

Controversies in Experimental Dermatology

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Who is really in control of skin immunity under *physiological* circumstances – lymphocytes, dendritic cells or keratinocytes?

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Abstract: Our views of the skin immunity theatre are undergoing constant change. These not only reflect paradigm shifts in general immunology and skin biology, but also have profound clinical implications, which call for strategic changes in dermatological therapy. Nowhere can this be witnessed at a greater level of instructiveness and fascination than when addressing the question posed by this new *Controversies* feature. Thus, after a very long period of dominance by T cells and Langerhans cells as 'lead actors' on the skin immunity stage, the lowly keratinocyte has recently made an astounding theatrical appearance as a key protagonist of the innate skin immunity system, which may control even acquired skin immune responses. Further enhancing dramatic complexity and tension, the mast cell has entered as an additional actor claiming centre stage, and the epidermal Langerhans cell has slipped in a surprise appearance as the chief agent of immunotolerance. May you, esteemed reader, enjoy the spectacle offered here by selected immunodermatology authorities who double as 'stage managers' pushing their respective favourite actors into the limelight. You get everything you may expect from a good performance – complete with the impresario's overture that lures you into the theatre and sets the stage, competing divas, recently discovered new talents and even the critic's digest while the performance is still ongoing. By the time the curtain drops, you will have reached your own, independent conclusions on how to answer the title question of this play – at least for the time being...

Prelude

The trouble with working at an environmental interface

What is the main task of the skin immune system? The task is to defend a critical border. The epidermis is a strong barrier. Covered by a three-dimensional self-sustaining brick wall of corneocytes embedded in a lipid-rich envelope, it consists of several layers of tightly connected keratinocytes. Keratinocyte cohesion is conferred by special structures, the desmosomes. Transmembranous des-

mosomal components of neighbouring cells, desmogleins and desmocollins, intercalate and form adhesive bridges. They are connected to intracellular molecules of the desmosomal plaques which in turn interact with components of the cytoskeleton (1).

The dermoepidermal basement membrane zone is the inner sheath of our cutaneous wall. The epidermis protects us against mechanical, physical, biological and chemical injury. The epidermis can tan and sense. It can also defend itself to a certain degree because it is equipped with an evolutionary old, but effective system of primary defense

which is referred to as innate immune system. But the real troops, the evolutionary younger, thymus-trained all-rounders brim-full with multifunctional cytokines come in via dermal vessels. This is necessary because many things can go wrong. A plethora of potentially harmful microorganisms waiting for a disruption of the barrier to sneak in, overthrow primary epidermal defense and enter the organism. Keratinocytes of the epidermal barrier get infected by viruses or are genetically damaged by UV irradiation and need to be eliminated.

The adaptive skin immune system is a learning, flexible, powerful surveillance programme that complements and extends epidermal barrier functions. The members most commonly sent to do the job are T cells. They are often accompanied by neutrophils, and sometimes monocytes. They are called in by and work together with local partners such as keratinocytes, macrophages, dendritic cells (DCs), and mast cells. Before entering the skin, they interact with endothelial cells of the dermal vessels.

But power is nothing without control. The majority of bacteria populating the skin need to be controlled, but not attacked. And the vast majority of foreign substances encountering the skin from the outside environment should be completely ignored because the allergy specialists are already working overtime hours. Several levels of T-cell control to achieve this can be distinguished.

1. *The level of doormen and soluble mediators of directed locomotion.* Before entering a target organ, T cells and other inflammatory cells interact with endothelial cells via selectins and adhesion molecules and, when attached to these cells, listen to the sirens' singing of chemotactic factors. In other words, a T cell will only find its place when expressing the appropriate set of homing and chemokine receptors and when the target organ provides the corresponding binding partners of these receptors.

2. *The level of the power button.* The type of effector function turned on in a specific immune situation is dependent on the type, functional status, surface molecule expression and cytokine repertoire of the antigen (Ag)-presenting cells that initiate T-cell activation.

3. Closely related to the latter – *the level of active counter-regulation.* The complex world of effector T cells is controlled by different T cells of presumably similar complexity specialized in suppressing immune responses. These CD4⁺ cells are likely to play a major role in immune regulation including the control of autoimmunity and the maintenance of peripheral tolerance (2). Among these cells, the naturally occurring CD4⁺ CD25⁺ regulatory T cells (Tregs), which are continuously produced in the thymus and may account for up to 10% of peripheral CD4⁺ cells, inhibit the activation of T helper (Th) cells in an Ag-independent manner by direct cell–cell contact. In contrast, type 1 Tregs (Tr1) are induced in the periphery under certain tolerogenic conditions and mediate Ag-specific tolerance via secretion of the immunosuppressive cytokines interleukin (IL)-10 and transforming growth factor- β .

In other words, if we ask which cells control skin immunity, basically, we must ask: which cells in the skin are in charge of controlling adaptive immunity? What are the cellular components and mechanisms that organize the influx, shape the functional repertoire and control the activity of the T-cell troops?

Before further pursuing these questions, some concluding remarks on the 'external surface problem' may be allowed. There are immunologically privileged sites such as the eye, the brain and the testis where foreign Ags may

persist for an extended period of time, providing unwanted refuge for pathogens and tumor cells. With important exceptions, such as the hair- and the nail-generating skin appendages, the skin is the opposite: the interplay of innate and adaptive immune mechanisms and the dermal capillary plexus as an autobahn network for immune cell entry make it a highly non-privileged site. The finger is always at the trigger. Is this the price we have to pay for the close immunological control of our external surface?

Not surprisingly, then, the skin is a common manifestation site of allergic, autoimmune and chronic inflammatory conditions. The broad spectrum of different reaction patterns seen under pathological circumstances throws some light on the complexity of defense programmes that have developed during evolution and the accordingly increased requirements for ever more complex regulation. These patterns are hard to classify and even harder to understand. Some are reminiscent of a physiological defense and repair programme such as the inflammatory process in psoriasis. Some are good representations of the type I–IV reaction patterns of the historical 1963 Gell–Coombs classification (3). Some, like the lichenoid reaction pattern, which is seen in such different conditions as drug reactions, skin manifestations of collagen vascular diseases, chronic cutaneous graft-versus-host disease and lichen planus disclose the limitations of our present ability to correlate physiology and pathophysiology, to predict a cutaneous immune programme from a provoking stimulus.

There is increasing evidence that critical components of the activating and suppressive elements of the immunological and non-immunological facets of our skin barrier are influenced by variations in the underlying genes including those involved in Ag presentation and regulation of cytokine production (4). But the properties of no other organ of the mammalian body, including its immune system, are likely to be shaped by environmental factors to the same degree as the skin.

Thus, the skin may best be regarded as an immunological site that paradigmatically reflects the excruciatingly difficult compromise between defense and tolerance, genetic control and environmental modulation, protection and disease.

With a little help from a friend – innate and adaptive immunities in the skin

A speciality of the skin is the strong representation of the innate immune system. The Nobel Prize-winning Toll-like receptors (TLRs) endow keratinocytes and DCs with the ability to respond to certain molecular patterns of potential pathogens with the production of defensive proteins, an effective first line of antimicrobial response, but also mediators that enhance the cutaneous influx of inflammatory cells and activate adaptive immune mechanisms (5).

Toll-like receptors, IL-1 and IL-18 signal through common pathways that culminate in the activation of nuclear factor- κ B and mitogen-activated protein kinases. Some of the TLRs seem to specifically promote IL-12 and IL-23 cytokine pathways and also tumor necrosis factor (TNF)- α that participate in the orchestration of Th1-type cutaneous immune reactions. The importance of these findings has been confirmed by the clinical observation that topically applied activating agonists of TLR7 such as imiquimod induce strong antiviral and also antitumoral immune

responses in the skin. On the other hand, inhibitors of protein–protein interactions that prevent the formation of complexes involved in TLR signalling have been shown to effectively reduce skin inflammation driven by T cells and neutrophils (6). We can conclude that, in the case of an external danger signal or a malignant threat of epidermal integrity, innate immune mechanisms link keratinocytes and (epidermal) DCs to adaptive effector functions primarily mediated by T cells and neutrophils.

Psoriasis – when innate immunity flexes its muscles

A confession at the outset: I do not believe that psoriasis is a primary autoimmune disorder. We get confused because at the time we see the fully manifested disease, a multitude of secondary inflammatory events obfuscate our view of the initiating disease process. Recent animal models, despite their limitations, have produced the data that helpless observers of the Koebner phenomenon have been looking for. If clinically unaffected psoriatic skin is transplanted onto mice lacking a functional T-, B- and NK-cell systems, the psoriatic phenotype develops within weeks (7). The manifestation of the phenotype in this model is obviously driven by members of the innate immune system – TNF- α producing dermal DCs; or, if you take a second look, interferon- α producing plasmacytoid pre-DCs (8). Or, manipulate keratinocytes to overexpress the proinflammatory signal transducer Stat3 and mechanically irritate them; CD4⁺ T cells will enter the skin and the mice develop a skin disease resembling psoriasis (9). Or, manipulate keratinocytes to not express Jun proteins and, as a consequence, overexpress chemotactic factors such as S100A8 and A9; neutrophils and macrophages get recruited to the epidermis and a psoriasis-like phenotype develops (10). These mice even develop arthritis which seems to require T and B cells and signalling through the TNF receptor type 1. All these models share the necessity for genetic manipulation or abrogation of potentially counter-regulatory immune mechanisms to allow manifestation of the psoriasis-like phenotype. As a simplifying extract of these observations (and a long list of findings in the human disease), we can conclude that psoriasis is likely to represent a defense and repair programme kicked off by epidermal innate immune mechanisms in individuals genetically predisposed to an increased responsiveness towards activating signals (bacterial infection, certain drugs and mechanical irritation) and/or impaired regulatory control. Accordingly, targeting ‘innate’ cytokines involved in the activation of adaptive immunity such as IL-12/23 and TNF- α should be a successful therapeutic strategy in psoriasis. And what do predisposed patients get when they apply imiquimod? They get psoriasis (11).

Pemphigus – losing the balance

Pemphigus has long been regarded as a classical cutaneous manifestation of humoral autoimmunity. Only in recent years, the role of autoreactive T cells and their regulatory counterparts has become more apparent, especially in pemphigus vulgaris (PV) (reviewed in Ref. 1). Desmoglein-specific Th type 1 and type 2 T cells have been identified that are activated in the context of PV-associated HLA-DR and -DQ molecules and seem to contribute to the production of IgG₁ and IgG₄ antidesmoglein antibodies,

respectively. Similar to autoantibodies, these autoreactive T cells are present not only in affected patients, but also in healthy carriers of the PV-associated HLA class II molecules. In other words, predisposed healthy individuals display the characteristics of autoreactivity, meaning the priming of auto-Ag-specific immune cells, but show no signs of tissue damage. One important difference is that desmoglein-reactive Tregs phenotypically and functionally resembling Tr1 cells were found in the majority of healthy carriers of PV-associated MHC class II alleles but in less than 20% of the investigated PV patients. Moreover, when the expression of *Foxp3*, a gene activated in Tr1 cells that encodes a transcription repressor critical for Treg development, is inhibited by antisense strategies, desmoglein-reactive Tr1 cells shift toward the dangerous Th2 phenotype and lose their suppressive capacity (12). We take this as evidence for a model of pemphigus pathophysiology, in which the ratio of desmoglein-reactive Tr1 to Th2 cells in carriers of PV-associated HLA class II molecules is critical for the maintenance of tolerance at the B-cell level and the prevention of the PV phenotype.

Which factors are now critical in the regulation of this T-cell effector balance? Based on the recent demonstration of a predominant role of TLR ligand-activated DCs over T-cell receptor signalling and cytokine milieu (IL-12, IL-23 and IL-18) in determining T-cell effector functions (13) and several other experimental findings (reviewed in Ref. 14), we speculate that the conversion of skin autoreactivity into overt skin autoimmune disease is controlled by innate immune mechanisms. Thereby we have converted pemphigus from an autoantibody disease to a disease of cutaneous innate immunity.

Conclusions

Let us contemplate, once again, the question posed here: ‘who is really in control of skin immunity under *physiological* circumstances – lymphocytes, DCs or keratinocytes?’ After a long night at the desk and some digressions into the world of skin pathophysiology, I vote for keratinocytes and DCs, the major cellular players of cutaneous innate immunity, as the decision-makers in the maintenance of the immunological integrity of our largest organ (Fig. 1).

This provokes the second question: which DCs? The more immature, possibly more tolerogenic epidermal DC, or the more mature, defensive effector function-inducing dermal DC? The question might be artificial. One of the most important functions of DCs besides the business of Ag presentation is migration. With regard to the skin, this primarily means migration from the epidermis to the dermis and to local lymph nodes, and presumably from the dermis and the peripheral blood to the epidermis. The investigations showing a strict separation of epidermal and dermal DCs may at least partially overlook the fact that they represent a static picture of a highly versatile cell system. On the other hand, there are several findings indicating that certain diseases such as atopic dermatitis are characterized by an abnormal population (and function) of epidermal DCs (15).

This evokes the – possibly more important – question as to the factors that, in turn, control DC development and function. At least in atopic dermatitis, there is intriguing evidence that keratinocytes play a major role as part of the developmental environment that governs the function of epidermal DCs (16).

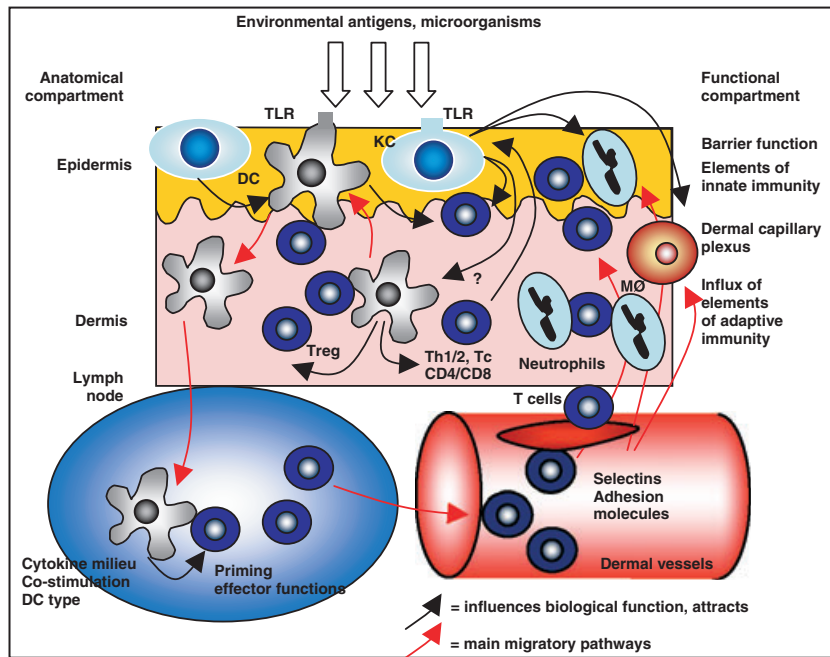


Figure 1. Schematic representation of the immunological hierarchy of skin immunity. Keratinocytes and epidermal DCs, the innate first line of our external surface, sense foreign danger signals and evoke adaptive immune responses. Cytokines released from infiltrating T cells orchestrate a variety of secondary inflammatory reactions and can in turn modulate keratinocyte function. Keratinocytes are also key components of the epidermal microenvironment that may shape the repertoire of epidermal DCs. Keratinocytes are therefore in a pole position in maintaining cutaneous immune homeostasis. For further explanation, see text. TLR, Toll-like receptor; KC, keratinocyte; DC, dendritic cell; Treg, regulatory T cell; Th, Tc, T helper and cytotoxic T cell, respectively; M ϕ , monocyte/macrophage.

As a final consequence, this concept allows for an even more blunt answer to the question posed: who is in charge? 'It's the keratinocyte, stupid!'

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Viewpoint 1

Body surfaces of multicellular organisms like plants, invertebrates and vertebrates, including man, are always covered with micro-organisms, but become surprisingly rarely infected. Furthermore, healthy skin bears a rather constant number of microbes at its surface ($0.1\text{--}10 \times 10^6$ organisms/cm²), which varies depending on the location (1). Textbook wisdom has it that resistance of healthy skin towards infection is mainly achieved by an intact physical barrier, together with a low pH near 5.5.

But is this sufficient? The physical permeability barrier, consisting of stratum corneum and lipid layers, consists of potential microbial nutrients, proteins and lipids. At special locations, such as axillae, in addition to high humid-

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ity, high temperature as well as a high iron content is observed. Despite these conditions – a veritable microbial growth paradise – one surprisingly finds only a rather restricted range of microbe numbers, and never an exponential bacterial growth. This cannot be explained with the physical permeability barrier as such, and suggests that, in addition, also a chemical ('antimicrobial') barrier may exist (2,3): with the discovery of numerous microbicidal and bacteriostatic compounds produced by human cells, mainly neutrophils (4) and epithelial cells (5) – in particular, keratinocytes (2) – it would make sense, when strategically optimal located keratinocytes play a major role in protecting us from infection.