Einwanderung von Langerhanszellvorläufern in die Epidermis: Entwicklung eines Modellsystems

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1. Introduction

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1.Introduction

Dendritic cells (DC), and thereby also Langerhans cells, are at the centre of a widespread interest in immunology today. Many research groups think of them as promising remedies in various fields, all connected with the misfunction, i.e. illness, of the immune system: allergy, autoimmunity, AIDS, tumour immunotherapy, transplantation and many others. Because of their unique features among the cells involved in an immune response scientists hope to be able to manipulate the immune system one day – with dendritic cells as tools – towards tolerance or a strong immune response, depending on the illness fought.

1.1 Historical aspects

Discovery

Dendritic cells were discovered in an attempt to understand and better define what were initially called "accessory cells". Clonal selection theory in the 70s stated, that antigens simply select and expand responsive lymphocytes – at that time it had not yet been discovered, that T cells recognize fragments of antigen presented on MHC molecules. Therefore, it was surprising that it was difficult to create immunity in tissue cultures: when attempts were made to stimulate purified lymphocytes with antigens and even mitogens, additional accessory cells were required. This phenomenon was studied in two main systems: the primary antibody response by mouse splenocytes (1) and the recall response to protein antigens by cells from primed guinea pigs (2,3) and humans (4,5).

The accessory cells were supposed to be macrophages. Macrophages, though, showed weak or absent functionality as accessory cells for the antibody response, and in cell biologic studies it could be shown that antigens they had taken up were thoroughly degraded (6,7). Therefore, an examination of adherent mouse splenocytes, the best source for accessory cells, was begun. One fraction of these cells was a cell that had not been described before, and it was termed "dendritic cell"(8,9).From today's point of view these dendritic cells must be considered as mature interdigitating DCs.

Spleen dendritic cells had unusual cell and nuclear shapes, and continually formed and retracted processes. Unlike macrophages, dendritic cells had scarce lysosomes, lacked Fc receptors, and were poorly endocytic *in vivo* and *in vitro* (9,10). They were bone marrow-derived, independent of T cells (present in nude mice), did not respond to either B- or T-cell mitogens, and had limited viability in culture (11).

Less than 1% of splenocytes were dendritic cells, but they could be enriched 10- to 20-fold by flotation of dense BSA (9,10,12). Non-phagocytic cells with a similar appearance to isolated dendritic cells were evident in sections of mouse spleen, primarily in the white pulp, rich in lymphocytes, rather than red pulp, rich in macrophages (8).

Dendritic cells were found to express high amounts of MHC class II, as shown by immunofluorescence and complement cytotoxicity with alloantisera and monoclonal antibodies (10,13). In quantitative binding studies this expression was much higher than on the prototype MHC class II-positive cells, B-lymphocytes (13).

In contrast, most macrophages in the peritoneal cavity and spleen of mice proved to be MHC class II negative (10). They only became MHC class II positive when exposed to lymphokines *in vitro* [interferon (IFN)-γ] (14,15) or when elicited during a cell-mediated immune response *in vivo* (16).
Finally, dendritic cells were proved to be the active accessory cells for the antibody response (17-20), whereas macrophages were inactive or suppressive (21).
The distinctive role of dendritic cells is to initiate immune responses of quiescent T lymphocytes. Once sensitised, the T lymphoblasts readily interact with other antigen-presenting cells, such as macrophages or B cells (20,22-24).
In summary these early studies showed that a novel cell type, distinct from macrophages, had been discovered.

1.2 Biology of dendritic cells – specific functional properties

There are three properties, that distinguish dendritic cells from all other cells involved in an immune response and that allow them to fulfil their task in such a "professional" way:

1. function as sentinel: acquisition of antigens in tissues
2. migratory function: migration to lymphoid organs
3. function as "nature's adjuvant": identification and activation of antigen-specific T cells.

These 3 functions are corresponding to different periods during the life cycle of a dendritic cell and happen in separate tissues in the body, as portrayed in simplified terms in table 1.
Only the main, basic functions and properties of dendritic cells are shown, necessary for a first understanding of the dendritic cell system. Other fields of dendritic cell activity, such as the stimulation of peripheral memory T cells or the induction of tolerance in the periphery or the thymus, are not mentioned.

Table 1: Functions of dendritic cells in correlation with their maturation stage, properties and localisation in the body (simplified). Legend: DC...dendritic cell, Φ...macrophage.

Maturation stage	Function	Properties	Localisation
immature DC	sentinel	intermediate antigen uptake as compared to Φ, high ability for antigen processing	stratified squamous epithelia, interstitium (exception: brain)
maturing DC	migration	upregulation of (peptide-loaded) MHC I+II and costimulators (CD40, CD54, CD58, CD80, CD86), migration to T-cell areas of the draining lymph nodes or spleen	afferent lymph (so called "veiled cells"), blood
fully mature DC	"nature's adjuvant"	optimal presentation of antigenic peptides and interaction with antigen-specific T cells for induction of an immune response	T-cell area of lymph node or spleen (so called "interdigitating DC")

Today the details of this course and its regulation are a quickly developing field of investigation.

1. Function as sentinel

Dendritic cells, and thereby Langerhans cells, arise from progenitors in the bone marrow (11,25-27). During their ontogeny, described in more detail in chapter 1.3, they migrate to non-lymphoid tissues where they reside as immature dendritic cells.
Immature dendritic cells can be found in all stratified squamous epithelia such as the skin, where they are named Langerhans cells (28), and – as cells similar in morphology to Langerhans cells – in epithelium of anus, pharynx, oesophagus, vagina (29-31) and cervix (32,33). They are also found in the epithelium of the airways of the lung (34-41), the intestine (42,43), and the iris and ciliary body (44,45). There, even if laying within the epithelium, they maintain contact with the subepithelial space.
Dendritic cells can also be found in the interstitial spaces of most organs, like for example heart, liver or kidney (46-48). Only the brain makes an exception (46). Dendritic cells resident in the dermis are also considered as interstitial dendritic cells (49). Furthermore in spleen and lymph nodes a sub-population of immature dendritic cells can be found.
This tissue distribution maximises the possibility of antigen capture and thereby the development of an immune response.

There is little information about the turn-over rates of dendritic cells in these different compartments: Langerhans cells in the epidermis have a half-life of at least one month (25), in the conducting airway epithelium a very short half-life of 2 to 3 days (50).
As to the phenotype dendritic cells in non-lymphoid tissues, a well studied example being Langerhans cells, are immature and still express some monocyte/macrophage-markers (Fc-receptor/CD32$^+$, complement-receptor/CD11b$^+$, F4/80$^+$, membrane bound ATPase$^+$, unspecific esterase$^+$, endogenous peroxidase$^+$, receptor for M-CSF/CD115$^+$) (51-53). MHC class I and II are expressed constiutively at high levels (intracellularly, not on the surface membrane), that are further upregulated and translocated to the cell surface (54) upon induction of maturation (55-59). Costimulatory molecules can be detected only at low levels if at all (52,60).
Also mechanisms for antigen uptake are active constitutively, specially macropinocytosis (61). Other mechanisms used are receptor-mediated molecule uptake (61-65) and phagocytosis of particles or micro-organisms, like latex (66), yeasts (67) or leishmania (68).
Even if dendritic cells are not so potent in phagocytosis as macrophages they are highly specialised in processing the antigen for a short period of time (69). Only fresh isolates from epidermis (70,71) and spleen (72) present native proteins. The specialisation for antigen processing is reflected in the presence of a large amount of suitable organelles in immature dendritic cells freshly isolated or cultured form skin, spleen, blood and bone marrow: endosomal compartments in which peptides derived from exogenous antigens likely gain access to newly synthesised MHC class II (54,73-76).
The constitutively active macropinocytosis and the effective antigen processing supply the basis for eliciting an immune response even with very low concentrations of antigen. So, while residing in non-lymphoid tissues dendritic cells are "actively awaiting" the moment of antigen contact and inflammation.

2. Migratory function

Antigens usually enter the body through its surfaces. The vast majority of T cells though is contained in peripheral lymphoid organs. To obtain a successful primary immune response the distance between body surface and T-cell area – for example of the draining lymph node – must be covered. Dendritic cells are specialised to fulfil this task and build a system that occupies discrete portions of nonlymphoid and lymphoid organs, interconnected by defined pathways of movement: via the blood to the T cell areas of spleen or via the afferent lymph to T cell areas of lymph nodes.
Langerhans cells are a well studied example for migration through the afferent lymphatics. After antigen encounter *in vivo* they begin to mature and to migrate, when appropriate inflammatory stimuli like GM-CSF, TNF-α or IL-1β are present (77-81). The cytokine requirements for maturation are likely to be complex and are still under investigation.
The maturing Langerhans cells begin to enter the afferent lymph, forming cords of "veiled cells" in dermal lymphatics (82-87). The importance of this pathway is underlined by the finding, that in certain primary responses, like sensitisation to skin transplants (88) and contact allergens (89) afferent lymphatics need to be intact.

Several chemokines are discussed to play a role in the attraction towards the lymphatic vessels. Some examples are RANTES, MCP-1, TNF-α, SLC and MIP-3β (90-93). Molecules, that apparently play a role in emigration of Langerhans cells from the epidermis, are E-cadherin, the mediator for adhesion to keratinocytes, that is down-regulated during maturation (94,95), CD44 (96), p-glycoprotein (MDR-1) (97), and alpha 6 integrins (98). Already this "simple" detachment from the tissue where dendritic cells reside and the movement towards the peripheral lymphoid organs requires high specialisation and the mechanisms involved are not yet fully elucidated.
Another pathway for migration is the blood. On this route dendritic cells gain access to the spleen. Evidence for this second pathway was obtained in heart transplant models, in which donor derived dendritic cells were found in the blood stream and spleen of the recipient (99,100). Alternatively, dendritic cells found in the blood stream could be migrating from the bone marrow to nonlymphoid tissues This point will be discussed later (1.3 Ontogeny of Langerhans cells).
Another interesting property of dendritic cells is that they thoroughly change their phenotype during migration: they mature (52,86,87,101-103). In vitro MHC class II molecules loaded with processed antigen are quickly upregulated and retained at the cell surface with high stability (104). This guarantees that dendritic cells remain immunogenic until they reach the peripheral lymphoid tissue and encounter T cells. To effectively cluster T cells and to activate them co-stimulatory molecules (CD40, CD54, CD58, CD80, CD86) are expressed at high levels. At the same time dendritic cells undergoing maturation lose the abilities they needed as immature cells: antigen capture and MHC biosynthesis are downregulated (104).
The development of dendritic cells summarised with "migratory function" describes a far-reaching change of phenotype, skills and position in the body. This surely makes these cells unique among all others and again shows their great extent of specialisation.

3. Function as nature's adjuvant

Once dendritic cells reach the peripheral lymphoid organs they are fully mature. Only afferent, but not efferent, lymph contains "veiled" cells (105-111). Nevertheless dendritic cells do not accumulate in the lymph node and probably quickly die of apoptosis if no fitting T cell clone could be bound (59,112,113). *In vivo* antigen-pulsed dendritic cells injected without any adjuvant cluster large numbers of antigen-specific $CD4^+$ T cells around them in the T-cell areas of peripheral lymphoid organs (114).
In vitro mature dendritic cells are unusually potent stimulators in the primary mixed leukocyte reaction (MLR): small numbers of dendritic cells pulsed with low doses of antigen stimulate strong T-cell responses (115,116). Enriched dendritic cells could be used in roughly 100 times smaller numbers than bulk leukocytes. Other MHC class II-positive cells, including B cells and macrophages, were weak or inactive (14,115,116). Potency in MLR stimulation is still the most convenient assay for demonstrating the function of mature dendritic cells, in which both $CD4^+$ helper and $CD8^+$ killer responses are induced (23,117).
These MLR findings provided the first clues to the physiologic role of dendritic cells.

Antigen presentation on MHC molecules (signal 1) alone is necessary, but not sufficient to initiate a response by resting T cells *in vitro*. Also B cells and macrophages can function as APCs (i.e. show signal 1), but these cell types lack the accessory or costimulatory molecules (signal 2) that are strongly expressed by dendritic cells. Once activated by dendritic cells, T cells can efficiently interact with other APCs in either the antibody response or the MLR (20,22). As to an antibody response it is worth to be reminded, that activation of T cells develops in two phases: a first step, in which priming is induced by dendritic cells, and a second step, in which the T helper cell interacts with B cells as the APCs to induce antibody formation. Also in the MLR priming of naive T cells requires antigen presented on dendritic cells, but once activated, T-cell blasts respond vigorously to antigen presenting B cells and reciprocally cause B cells to proliferate and make antibody *in vitro* (20).
Dendritic cells strongly express a broad spectrum of second signals that mediate T-cell binding and costimulation: CD40, that interacts with CD40L on T cells and enhances maturation, CD54 [intracellular adhesion molecule (ICAM)-1], CD58 [lymphocyte function associated antigen (LFA)-3], and B7s (CD80/B7-1, CD86/B7-2) (100,103,118,119). Especially CD54/ICAM-1 and CD86/B7-2 are expressed at higher levels on dendritic cells than on others (120).
The number of interactions between MHC-peptide and T cell receptor (TCR) that are required to stimulate a T cell is decreased in the presence of costimulators (121). As to the *in vivo* situation costimulatory molecules probably play a crucial role in promoting an immune response at the beginning of an infection, because only low concentrations of antigen are present and the number of MHC-peptide molecules on dendritic cell surface is lower than in artificial *in vitro* systems. When an organism can eliminate the antigen, for example bacteria, quickly after infection the manifestation of a disease can be avoided, what in turn can be crucial for its survival.
Dendritic cells can bind T cells loosely in an antigen-independent fashion. This may provide the opportunity for small amounts of MHC-peptide to be recognised (122,123). In current opinion, the couples of molecules function in a cascade-like manner to optimise the APC-T cell interaction, even if the mechanisms involved are not yet fully understood. After a first loose contact the TCR can recognise the antigen on the MHC molecule. Then ICAMs solidify the cell-cell binding and successively the other costimulators (B7-2, CD40) come into play (124-126). This interaction has the characteristics of a dialogue, as each of the partners sends and receives information and signals.
One of the signals that is received by dendritic cells is to produce cytokines and to stay alive for further stimulation of T cells. Maturing dendritic cells secrete high levels of IL-12, which enhances the formation of Th1 type helper and killer cells (127,128), and are resistant to the effects of IL-10 (129). IL-10 can induce apoptosis in developing dendritic cells, what can be prevented by the addition of TNF-α or CD40L (130). The life span of dendritic cells can be prolonged by interaction of CD40 (on mature dendritic cells) and CD40L (on activated T cells) (126), and by TRANCE receptor (on dendritic cells) and TRANCE (on activated helper and cytotoxic T cells (131).

In a vaccination it is necessary to add artificial substances, like oils in complete Freund's adjuvans or colloidal aluminium salts, to induce immunity to a certain antigen. A postulated mechanism of action of adjuvants is to enhance the expression of costimulators on macrophages and other APCs. Dendritic cells can activate naive and quiescent T cells, both $CD4^+$ T helpers (132-135) and $CD8^+$ T killers (136-141) *in vivo,* and thereby initiate a primary immune response, in the absence of any other adjuvant.
These findings led to the conclusion, that dendritic cells were acting as "nature's adjuvant" (113).
To denominate all cells that have both, antigen-presenting and costimulatory functions the term "professional APC" is now used.

1.3 Ontogeny of Langerhans cells

Since Katz *et al.* and Frelinger *et al.* in 1979 demonstrated that Langerhans cells derive from a mobile pool of cells that originates in the bone marrow (25,26), many attempts have been made in humans and rodents, especially mice, to establish intermediate stages and the ontogenetic relationship between Langerhans cells and other hematopoietic cell lineages. Even if a broad variety of approaches have been undertaken a definitive ontogenetic pathway or even culture conditions that give rise to pure Langerhans cell populations have not yet been identified. In the following I want to give a short overview of our current thinking.

1.3.1 Human system

There are some clear advantages about the identification of Langerhans cells in the human as compared to the widely used murine system. First of all, antibodies exist, that selectively stain human Langerhans cells, like "Lag", an antibody that recognises a Langerhans-associated granule antigen of unknown function, and Langerin, an endocytic receptor involved in the induction of Birbeck granules (142,143). No such specific antibody exists for the murine system. The murine homologue for Langerin has just been cloned and the first mAb´s are becoming available (S. Saeland, Schering-Plough, Dardilly, France, personal communication) Furthermore Birbeck granules of human Langerhans cells in culture remain stable for long periods, whereas Birbeck granules of murine Langerhans cells disappear within 72 hours when cultured with keratinocytes or keratinocyte-supernatant (52). Therefore, in the mouse the only unequivocal markers for the identification of Langerhans cells are missing, and the approaches to find answers to the above mentioned questions are far behind those in the human system.

Generation of Langerhans cells from $CD34^+$ progenitors

The first ones to establish culture conditions for the generation of Langerhans cells were Caux *et al.* in 1992 (144). $CD34^+$ hematopoietic progenitor cells (HPC) isolated form **human umbilical cord blood**, cultured in serum-containing medium supplemented with GM-CSF and TNF-α for 12 days, gave rise to a $CD1a^+$ population (20 – 50% of all $CD34^+$ HPC) of dendritic/Langerhans cells, of whom 1 out of 5 cells contained Birbeck granules as assessed by electron microscopy (~ 4 – 10% of $CD34^+$ HPC).

Four years later, in 1996, Strobl *et al.* presented a serum-free culture system of $CD34^+$ HPC (145). The medium was supplemented with GM-CSF, SCF, TNF-α and TGF-β1, the latter being essential for the appearance of Lag^+ Langerhans cells (yield 21% of all CD34+ cells), that regularly showed Birbeck granules.
In the same year Caux *et al.* extended our understanding of Langerhans cell development by their discovery of two apparently independent pathways, only one of which led to Langerhans cells, namely the $CD1a^+/CD14^-$ subpopulation of $CD34^+$ HPC (146). From this subpopulation, after 12 – 14 days of culture in serum-containing medium, supplemented with GM-CSF and TNF-α, a subset expressing E-cadherin, Lag (19 – 34% Lag^+) and Birbeck granules (31 – 56% BG^+) arose (12 – 14% Lag^+ of the total $CD34^+$ population). When these results are compared with those of Strobl (145) TGF-β1 seems to increase the total percentage of Langerhans cells and to push development of progenitors towards this special cell type *in vitro*. The other pathway, $CD14^+/CD1a^-$ cells, gave rise to $CD1a^+$ dendritic cells lacking E-cadherin, Lag and Birbeck granules, that were supposed to be dermal dendritic cells.
In 1997 Strobl *et al.* published another paper about serum-free culture of Langerhans cells (147). In this system the culture medium was supplemented with flt3-ligand in addition to TGF-β1 and the above mentioned cytokines (GM-CSF, SCF, TNF-α). The addition of flt3-ligand only slightly increased the percentage of Lag^+ cells (from 19% to 21%) and thereby seemed not to be crucial for the development of Langerhans cells.
Of much more interest are two recently published papers, one from Jaksits *et al.* (148) and one from Caux *et al.* (149), that further describe the two different developmental pathways of Langerhans cells Caux *et al.* have already described in 1996 (146).
Jaksits *et al.* found, that the addition of TGF-β1 to a serum-free culture medium further supplemented with GM-CSF and TNF-α, was only necessary for the development of Langerhans cells (Lag^+, BG^+) from $CD14^+/CD1a^-$ precursors (the first six days cells were grown in a serum-containing medium, then sorted for CD14 or CD1a and transferred to a serum-free culture medium conditioned with defined cytokines). The $CD1a^+/CD14^-$ population could develop into Langerhans cells independently of TGF-β1 (serum-free medium supplemented with GM-CSF and TNF-α). As to the yields of Langerhans cells in both pathways, the majority of cells were Lag^+ and 10 – 20% showed Birbeck granules, but the $CD14^+/CD1a^-$ precursor population underwent one more cell cycle as compared to the $CD1a^+/CD14^-$ precursor population, and thereby seems to be the major "route" for the development of Langerhans cells. Basically the findings of Caux et al. suggested the same (149).
These newer results in the human system strongly add to the importance of TGF-β1 for the development of Langerhans cells. The finding, that TGF-β1 knock-out mice are devoid of Langerhans cells, but not of other dendritic cell types, reflects the importance of this cytokine for Langerhans cell-development also in the murine system (150,151).
Parallel to the culture systems that used $CD34^+$ HPC from human cord blood another source of $CD34^+$ HPC was examined, the **human peripheral blood**. The first ones to describe such a culture system were Mackensen *et al.* (152). The blood was taken from cancer patients, $CD34^+$ HPC were enriched and then cultured in serum-containing medium supplemented with

a whole armada of cytokines (IL-4, GM-CSF, SCF, erythropoietin, IL-1β, IL-3, IL-6) for 15 days. The yields of Langerhans cells at first glance were impressing: over 75% of the sorted $CD1a^+$-population were Lag^+; but the $CD1a^+$ cell population made up approximately 10% of all nucleated cells on day 15 of culture. This system was not further examined, maybe because of the scarce availability of this specific source of $CD34^+$ HPC (cancer patients with solid tumours that are undergoing an autologous peripheral blood precursor cell-transplantation) and of the low total yield of Langerhans cells.
The year after another culture system for Langerhans cells was published: Strunk *et al.* developed a procedure for the development of human dendritic and Langerhans cells from $CD34^+$ HPC isolated from buffy coats and leukapheresis products of healthy volunteers (153). $CD34^+$ HPC were cultured for 14 – 21 days in serum-containing medium supplemented with GM-CSF, TNF-α and IL-4. The cell population that arose after that time was approximately 6% Lag^+ and 24% $CD1a^+$. IL-4 decreased total cell numbers, but increased the percentage of $CD1a^+$ cells. In the paper following this work Strunk *et al.* characterised two different subpopulations of $CD34^+$ HPC derived from human peripheral blood of healthy volunteers by the marker cutaneous lymphocyte-associated antigen (CLA) (154). This marker is a skin homing receptor and thereby strongly suggests that the destiny of dendritic cells expressing this marker might be dermis or epidermis. After 10 – 18 days of culture in serum-containing medium supplemented with GM-CSF and TNF-α only the CLA^+ subset expressed Birbeck granules and Lag (12% - 22,5% $CD1a^+$, 5 – 20% Lag^+; 4 – 7.5% BG^+ of $CD34^+/CLA^+$ population). The CLA^- subset never showed Lag reactivity nor Birbeck granules. These results indicate another good and useful marker for Langerhans cell development in the human system: CLA.
All culture systems suggested, that commitment to the Langerhans cell lineage happens at an early stage during the precursors life.

Generation of Langerhans cells from other progenitors from human peripheral blood

Two more publications in this field seemed to be of particular interest to me. One is a paper published by Geissmann *et al.* in 1998, in which it could be demonstrated that Langerhans cells also arise from $CD14^+$ peripheral blood monocytes (155). These cells differ from the above mentioned $CD14^+$ cells as they are not derived from $CD34^+$ hematopoietic progenitor cells. The $CD14^+$ peripheral blood monocytes were isolated from peripheral blood mononuclear cells (PBMC) by negative magnetic depletion and cultured for 5 – 7 days in serum-containing medium supplemented with GM-CSF, IL-4 and TGF-β1, the latter being again essential for the generation of Langerhans cells. Of the thereby generated cells 26 – 33% were BG^+ and 20 – 40% Lag^+ (82% E-$cadherin^+$, 93% $CD1a^+$, 80 – 90% CLA^+). Monocyte derived-dendritic cells obtained in the presence of GM-CSF and IL-4 could be further induced to differentiate into E-$cadherin^+$, CLA^+ and BG^+ Langerhans cells in the presence of GM-CSF, IL-4 and TGF-β1, thus confirming that Langerhans cells and dendritic cells can originate from the same population of $CD14^+$ monocytes and share a common precursor.

The other publication that deserves a close look is the paper published in 1999 by Ito *et al.* (156). $CD4^+/CD3^-/CD14^-$ cells were sorted from peripheral blood mononuclear cells (PBMC) and cultured in serum-containing medium supplemented with GM-CSF, IL-4 and TGF-β1 for 1(!) – 6 days. Already after 24 hours of culture a defined subset of dendritic cells ($CD1a^+/CD11c^+$) started to upregulate E-cadherin and CD1a and to express Langerin. This upregulation and expression increased during the whole time of culture (6 days), leading to a 20 – 25% BG^+ population. Only the $CD1a^+/CD11c^+$ fraction gave rise to Langerhans cells and because of the very short time needed to acquire the specific phenotype was supposed to be a direct precursor of Langerhans cells in peripheral blood.

Langerhans cell ontogeny

Only few publications describe results about Langerhans cell ontogeny in the human system. First hints concerning the arising of Langerhans cells from bone marrow progenitors came from a case report by Volc-Platzer et al. (157) and the paper published by Perreault *et al.* in 1984 (158). As to the latter, the number of Langerhans cells in the epidermis was determined by fluorescent immunolabelling before and after bone marrow transplantation. Directly after transplantation numbers of Langerhans cells decreased and reached a nadir around day 11. Normal values were gained 120 – 365 days post transplant. Considering the experimental evidence in the murine system (25,26) it could be concluded that Langerhans cells in humans originate from the bone marrow. As to the development of Langerhans cells in the epidermis of the human fetus Foster *et al.* described the appearance of $HLA\text{-}DR^+/ATPase^+$ Langerhans cells in human fetal skin already ˆ ı 6 – 7 weeks estimated gestational age, the earliest time point examined (159).
Because of evident difficulties as to experimental material and ethical aspects *in vivo* studies in humans were not extended much further.

1.3.2 Rodent systems

As mentioned above there are clear disadvantages in the rodent systems, taking the murine system as example, in clearly identifying Langerhans cells *in vitro*. As usually in life, though, there are also clear advantages: *in vivo* experiments can be performed easily and the availability of progenitor cells usually is not a limiting factor. Therefore, there are many publications about examination of the ontogeny of Langerhans cells during gestation and a few about immigration of Langerhans cells into the epidermis.

Langerhans cell ontogeny

First of all, as already mentioned, Katz *et al.* and Frelinger *et al.* showed that Langerhans cells are bone marrow derived (25,26).
It could be shown, that in fetal epidermis only very few Ia^+ Langerhans cells could be detected, but Ia^- cells expressing Langerhans cell and macrophage markers (Fc- and C3bi-receptor, F4/80, ATPase) were found before birth (160). These Langerhans precursor cells quickly became Ia^+ after birth and reached levels superior to LC-densities in adult mice on day 7 post partum (160,161). Densities afterwards decreased, but normal values were not detected within 6 weeks. In fetal rats the spreading of Langerhans precursor cells from cranial

to caudal was described (162). Mature Ia^+, BG^+ Langerhans cells appeared from day 21 of gestation on.
Data obtained in these experiments strongly suggested for a major contribution of the epidermis to the maturation of Langerhans cell precursors (163).

Langerhans cell immigration into the epidermis

A field that has only recently became a focus of interest is the immigration of Langerhans cells or Langerhans cell precursors into the epidermis.
As to the migration of fully differentiated Langerhans cells it was shown that when injected intravenously they preferentially migrate to the skin (164). The migration rate is not changed by UVB-irradiation or by working in an allogeneic system. The migrated Langerhans cells are functional and can induce contact hypersensitivity.
When the skin is depleted of Langerhans cells by daily topical application of clobetazole propionate and is then put, epidermal side down, onto a culture medium that contains syngeneic epidermal cells, and thereby fully differentiated Langerhans cells, the latter actively and strongly immigrate into the epidermis (165). In contrast to the above-mentioned intravenous injection experiments semi-syngeneic or allogeneic Langerhans cells hardly immigrated at all. Anti-IL-6– and anti-TNF-α– antibody markedly decreased the numbers of immigrated Langerhans cells by 45 – 60%, what appears to be in contrast with the pro-inflammatory and emigration-enhancing function attributed to these two cytokines.
When the pattern of repopulation with Langerhans cells after mild heat injury is assessed *in vivo*, and thereby the immigration of Langerhans cell precursors, precursors coming from the blood or the dermis reconstitute the epidermal population (166). Langerhans cells residing in the epidermis are neither able to divide nor to migrate to depleted areas.
Another interesting situation is the inflamed skin. Immature bone marrow-derived dendritic cells tether and roll on P- and E-selectin *in vitro* and on murine dermal endothelium *in vivo* (intravital microscopy) (167). They are preferentially recruited to inflamed skin (upregulation of P- and E-selectin in neighbouring endothelia) and were shown to extravasate *in vivo*.
Preincubation of dendritic cells with TGF-β1 further enhanced migration of immature bone marrow-derived dendritic cells to inflamed skin (168). In this work epidermis and Langerhans cells and dermis and dermal dendritic cells were not evaluated separately.

Generation of Langerhans cells from bone marrow-derived progenitors

The only data available on such attempts were the unpublished results of Dr. Franz Koch, presented in a talk at the ÖGAI-meeting 1999. By evaluation with electron microscopy bone marrow-derived precursor cells, cultured with GM-CSF alone for 13 days, were found to differentiate into Langerhans cells. The impressively high rate of 60% of total cells were Birbeck granule-positive.

When the research mentioned in this chapter is considered it becomes evident that the conditions for Langerhans cell development and immigration *in vivo* in the mouse are a promising field of investigation. To find models in which different cytokines, chemokines and culture conditions can be tested is essential for elucidation of the processes that happen in a

healthy organism. This would contribute to a better understanding of this cell type and thereby facilitate its manipulation and specific use.