhanced green fluorescence protein (EGFP) and luciferase. Deletion contructs were used to characterize the active promoter. Several cell lines were transfected and analysed for reporter gene expression, indicating that cell type specificity of fascin promoter reflects expression pattern of fascin *in vivo*. The murine EGFP reporter construct was used to restrict expression to LC following bioballistic transfer of expression constructs to murine epidermis by gene gun transfection. Compared with transfections of antigens under control of ubiquitously expressed promoters the LC-targeted expression of transgenes will allow for analysis of the importance of the route of antigen delivery for the outcome of the immune response.

P61

In Situ Characterisation of Dendritic Cells in Human Placenta and Decidua

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Dendritic cells (DC) are sentinel cells of the immune system and located in surface tissues such as skin and mucosa, where the organism is threatened by infectious agents. The human decidua, despite its proposed immunosuppressive function, hosts a variety of immunocompetent CD45 cells such as natural killer cells, macrophages, T cells and, as recently described by us, CD83+ DC. We have now undertaken a more detailed immunhistochemical characterization of the HLA-DR+ antigen presenting cells (APC) present in fetal placenta and maternal decidua. Using mAbs against the monocyte/macrophage markers CD14 and CD68, the DC marker CD83 as well as the novel lectin type molecule DC-SIGN we found that HLA-DR positive APC in decidua can be divided into 3 populations: a majority of undetermined CD14+/CD68–/CD83–/DC–SIGN+ cells, a minority of CD14+/CD68+/CD83–/DC–SIGN– macrophages and few CD14–/CD68–/CD83+/DC–SIGN+ DC. In contrast to maternal decidua, CD14+ cells within early placental villi were generally negative for DC–SIGN as well as for CD83. Interestingly, in mature placenta at term most of the CD14+ cells expressed DC–SIGN, but still were CD83 negative. In summary, this is the first identification of CD83–/CD14+ APC at the feto–maternal interface that express the HIV-binding lectin DC–SIGN. Expression of DC–SIGN on fetal APC seems to be up-regulated during pregnancy. The functional role of DC-SIGN+ APC not belonging to the CD14–/CD83+ DC lineage awaits further analysis.

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In Vitro and _In Vivo_ Analysis of RelB-Deficient Dendritic Cells

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The Rel/NF-κB family member RelB is expressed in specific regions of lymphoid organs. RelB-deficient mice ($relB^{-/-}$) have a complex phenotype including multiorgan inflammation, myeloid hyperplasia, and multifocal defects in immune responses. Interdigitating dendritic cells (DCs) express high levels whereas Langerhans cells (LCs) express only very low levels of RelB, correlating with reduced DC populations in lymphoid organs but normal numbers of LCs in the skin of $relB^{-/-}$ mice. RelB expression increases during DC maturation and strong kB-binding activity can be induced by different stimuli triggering terminal differentiation of DCs *in vitro*. To investigate the role of RelB in DCs we compared DCs generated *in vitro* from wild-type and $relB^{-/-}$ bone marrow (BMDCs). So far, BMDCs from control and $relB^{-/-}$ mice did not reveal clear differences in maturation, antigen uptake, and MLRs. Also, PKH26-labeled $relB^{-/-}$ BMDCs injected into wild-type mice were able to migrate to the draining lymph node. We previously demonstrated that $relB^{-/-}$ mice show poor CHS responses. Since the development of lymph nodes is impaired in $relB^{-/-}$ mice we injected BMDCs after haptenization with TNBS into wild-type recipients. In these adoptive transfer experiments, both control and $relB^{-/-}$ BMDCs showed a similar capacity to induce an ear swelling response. Experiments to test the ability of freshly isolated LCs from $relB^{-/-}$ mice to migrate and to induce immune responses will be presented.

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Two Subsets of Spleen DC in Rat: Phenotype and Function

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We previously shown that freshly extracted OX62+ DC from rat spleen contain two subsets, one CD4+ and one CD4–. The CD4–, but the not the CD4+ subset of spleen DC exhibited a cytotoxic activity against selected tumoral targets cells. Here we extended our study on the function of rat spleen DC subsets. Three color FACS analyses of unsorted low density cells showed a CD4+ DC:CD4– DC ratio of 1.5:1–1:1. Unlike CD4– cells, CD4+ spleen DC co-express CD5, SIRP and Thy1, express higher levels of CD11c and lower levels of the OX62 integrin than CD4– DC. Giemsa staining of cytospun revealed a myeloid and lymphoid-related morphology of CD4– and CD4+ DC, respectively. However, cell morphology heterogeneity was observed in the CD4+ subset of spleen DC. CD4– DC exhibited an extremely short half life *in vitro* but could be rescue from cell death by CD40 cross linking, GMCSF, IL-3 or PolyI:C. These stimuli had no effects on *in vitro* survival of CD4+ DC. Moreover, unlike CD5 and SIRP, the expression of CD4 seems to be lost in culture by at least a subset of CD4+ spleen DC. Upon stimulation by CD40L of LPS, very low levels of IL-10 and IFNg was detected in the supernatant of both CD4+ and CD4– DC. In contrast, IL12p40 production was strictly restricted to CD4– DC *in vitro*. The influence of these two spleen DC subsets on Th and CD8+ T cells differentiation *in vitro* and *in vivo* is currently under investigation.

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Differentially Expressed Molecules in Dendritic Cells: Integrin Adhesion Dependent Membrane Translocation of CYTIP-1, an Up-Regulated Novel Cytohesin-1 Interacting Protein

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Dendritic cells grown from $CD14^+$ precursors to both stages of maturation were used in a differential display of mRNA to identify molecules differentially expressed during DC maturation. A novel cytoplasmic molecule, CYTIP-1 (Cytohesin-Interacting Protein) was up-regulated during DC maturation. Sequence analyses reveales two protein–protein interaction domains: a coiled-coil domain and a PDZ domain. We show that CYTIP-1 specifically interacts with cytohesins. DC express only cytohesin-1, which is simultaneously up-regulated during maturation of DC and is the putative binding partner of CYTIP-1 in DC. Immunoprecipitation studies confirm binding of cytohesin-1 to CYTIP-1. Mapping of the binding sites reveals the coiled-coil domain of cytohesin-1 to interact with the coiled-coil domain of CYTIP-1. Cytohesin-1 after translocation to the plasma membrane interacts with the αLβ2 chain of LFA-1 to strengthen its adhesiveness to ICAM-1. Specifically after integrin ligand binding CYTIP-1 also localizes to the plasma membrane, suggesting a role for CYTIP-1 in the integrin adhesion dependent functions of cytohesin-1. LFA-1 – ICAM-1 binding is one of the interactions of mature DC to interact with T-cells in an immune response. Up-regulation of CYTIP-1 therefore may be involved in the regulation of this interation.

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Molecular Identity and Function of Langerhans Cell-Associated Ecto-ATPase/ADPase Activities

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Although ecto-nucleoside triphosphate diphosphohydrolase (NTPDase) activities have been widely used as useful markers of epidermal Langerhans cells (LC), the molecular identity or physiological function of these activities remain unknown. CD39, which was originally described as an obscure activation marker expressed by B cells, dendritic cells, and endothelial cells, has been recently identified as a vascular NTPDase. We detected CD39 mRNA and/or protein expression almost exclusively within the IA^+ population in mouse epidermis and in LC-derived XS52 and XS106 lines, but not in keratinocyte or epidermal gd T cell lines. Moreover, CD39-deficient mice showed normal surface densities of IA^+/$DEC205^+$ LC, whereas these $CD39^{-/-}$ LC lacked apparent ecto-NTPDase activities. Thus, we conclude that CD39 is responsible for LC-associated ecto-NTPDase activities. With respect to function, $CD39^{-/-}$ mice developed significantly ($p < 0.01$, $n = 10$) exacerbated inflammatory responses to topically applied croton oil, benzalkonium chloride (BAC), and ethyl phenylpropiolate (EPP) than did $CD39^{+/+}$ mice. Local administration of soluble NTPDase reduced the extent of croton oil-induced ear swelling in $CD39^{-/-}$ mice to the normal level, supporting the anti-inflammatory role for CD39. Exposure of Pam 212 keratinocytes to the same chemicals released biologically relevant ATP and ADP into culture media. Addition of CD39-expressing XS52 cells in keratinocyte cultures significantly reduced ATP levels in the media. These results reveal a novel pathogenic mechanism of irritant contact dermatitis, as well as a previously unrecognized protective role for LC-associated CD39 against environmental insults via phosphohydrolysis of otherwise pro-inflammatory nucleotides released by neighboring keratinocytes.

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Phenotypic and Functional Characterisation of DC in Afferent Lymph Draining Ovine Skin

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We have used a pseudoafferent lymphatic cannulation model, draining the skin of sheep, to characterise dendritic cells (DC) within this compartment into two major populations. The major subpopulations are CD11c+, MYD-1+, CC81–, WC10– or CD11c–, MYD-1–, CC81+, WC10+ and can be further subdivided by the expression of CD4 and CD8. These distinct phenotypic DC populations express high levels of the maturation markers CD83, CD80/CD86, CMRF-56 and MHC II and are capable of stimulating naïve allogeneic lymphocytes. Differences between the populations are unlikely to be due simply to stages of maturation in the afferent lymphatics. Antigen uptake studies *in vitro* showed that all DC populations retained phagocytic functions normally associated with immaturity including receptor mediated phagocytosis of FITC-dextran and latex beads, but not uptake of Lucifer Yellow by fluid phase endocytosis. Within 24 h of intra-dermal injection of particulate antigen (1 mm latex beads), DC carrying beads were found in the afferent lymph. A variable number of beads were found in each of the DC populations suggesting none are specialised for efficient multiple bead uptake. To determine the kinetics of migration of the blood DC precursors through skin, a cohort of whole blood was labelled with a long-term tracking label, CFSE, *in vitro* and reinfused. The number and phenotype of labelled cells migrating into afferent lymph was monitored daily by flow cytometry. Labelled DC of each subset migrating into afferent lymph peaked within 24–72 h of labelling, which is in keeping with rapid turnover of DCs within the different subpopulations.

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Role of Langerhans Cells in Corneal Graft Rejection

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Antigen-presenting cells in the cornea are Langerhans cells (LC), normally present at corneal periphery. Centripetal migration of LCs toward graft seams to be crucial in graft rejection, and TNF-α might induce this migration. We aimed to determine whether LC activity suppression by sTNFRI might improve corneal graft success rate. To check whether TNF-α can induce centripetal LC migration, mice corneas (n = 10 per group) were centrally injected with 10 ng per ml or 1 μg per ml of TNF-α LS in the central cornea were counted by immunofluorescence assay after 1 week. Secondly, donor corneas (2 mm) from C57Bl/6 mice were grafted into naive and presensitized Balb/c recipients. LS number at donor/recipient junction was determined 14 days after surgery. Balb/c (fully disparate) and B10.D2 (minor disparate) eyes bearing C57Bl/6 corneal grafts were topically treated (4 × day) with placebo or TNF-antagonist – soluble TNF receptor I. Grafts were considered rejected as already described (*Sonoda, Transpl 92*). Graft success rate was compared during 8 postoperative weeks. Intracorneal TNF-α injection induced dose-dependent centripetal LS migration (42 LC per mm^2, 55 LC per mm^2). Number of LCs at graft rim was significantly lower in naive corneal graft recipients (48 per mm^2) as compared to sensitised corneal graft recipients (98 per mm^2, $p < 0.05$). Topical sTNFRI treatment improved corneal graft success rate in minor-only disparate graft recipients (60% vs. 30% in placebo, $p < 0.05$). Corneal Langerhans cells are activated both by TNF-α injection and corneal transplantation. sTNFRI has suppressive effect on corneal LCs and can attenuate immune response to minor H alloantigens.

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Langerhans Cells in Corneal Graft Rejection

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The aim of this study was to evaluate the role of donor Langerhans Cells (LC) in allorecognition of corneal grafts. We used mice in which minor HYA was involved (C57BL/Go female recipients-C57BL/Go male donors. The median survival times (MSTs) of test skin grafts were estimated in recipients that were three weeks earlier pretreated with fresh cornea graft or superficially cauterized cornea graft. The presence of conglomerates of LCs were correlated with skin graft survival. The human corneo-scleral rings (remnant after excision of the corneal transplant) were analysed for the number of donor LC conglomerats, and they were correlated with survival of corneal grafts in human patients. In central part of intact mice corneas we did not found LC conglomerats while they appeared in cauterized corneas. C57BL/Go female rejected faster test graft after senzitization with cauterized cornea graft (MST 10 days) comparing with sensitization with normal cornea (MST 17 days). In experiment with human corneas we did not find correlation between number of donor LC cells in corneo-scleral ring and rejection rate of transplanted corneas, instead inflammation and neovascularisation of recipient eye were predominant reason for corneal rejection. Although donor derived Langerhans cells can induce corneal allograft rejection (direct pathway) it is most probable that the majority of corneal graft rejection is in conjunction with recipient derived LC which are mobilized in the presence of risk factor of the recipient eye (indirect pathway).

P69

IL-12 Production of Mouse Langerhans Cells: Regulation by TGF-β for the Induction of TH1 Cells

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IL-12 is a cytokine known to serve as a powerful mediator for Th1-type differentiation of T helper cells both *in vitro* and *in vivo*. We have previously shown that TGF-β enhances IL-12 production of anti-CD40 mAb-stimulated LC. In this study, we investigated the ability of anti-CD40 mAb/TGF-β-treated LC to prime antigen-specific T cells *in vitro*, and examined the cytokines that they induced in naive T cells. A highly purified population of LC (> 95%) was prepared from BALB/c mouse skin by the panning method using anti-I-A^d mAb. LC were incubated overnight in the presence of OVA peptide [323-339] 1) with anti-CD40 mAb or 2) with anti-CD40 mAb plus TGF-β. LC were then washed and co-cultured with naive $CD4^+$ $CD62L^{high}$ T cells isolated from the spleen of OVA323-339-specific TCR-transgenic BALB/c mice (OVA23-3). The culture supernatants were collected after 24 h for measurement of IL-2 and after 72 h for measurement of IL-4 and IFN-γ. Anti-CD40 mAb/TGF-β-treated LC induced in T cells greater levels of IFN-γ and lower levels of IL-4 than anti-CD40 mAb-treated LC did. However, there were no significant differences in IL-2 production of T cells primed by either LC. Thus, our *in vitro* data provide further evidence that TGF-β, together with CD40 ligation, induces Th1 responses in the skin.

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Polarized Dendritic Cells Mediate CD8 help for $CD4^+$ T cells: Helper Function of $CD8^+$ T Cells in the Development of Th1 Responses

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Th1 responses are essential in resistance against most intracellular pathogens and cancer. We show that the induction of IL-12 production in DC during the priming of naive T cells and the resulting development of Th1 cells critically depend on the cooperation between CD40L-expressing naive $CD4^+$ T cells and IFN-γ-producing naive $CD8^+$ T cells. The interaction of DC with $CD8^+$ T cells activates DC and results in their development into type-1-polarized effector DC that produce elevated amounts of IL–12 upon subsequent interaction with $CD4^+$ T cells and induce strongly-polarized Th1 responses. In contrast, DC maturation induced by either bacterial LPS or stromal-type inflammatory cytokines, such as TNFα and IL-1β, exhausts their IL-12-producing capacity. The currently described helper function of $CD8^+$ T cells helps to explain preferential induction of Th1 responses against intracellular pathogens and contact allergens, which are effectively presented in context of MHC class I. The ability of DC to receive imunomodulatory signals from $CD8^+$ T cells, a classical effector T cell subset, represents a novel mechanism of immunoregulation and may help in designing improved DC-based immunotherapeutic strategies.

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Antigen-Presenting Function Perturbation by UVB Irradiation of Human Epidermal and Monocyte-Derived Langerhans Cells

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Exposure of the human skin to UV radiation causes an imbalance of its homeostasis and a reduced cutaneous immunity. The immunosuppression brought about by UVB is assumed to result in particular from the direct effects of UVB on the antigen-presenting function of the Langerhans cells (LCs), adhesion and co-stimulatory molecules being of crucial importance for the interaction of antigen-presenting cells with immune cells, especially T lymphocytes. The aim of the present study was to determine the differential effects of UVB on Langerhans cells antigen-presenting function by means of two models: a human skin explant model irradiated with increasing levels of UVB (312 nm; 0.5–1 J per cm^2): after irradiation, LCs freshly isolated were tested for their allostimulating capacity in mixed lympho-epidermal culture (MLEC). UVB inhibited the lymphocyte proliferating response which was evaluated in the presence of ^3H thymidine : 0.75 J per cm^2 caused a 43% inhibition of the response; a human monocyte-derived Langerhans cells (MoLCs) model: monocytes were cultured for 6 days in supplemented RPMI-1640, containing 50 ng per ml IL4, 100 ng per ml GM-CSF, 4 ng per ml TGFβ. *In vitro* UVB irradiation of MoLCs to increasing doses (0.005–0.03 J per cm^2) perturbed their allostimulating capacity in MLEC (0.03 J per cm^2 caused a 34% inhibition of the response) and modulated their functional expression of HLA-DR, CD54 and CD86: MoLCs were stained for four-color analysis with the corresponding different monoclonal antibodies and analyzed on a double laser equipped FACSCalibur flow cytometer. Better knowledge of mechanisms involved in UV-induced immune suppression is essential for developing new strategies aimed at photoprotection and cancer prevention. Our results can be used and expanded to quantify UV-mediated Langerhans cell damage and the degree of immune photoprotection provided by various agents or sunscreens.

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Comparative Determination of Sunscreen Immune Protection Factors by Using Either Human Skin Explants, Langerhans Cell or Monocyte-Derived Dendritic Cell Suspensions

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The immunosuppressive effect of UVB has long been recognized and is thought to predispose to the development of skin cancers. Sunscreens are generally accepted to protect the skin against erythema and photoaging but their ability to protect against immunosuppressive effects is still debated and the need to determine sunscreen immune protection factors (IPF) is increasing. In the present study, three *in vitro* models have been developed to determine sunscreen IPF. First, human skin explants were treated or not with sunscreens with different sun protecting factors (SPF 4 and 15) and irradiated with increasing doses of UVB before epidermal cells were recovered by trypsinization. Alternatively, human enriched Langerhans cell (LC) or day 6 monocyte-derived dendritic cell (DC) suspensions were irradiated through a piece of translucent strip recovered or not with the sunscreens. The allostimulatory function of the different cell suspensions was then analysed in a 5-day mixed lymphocyte reaction. Results showed that in any experimental conditions, the UVB dose providing 50% (D50%) immunosuppression in the presence of sunscreens was significantly higher than that obtained with their respective vehicles, thus demonstrating the sunscreen immunoprotective effect. IPF were determined by the ratio of the D50% value in the presence of sunscreen to that of vehicle alone. IPF values for SPF 4 and 15 sunscreens were: 5.1 ± 2.7 and 9.8 ± 5.6; 6.2 ± 2.5 and 10.5 ± 2.9; 4.0 ± 1.0 and 10.5 ± 3.4 using human skin explants, LC or monocyte-derived DC, respectively. In conclusion, IPF values for sunscreens were very similar whether we used human skin explants or reduced the model to either LC or monocyte-derived DC suspensions. Furthermore, IPF ranked according to the sunscreen SPF values. Although investigating only one aspect of immunosuppression, i.e. the inhibition of DC allostimulatory function, these models proved to be very useful in determining sunscreen IPF.

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IL-4 Mediated Suppression of Prostaglandin E2 Production in Human Monocyte-Derived Dendritic Cells: Transcellular Arachidonic Acid Metabolism Enhances Priming of Th2 T cells

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Prostaglandin E2 (PGE2) is a member of the eicosanoid family of arachidonic acid derivatives which is liberated from membrane phospholipids by phospholipases. Eicosanoids have been implicated in the regulation of inflammatory responses and T helper (TH) cell development. We show here that IL-4, which is required for dendritic cell development from monocytes, suppresses PGE2 production by human monocyte-derived dendritic cells (moDCs). MoDCs, however, efficiently converted exogenous arachidonic acid into PGE2. PGE2 production from exogenous arachidonic acid further reduced IL-12 biosynthesis in LPS-matured, ''exhausted'' moDCs in response to CD40 ligation and enhanced their ability to induce IL-4 producing Th cells in mixed leukocyte reactions at a low moDC:T cell ratio. Our data together with previous reports suggest that cytokine exhaustion, declining availability of antigen and accumulation of free arachidonic acid followed by ''transcellular'' metabolism are important for Th2 development. These conditions all apply for the late phase of inflammatory and immune responses.

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Interleukin-3 Modifies the Capacity of Human Monocyte-Derived Dendritic Cells to Induce Helper T Cell Responses: Shift Towards a TH_2 Cytokine Secretion Pattern

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Human monocyte-derived dendritic cells (DC1) were described to induce TH_1 differentiation, whereas dendritic cells (DC2) derived from plasmacytoid cells induce TH_2 differentiation. We wondered whether human monocyte-derived dendritic cells cultured in different cytokine microenvironments display different TH-inducing patterns. The standard method to generate dendritic cells from monocytes is culture with GM-CSF + IL-4; standard approach to obtain DC2 is culture in the presence of IL-3. Human $CD14^+$ monocytes (pDC1) express not only GM-CSF-receptors (CD116) but also IL-3-receptors (CD123). Therefore, we cultured dendritic cells with IL-3 (i.e. instead of GM-CSF) and IL-4. Maturation of both subpopulations (i.e. control GM-CSF + IL-4 DCs and experimental IL-3 + IL-4 DCs) was induced with a cytokine cocktail of TNFα, IL-1β, IL-6 and PGE_2. Yields of these two populations were almost identical; they showed similar morphology (''veils'') and phenotype. Only CD1a was differentially expressed: ''GM-CSF cells'' expressed high levels whereas ''IL-3 cells'' were negative. Also, the immunostimulatory capacity in the mixed leukocyte reaction was equal. When these two mature dendritic cell populations were stimulated with CD154 and IL-12 p70 was measured by ELISA, ''IL-3 cells'' produced significantly less IL-12 than ''GM-CSF cells''. In co-culture experiments with naive allogeneic $CD4^+$ T cells these ''IL-3 cells'' induced T cells to produce significantly more IL-5 and IL-4 and less IFN. as compared to stimulation with conventional ''GM-CSF cells''. These results indicate that a different cytokine environment during differentiation of pDC1 can change the nature of dendritic cells from a TH_1-inducing antigen-presenting cell to a TH_2-inducing antigen-presenting cell.

P75

Differential Regulation of Human Blood Dendritic Cell Subsets by Interferons

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Based on the relative expression of CD11c, two types of DC precursors have identified in human peripheral blood. A $CD11c^+$ population is myeloid-DCs, whereas a $CD11c^-$ population, sometimes called type I interferon-producing cells but better known as plasmacytoid DCs, is lymphoid-DCs. Here, we investigate the effects of IFN-α/β and IFN-γ, as well as other cytokines, on $CD11c^+$ and $CD11c^-$ DC subsets, directly isolated from the peripheral blood. Both IFN-γ and IFN-α, rather than GM-CSF, were the most potent cytokines for enhancing the maturation of $CD11c^+$ DCs. Incubation of $CD11c^+$ DCs with IFN-γ also resulted in increased IL-12 production, and this IL-12 allowed DCs to increase Th1 responses by alloreactive T cells. In contrast, IFN-α did not induce IL-12 but rather augmented IL-10 production. IFN-α-primed matured $CD11c^+$ DCs induced IL-10-producing regulatory T cells; however, this process was independent of the DC-derived IL-10. On the other hand, IFN-α, by itself, neither matured $CD11c^-$ DCs nor altered the polarization of responding T cells, although this cytokine was a potent survival factor for $CD11c^-$ DCs. Unlike IFN-α, IL-3 was a potent survival factor and induced the maturation of $CD11c^-$ DCs. The IL-3-primed $CD11c^-$ DCs activated T cells to produce IL-10, IFN-γ and IL-4. Thus, $CD11c^+$ and $CD11c^-$ DC subsets play distinct roles in the cytokine-network, especially their responses to interferons. In exerting the unique and integral roles to prime naive T cells, DC system not only shows heterogeneity in terms of cellular lineage, but also has plasticity to induce different Th cell development depending on the cytokine milieu