The skin immune system
Its cellular constituents and their interactions

Jan D. Bos and Martien L. Kapsenberg

The term immunodermatology describes the systematic investigation of the complex mechanisms of the 'skin immune system' in health and disease. In this review Jan Bos and Martien Kapsenberg discuss the skin's vascular and lymphatic systems and the various cells which participate in the immune response. These include Langerhans' cells, indeterminate cells, veiled cells, endothelial cells, mast cells, tissue macrophages and 'homing' T lymphocytes, which are all present in skin under physiological conditions.

Immunodermatology is primarily directed towards an understanding of immunological mechanisms operating in skin diseases. Few investigations have been carried out on healthy skin. The skin functions as a general (physico-mechanical) barrier but also synthesizes a large number of different biologically active substances. The skin is the largest organ of the human body and functions as a general defence system, while the immune system has evolved as a complementary line of defence. This intimate relationship justifies studies of the cells which exert skin immune functions under physiological conditions.

It might well have been Kondo in 1922 who first described intra-epidermal lymphocytes. Fichtelius, Groth and Liden suggested that the skin was a 'first level lymphoid organ'. They hypothesized that 'the lymphocytes reaching the epidermis may to a large extent be non-competent lymphoid cells which become competent during or shortly after a stay in the epidermis or close to it in the corium'.

Streilein coined the term 'skin-associated lymphoid tissues' (SALT) to describe: Langerhans' cells with their antigen-presenting properties; epidermotropic recirculating T-lymphocyte subpopulations (homing T lymphocytes); keratinocytes producing epidermal thymocyte-activating factor (ETAF; Refs 4, 5); and the skin draining lymphoid organ. They hypothesized that 'the lymphocytes reaching the epidermis may to a large extent be non-competent lymphoid cells which become competent during or shortly after a stay in the epidermis or close to it in the corium'.

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The skin immune system

Other cells to be included are mast cells, tissue macrophages, granulocytes, indeterminate cells, veiled cells, vascular endothelial cells and afferent lymphatic endothelium starting in the dermis. All these cells taken together with those comprising SALT, but excluding lymph node tissue, form an intricate and complex system that we propose to call the 'skin immune system' (SIS).

The number of different cell types present in normal skin and exerting immunological functions at least equals the number of cell types that until now are regarded as having no immunological role (Table 1). Cells not involved in the immune response include eccrine and apocrine gland cells with their myoepithelial and duct cells, melanocytes, fibroblasts and fibrocytes, pericytes of the vasculature, smooth muscle and nervous tissue cells, and Merkel cells.

T cells and their subsets

It has been suggested that of the circulating T-cells, an as yet to be quantified number infiltrate the healthy skin and reside there for an undefined period ('homing' T cells). This seems justified by the simple observation that normal skin always contains some scattered T lymphocytes, especially around and above the superficial venous plexus, and rarely intra-epidermally (J.D. Bos et al., unpublished). (Epidermotropism of T cells becomes prominent in many dermatological disease states ranging from the rare cutaneous lymphomas to common benign inflammatory skin diseases such as eczema and psoriasis.

Many questions remain to be answered about the homing T cells in skin. It has not been proved that they are 'homing'. Are they instead being recruited? Do they differ from their counterparts in other healthy connective tissues? Are there any functional or immunophenotypic characteristics by which the homing T cells of SIS differ from the scattered T cells of other connective tissues?

Dendritic antigen-presenting cells

As well as phagocytosing monocytes/macrophages there is another class of HLA-DR+, non-phagocytosing antigen-presenting cells. These include Langerhans' cells (LC), indeterminate cells, veiled cells, interdigitating cells and dendritic reticulum cells. Their subtypes, normal habitat and known markers are summarised in Table 1. The suggested migration pathways of the 'skin family' of dendritic cells present under physiological conditions in man are depicted in Fig. 2.

In mouse epidermis, a new type of dendritic cell was recently described and Romani et al. have provided immunophenotypic and morphological evidence that

Table 1. Overview of skin immune system cells and cells not primarily involved in immune response

<table>
<thead>
<tr>
<th>Skin immune system (SIS) cells</th>
<th>Non-immune response related cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratinocytes</td>
<td>Melanocytes</td>
</tr>
<tr>
<td>Langerhans' cells</td>
<td>Merkel cells</td>
</tr>
<tr>
<td>Indeterminate cells</td>
<td>Fibroblasts/ocytes</td>
</tr>
<tr>
<td>Veiled cells</td>
<td>Eccrine gland cells</td>
</tr>
<tr>
<td>Tissue macrophages (histioocytes)</td>
<td>Duct cells</td>
</tr>
<tr>
<td>Neutrophilic granulocytes</td>
<td>Myoepithelial cells</td>
</tr>
<tr>
<td>Mast cells, connective tissue type</td>
<td>Apocrine secretory cells</td>
</tr>
<tr>
<td>Mast cells, mucosal type</td>
<td>Sebocytes</td>
</tr>
<tr>
<td>Vascular endothelial cells</td>
<td>Smooth muscle cells</td>
</tr>
<tr>
<td>Lymphatic endothelial cells</td>
<td>Neural receptor cells</td>
</tr>
<tr>
<td>'Homing' T cells</td>
<td>Pericytes</td>
</tr>
</tbody>
</table>
these Thy1+ dendritic cells are related to natural killer cells. Their possible human equivalent has as yet not been discovered.

Langerhans' cells

Probably the most important SIS-related cell is the Langerhans' cell (LC), an accessory, antigen-presenting cell that is strongly HLA-DR and T6 (CD1) positive. LC express low densities of the T4 (CD4) antigen in physiological conditions, as well as pathological conditions. They have surface receptors for C3 and the Fc fragment of IgG and are bone-marrow derived. So-called 'Birbeck granules', which are T6 (CD1) positive, can be found intracellularly by electron microscopy, but the function of these structures is as yet unknown. Isolated human epidermal LC produce interleukin-1 (IL-1). Large numbers of LC form a regular and almost closed network of dendrites within the spinal cell layer of the epidermis (Fig. 3). A simple calculation suggests that not less than $1 \times 10^9$ LC are normally present in the epidermis of a human being (Table 3 and Ref. 20). This high number underlines the antigen-presenting potential of the dendritic cells of skin.

One may hypothesize that LC continuously leave the epidermis, presumably through the lymphatics, being replaced by circulating LC precursors. LC have a mean stay of approximately three weeks within mouse epidermis and applying this figure to man would result in a daily repopulation number of about 45 million LC for the entire skin (Table 3). Repopulation studies in patients treated by phototherapy (PUVA) also resulted in a mean repopulation period of three weeks. However, these figures represent abnormal conditions. Chernielewski, Vaigot and Prunieras gave evidence for intra-epidermal cycling of LC. In-vitro analysis of the cell cycle stage of LC indicated that 1.3–3.3% and 1.0–2.5% of epidermal LC were in S and G2/M phase, respectively. These studies do not substantially differ from earlier findings in which [3H]thymidine incorporation labeling indices were found to be very low. Nevertheless, at least part of epidermal LC may be replaced by division from an intra-epidermal LC pool.

For technical reasons we are unable to reproduce this figure in colour. See the July/August issue of Immunology Today for full colour illustration.
Table 2. The family of skin dendritic antigen-presenting cells

<table>
<thead>
<tr>
<th>Dendritic antigen-presenting cell</th>
<th>Normal habitat</th>
<th>Fc-rec</th>
<th>C3-rec</th>
<th>HLA-DR</th>
<th>T6</th>
<th>RFD1</th>
<th>M241</th>
</tr>
</thead>
<tbody>
<tr>
<td>Langerhans’ cell</td>
<td>Stratified squamous epithelia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Intermediate cell</td>
<td>Epidermis/dermis</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Interdigitating cell</td>
<td>T-cell areas RES</td>
<td>?</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Veiled cell</td>
<td>Skin-draining lymph vessels</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

RES = reticulo-endothelial system

Indeterminate cells

Breathnach defined the dendritic nonkeratinocytes found in normal epidermis and lacking Birbeck granules, melanosomes or Merkel cell granules, as the ‘indeterminate cells’. They are HLA-DR+. Similar cells occur within the normal papillary dermis and are T6+, as are LC (Ref. 27). Transitional forms of dermal indeterminate cells and epidermal LC have been noted. Monoclonal anti-M241 reacts with a minority of LC but with a substantial number of other dermal dendritic cells with morphological features of indeterminate cells. Their exact position in the migration pathways of skin dendritic cells is as yet unclear. Some of the possibilities are depicted in Fig. 2.

Veiled cells

Afferent lymph, as described above, contains a group of LC resembling cells known as veiled cells. In the rabbit there are an average of 72,000 veiled cells per ml of skin-draining lymph. Veiled cells are strongly HLA-DR+, bear Fc(IgG) and C3 receptors and have no surface immunoglobulins. They have as yet not been demonstrated in situ in (normal) human skin lymphatics but can be presumed to be present.

Interdigitating cells

Interdigitating cells are normally present in the T-cell areas of the reticulo-endothelial system and carry the RFD1 antigen (as identified by a monoclonal antibody). They are not present in normal skin but RFD1+ dendritic cells have been detected in the inflammatory infiltrates of psoriasis and leprosy and in the lesional skin of atopic dermatitis and pityriasis rosea. Juxtaposition with T cells, a phenomenon also known as periploesis, was always found and this combination may be indicative of T-cell-mediated immune reactions.

Dendritic reticulum cells

These cells are normally present in the B-cell areas of lymph nodes and spleen but are not present in normal skin (unpublished observations). In comparison with interdigitating cells, they are rare in pathological conditions. This is not surprising because B-cell accumulations are rare in dermatological disease. We have observed DRC+, HLA-DR+ dendritic reticulum cells in two cases of lymphocytoma cutis, a benign disease in which some subtypes show more or less symmetrical intradermal infiltrations resembling ectopic lymph nodes (unpublished observations).

Dendritic reticulum cells are distinct from the other members of the dendritic cell family because they are mesenchymal and not bone-marrow derived. For this reason they have not been included in Tables 1 and 2, nor in Fig. 2.

Mast cells

Mast cells have an important potential for regulating vasculature and possibly have an important role in T-cell trafficking (see below). Mast cells may be divided into type I (mucosal) and type II (connective tissue) cells. ‘Mucosal’ and ‘connective tissue’ are misleading terms because both types occur in mucosa and in the connective tissue of the dermis (T. S. Orr, unpublished). Type I mast cells are small and unlike type II cells contain no heparin. Distinction of subtypes may be obtained by an Alcian blue-Safranin staining sequence, or by electron microscopic techniques.

The number of mast cells in normal human skin is quite high and has been estimated as 5120–9472 per mm3 of dermis (mean 7225) (Ref. 40). They play an obvious role in type I immediate hypersensitivity reactions such as immunological contact urticaria.

Tissue macrophages

Within the papillary dermis, there are dense populations of as yet unquantified monocytes/macrophages.
These cells, also known as tissue histiocytes, are scattered, in addition, through the reticular dermis. Their role under physiological conditions may be as scavengers of debris and the degraded cell (granulocyte) substances that are continuously produced. They may also have antigen-presenting functions, although such a capability has as yet not been demonstrated in situ in skin.

**Skin neutrophilic granulocytes**

Neutrophils have been said to leave the blood after an average intravascular sojourn of 10 h. They do not seem to return to the blood after leaving it but their fate after entering the tissues and body cavities is not yet clear. In normal skin, neutrophils are certainly not frequent extravascularly. Their number within the postcapillary venules is high since blood flow there is decreased, and granulocytes participate in immune complex clearing under normal conditions.

**Skin vasculature and lymphatic drainage**

Blood and lymphatic vasculature connect SIS to the remainder of the body’s immune system. Skin vasculature has three levels, depicted in Fig. 1 and reviewed in Ref. 42. It is around the superficial plexus and adjacent to the capillary loops that most SIS-related cells are present under physiological conditions. The postcapillary venules of the upper plexus, with a diameter of 10–20 µm, are the sites of inflammatory cell emigration, histamine-induced vascular permeability and immune complex deposition under different pathological conditions.

Endothelial cells of these postcapillary venules in skin are HLA-DR+ and can act as antigen-presenting cells in vitro. It is evident that endothelial cells of at least part of the skin vasculature exert important functions, to be further elucidated, in the immunophysiology of the skin.

Skin lymphatics start with open ends within the papillary dermis, form sinuses and are initially only lined by a single layer of endothelial cells. Later on they have valves and may, in the subcutaneous layers, acquire a smooth muscle wall. Their organization is similar to that described for the vascular network with upper and lower dermal plexuses (Zhdanov, cited in Ref. 42). Lymph flow from the skin is one-directional towards the skin-related lymph nodes. Skin draining lymph nodes are especially prominent in the body folds such as the elbows, knees, and the cervical, axillary and inguinal regions.

**SALT and SIS**

Several questions about the concept of SALT may be asked. Inclusion of keratinocytes in SALT may be justified by several observations in addition to their production of the interleukin-1-like factor ETAβL. Keratinocytes may play a role in T-cell differentiation. Epidermal cell culture supernatant was shown to induce terminal deoxynucleotidyl transferase, an early T-cell differentiation marker, on immature human bone-marrow cells and T cells and epidermal cells bound antibodies to serum thymic factor and thymopoietin.

Epidermal keratinocytes may therefore have some relationship to thymic cortical epithelial cells in T-cell differentiation but proof for intra-epidermal T-cell differentiation in vivo is still lacking. So-called epidermotropic recirculating T-lymphocyte populations are rare, in our experience, as intra-epithelial T cells are uncommon in normal skin (manuscript in preparation). Perhaps the more commonly encountered T cells in normal dermis are a pool distinct from T cells in other connective tissues.

LC form part of a widely distributed family of dendritic accessory antigen-presenting cells, and the organ specificity of this cell for stratified squamous epithelial tissues does not necessarily imply a distinct and tissuespecific type of immune function. High endothelial venules (HEV) that are supposed to be necessary for the direction of organ-specific T-cell migration have not been described in healthy skin. HEV-like vasculature has, to our knowledge, only been described in a rare skin disorder called angiolymphoid hyperplasia with eosinophilia, also known as Kimura’s disease.

It is clear that other mechanisms for directing T-cell traffic must operate in skin, possibly through antigen-specific, T-cell-derived factors bound to mast cells (see below). Skin-draining lymph nodes do not receive skin lymph only, and the cells comprising SIS cannot be regarded as lymph-node tissue. It is for these reasons that we define the immune response related cells operating in skin as the SIS.

**SIS dynamics**

With SIS, lymph nodes play an important role in tolerance as well as in immunization and sensitization mechanisms. Paracortical T-cell areas which contain dendritic antigen-presenting cells and HEV which direct T-cell migration are essential in the functional cooperation between lymph nodes and SIS.

LC function in vitro as stimulatory cells in epidermal cell-induced autologous and allogeneic T-cell activation and in the generation of epidermal cell-induced cytotoxic T-cell responses. They also function as antigen-presenting cells in in-vitro T-cell activation by
protein antigens. LC also play a role as stimulating cells in graft rejection in vivo.

The role of LC in contact hypersensitivity, a classical model of delayed hypersensitivity, is especially interesting. Sensitization starts with regular exposure of the skin to a hapten. Substances leading to allergic contact reactions generally have a molecular weight of less than 500. They first may bind to a carrier before they can be immunogenic, or they may bind directly to as-yet undifferentiated antigen-presenting cells membranes. The hapten-carrier complex is called anti- gen or immunogen.

Although the exact localization of primary sensitization is still debatable, experiments have demonstrated that an animal can only be sensitized on a prepared skin flap when the connecting part contains, next to essential arteriolar and venous vessels, also the draining lymphatics. It nevertheless remains unclear whether it is the hapten, the sensitized T cell already within the skin ("peripheral sensitization"), or the LC-hapten complex that is carried to the lymph node. Recent evidence provided by Knight et al indicates that hapten-carrying dendritic cells can sensitize unprimed syngeneic mice.

It is thus most probably the LC-hapten complex that is involved in presentation of hapten to cortical T cells. These become activated and are induced to proliferate and differentiate. Effector cell precursors proliferate and some of them differentiate into memory cells, whereas others become effector cells. Suppressor cell precursors are simultaneously induced to proliferate and they stop the further production of effector cells.

The evidence from contact sensitization, a pathological model of delayed hypersensitivity, may apply to the normal situation, where immunization with a subsequent induction of a state of tolerance may take place continuously. Streilein has suggested that SALT represents a physiological mechanism created to deal with neoepitopes, events taking place continuously within the skin due to ultraviolet radiation and the presence of potentially oncogenic viruses and carcinogenic agents. This role for LC may be significantly extended as follows. Under physiological conditions LC continuously present skin contacting antigens to the T cells in the lymph nodes. In fact, a large number of the 45 million available human LC will, every day, move through the lymphatics (presumably as veiled cells) to the regional skin-related lymph nodes. In this way, the SIS is continuously made aware of its antigenic microenvironment. Small abrasions and erosions of the physico-mechanical skin barrier that normally occur at microscopic level result in intra-epidermal injections of antigenic materials. Other water-soluble compounds will penetrate into the spinal layer and be taken up by LC. When other compartments such as neutrophils and tissue macrophages fail to clear these antigens, they are further processed by the continuously boosted T cells, without any clinical symptoms or signs.

In mice, mast cells may play an important role in delayed-type hypersensitivity (elicitation of allergic contact dermatitis). During sensitization, antigen-binding T-cell factors are produced and released into the circulation. These reach the skin mast cells which become sensitized. During elicitation, these antigen-binding T-cell factors bind the antigen and the mast cells react, releasing serotonin but without histamine release or degranulation. Serotonin induces, via receptors on post-capsular venules, vasodilatation and the formation of interendothelial spaces which facilitate the entry of T-cells, among them antigen-specific T cells, into the elicitation site. It is only then that the antigen-presenting, MHC class II restricted stage of delayed-type hypersensitivity develops, characterized by the development of a mononuclear cell infiltrate.

Whether these antigen-binding T-cell factors play a role in normal human skin is as yet unclear. If they do, LC presentation of immunogens to these mast-cell-bound, T-cell-derived factors seems possible.

The skin's micro-anatomy and immunohistology thus show a complicated and fine-tuned organization of many different immunologically active cells. Probably the most important cells within SIS are those comprising the family of skin dendritic cells. Together with recirculating and/or recruited T cells they act in the specific immune responses.

Non-specific cells such as granulocytes and tissue monocytes cooperate with specific recirculating T cells in antigen clearing. T-cell migration may be directed by mast cell release of vasoactive agents through antigen binding via immunogen-specific T-cell factors present on mast cell membranes. As well as Fc-receptor-bearing and C3 receptor bearing dendritic cells and macrophages, the vascular endothelium of the upper dermal plexus may also take part in antigen presentation.

It seems evident that the SIS form an ideal and moreover easily accessible model for the study of immunology of health and disease.

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