Mechanisms of immune-mediated skin diseases: an overview

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Abbreviations:
AD: atopic dermatitis;
AEAC: anti-endothelial cell antibodies;
AFA: anti-fibroblast antibodies;
DC: dendritic cells;
EGF: epidermal growth factor;
MMP: metalloproteinase;
SLE: systemic lupus erythematosus;
SS: Sjögren's syndrome;
SSc: systemic sclerosis;
Tr cells: T regulatory cells.

ABSTRACT

The skin is a frequent site of pathological immune responses that can take place in the dermal and/or the epidermal compartments. These immunopathological reactions often occur towards innocuous antigens and may be the result of T cell-dependent and/or autoantibody-dependent mechanisms. Defective immune regulation is increasingly recognized as very relevant in many skin and systemic immune-mediated disorders. In some instances (e.g., psoriasis and atopic dermatitis) genetic predisposition can affect also the capacity of keratinocytes to initiate or perpetuate inflammatory responses. A more precise understanding of the molecular and cellular mechanisms underlying each disorder may allow the identification of novel targets for more effective therapeutic strategies.

Introduction

Human skin is the largest organ of the body and provides a protective barrier against a variety of potential environmental threats. The skin has different systems of defence, which include its physical and chemical properties, and the production of antimicrobial substances. Another defence mechanism relies on the capacity of the skin to act as a complete "immunological" organ (1). In fact the skin provides a complex microenvironment where several cell populations actively participate in the initiation and regulation of inflammatory and immune responses. Cutaneous dendritic cells (DC) serve as dominant antigen presenting cells in the induction of T cell mediated immune responses and subsequent reactivation of T cells (2). Under homeostatic conditions, however, DC are primarily involved in the maintenance of immune tolerance to self and innocuous non-self antigens. T lymphocytes with specificity for antigens entered through the skin acquire a propensity, based on the expression of specific homing receptors, to recirculate in the skin. Keratinocytes have the capacity to secrete an array of cytokines and chemokines very important for the regulation of DC functions, and the recruitment and activation of inflammatory cells; in addition, keratinocytes can directly modulate T cell activation by expressing on their surface adhesion and MHC class II molecules. In some instances, the complex interplay between these skin components is distorted or the skin becomes target of systemic autoimmune responses resulting in different types of immune-mediated diseases with prominent skin manifestations.

CD40/CD40 ligand signaling and T regulatory cells in immune-mediated skin diseases

Autoimmune diseases play an increasingly important role in western health organizations. Although rarely lethal such disorders can cause significant morbidity. Autoimmunity is characterized by the presence of autoreactive T cells and/or autoantibodies. The generally accepted hypothesis for the development of autoimmune disorders, including skin autoimmunity, is that genetic predisposition and environmental factors contribute to induction as well as progression of disease. The skin is an organ where interaction with the environment (sunlight, heat, cold, microbes) induces frequent immune reactions. The major cellular components of the epidermis are keratinocytes, which have an important barrier function. Moreover, keratinocytes can upon stimulation produce a number of different soluble mediators and thereby modify cutaneous as well as systemic immune responses. The principle antigen-presenting cell within the epidermis are Langerhans cells that take up antigens, emigrate from the skin to the draining lymph nodes to interact with naïve and antigen-experienced T cells. T cells

with skin homing receptors, such as the cutaneous lymphocyte-associated antigen (CLA), can migrate to the dermis and epidermis to exert different effector functions. For the induction of effective T cell activation by antigen-presenting cells two signals are involved (3). Signal 1 is constituted by the interaction of the T cell receptor with MHC class I/II molecules. But also signal 2 has to participate in this cell-cell interaction. Signal 2 consists of co-stimulatory surface receptors (e.g. B7- and TNF-family members), adhesion molecules (e.g. integrins), and cytokines (e.g. IL-12, IL-6, TNF-α). An important co-stimulatory receptor-ligand pair within the TNF superfamily is the interaction of CD40 expressed on antigen-presenting cells with CD40-ligand (CD40L, CD154) on activated T cells. Besides T cell activation CD40-CD40L interaction is also involved in inducing an immunoglobulin class switch from IgM to IgG in B cells. Hence, CD40-CD40L signaling plays an important role during the communication between antigen-presenting cells, T-, and B cells during immune responses. Since soluble CD40L was detectable in the serum of systemic lupus (SLE) patients it was suggested that this signaling pathway participated in the development of systemic autoimmunity. Accordingly, increased CD40L serum concentrations were found in those patients with active disease. Additionally, T cells from patients with SLE expressed CD40L suggesting that, indeed, CD40-CD40L signaling plays a role in lupus development. This hypothesis is further strengthened by findings in CD40L transgenic mice overexpressing CD40L in basal keratinocytes (4). These transgenic mice develop spontaneously autoimmune dermatitis and subsequently a systemic autoimmune disease, including autoreactive T cells, autoantibodies and internal organ involvement. Therefore, therapeutic intervention of CD40-CD40L interaction might be useful for the control of autoimmune disorders.

Stimulation of T cells in healthy individuals is controlled by several mechanisms including activation-induced cell death (AICD) and/or active suppression. Suppressor or regulatory T cells are critically involved in the inhibition of effector T cells. Suppressor T cells have been described already in the early 1970s, however, their existence has been questioned for the following decades due to lack of molecular marker suitable for characterization (5). In 1995 Sakaguchi and co-workers identified $CD4^+CD25^+$ T cells with potent suppressor activity (6). This finding has since sparked intensive research on the development and function of suppressor T cells, which is now one of the most competitive fields in immunology. $CD4^+CD25^+$ suppressor T cells develop in the thymus and constitute 6-9% of the peripheral $CD4^+$ T cell population (7-9). Suppressor $CD4^+CD25^+$ T cells seem to develop as a T cell line and the transcription factor that controls lineage commitment is *Foxp3* (10). Accordingly, mice with natural mutation in the *Foxp3* gene or with *Foxp3* deletion do not develop suppressor T cells and develop severe autoimmune symptoms. IL-2 is an important growth factor for $CD4^+CD25^+$ T cells since IL-2- or IL-2Rβ chain-deficient mice show low to no detectable numbers of suppressor T cells in secondary lymphatic organs. Perhaps the expression of CD25 (α chain of the IL-2 receptor) reflects this need for IL-2. $CD4^+CD25^+$ T cells can suppress the activation of $CD4^+CD25^-$ T cells via cell contact dependent and/or independent mechanisms. The contact-dependent suppressor mechanisms include the secretion of granzyme B whereas production of immunosuppressive cytokines, such as IL-10, TGF-β appear to mediate cell contact-independent inhibition. In the past years several other surface receptors ($CD45RB^{low}$, CTLA-4, GITR, Neuropilin-1, CD62L, etc.) have been identified on suppressor T cells and thereby it became increasingly clear that several subpopulations of suppressor T cells exist. Analysis of the T cell receptor (TCR) repertoire revealed that effector and suppressor T cells have a similar TCR diversity, which means that both effector as well as suppressor T cells are also reactive to self. Upon stimulation via the TCR suppressor T cells become activated and it is currently believed that suppressor function occurs in an antigen-unspecific fashion.

In the past years a growing body of evidence suggested that suppressor T cells play a role in certain human autoimmune diseases. In patients suffering from psoriasis reduced numbers of peripheral $CD4^+CD25^+$ were detectable compared to healthy controls (11). In addition, isolation of $CD4^+CD25^+$ from lesional skin displayed decreased suppressor activity suggesting that either low numbers and/or inferior suppressor activity participate in psoriasis progression. $CD4^+CD25^+$ regulatory T cells maintain immune tolerance to nickel in healthy, non allergic individuals (12). In patients suffering from pemphigus vulgaris, a rare acquired bullous autoimmune disorder, autoantibodies against the desmosomal antigen desmoglein-3 (Dsg-3) mediate disease. However, Dsg-3 reactive T cells have been isolated from patients which can also contribute to disease induction since T cells can provide help to B cells for the production of (auto)antibodies (13). Interestingly, $Dsg\text{-}3^+$ T cells can be also detected in HLA-matched healthy individuals. Those volunteers also presented with normal numbers of peripheral T regulatory type-1 (Tr1) cells, a distinct suppressor T cell subset. In contrast, Tr1 cells were detectable only in low numbers with reduced function in pemphigus patients suggesting that effective active control mechanisms are impaired in these patients. Together, these findings suggest that patients suffering from autoimmune disease would profit from activation of suppressor T cells perhaps even in an antigen-specific fashion.

Keratinocytes in chronic inflammatory skin diseases

Substantial evidence accumulated during the past years has indicated that keratinocytes play an active role in the generation and expression of protective immune responses and immunopathological reactions. Keratinocytes can upregulate or generate a number of cytokines and cytokine receptors that influence their immunological responses. Both IL-1α and IL-1β are produced

and stored within keratinocytes in large amounts and are abundantly released following cell damage. Exposure to the bioactive forms of IL-1 stimulates the release of other cytokines by keratinocytes, including GM-CSF (14). GM-CSF is an important inflammatory mediator that is produced by keratinocytes in pathologic conditions, known to be essential for DC development and deeply involved in the regulation of DC functions (15). GM-CSF is also a strong mitogenic stimulus for human keratinocytes, and is considered an autocrine factor responsible for keratinocyte proliferation in human hyperproliferative skin disorders. Indeed, GM-CSF expression is strongly up regulated by a number of T cell-derived cytokines, including IFN-γ, TNF-α, IL-17 and IL-4. Keratinocytes constitutively express the receptors for a broad array of cytokines, known to crucially orchestrate the development of inflammatory and immune responses in the skin. These cytokines are also effective stimuli for the expression of numerous chemokines, which actually initiate and sustain the orientated migration of distinct leukocyte subpopulations (16). IFN-γ, in synergism with TNF-α or IL-1, up-regulates CXCL8/IL-8 and CCL27/ CTACK, and induces CCL2/ MCP-1, CXCL1/Gro-α, CCL20/MIP-3α, CCL5/RANTES, CCL22/ MDC and CCL1/I-309, with the latter two chemokines being produced at lower levels and with a delayed kinetics. Most importantly, keratinocytes respond to these cytokines with a massive release of the CXCR3 ligands CXCL9/MIG, CXCL10/IP-10 and CXCL11/ITAC, the most effective chemoattractants of type 1 T cells. These lymphocytes are the main effectors of tissue damage in T cell-mediated skin disorders, due to direct cytotoxicity and further release of pro-inflammatory cytokines and chemokines (17).

Atopic dermatitis (AD) is a chronic inflammatory disease that results from complex interactions between genetic and environmental factors. An altered lipid composition of the stratum corneum is responsible of the xerotic aspect of the skin, and may determine a higher permeability to allergens and irritants. Specific immune responses against a variety of environmental allergens are also implicated in AD pathogenesis with a bias towards Th2 immune responses. Keratinocytes of AD patients exhibit a propensity to an exaggerated production of distinct cytokines and chemokines, a phenomenon that can be relevant in promoting and maintaining inflammation. In particular, they displayed overproduction of spontaneous as well as induced GM-CSF both at the mRNA and protein level, when compared to healthy control keratinocytes (15, 18). Enhanced GM-CSF expression could be correlated with the increase of activator protein 1 (AP-1) specific activity (19). *In vitro* studies have also shown that keratinocytes from AD patients produced increased amounts of CCL5/RANTES, but reduced levels of CXCL10/IP-10, in response to IFN-γ or TNF-α when compared to keratinocytes from normal controls or psoriasis patients (20). Numerous functional polymorphisms in the regulatory/coding regions of clusters of cytokine/chemokine genes, including CCL5/RANTES, have been found in AD patients, which could be implicated in overproduction by keratinocytes. Psoriasis is a skin disorder characterized by epidermal abnormalities and a prominent inflammatory cell infiltrate. T cell-derived IFN-γ is overexpressed in psoriasis, consistent with the predominant Th1 immunopathology. In psoriatic lesions, under the influence of IFN-γ and TNF-α, keratinocytes produce high levels of cytokines, chemokines and adhesion molecules, which further amplify the inflammatory response. The genetic predisposition to psoriasis may include an altered control of inflammatory gene expression in keratinocytes. In particular, psoriatic keratinocytes may have intrinsic defects leading to exaggerated synthesis of certain chemokines such as CXCL8/ IL-8, CCL2/MCP-1 and CXCL10/IP-10[21] and display also increased expression of CXCR1, which can autocrinely mediate an increased proliferative response to CXCL8/IL-8. Moreover, psoriatic keratinocytes activated *in vitro* with IFN-γ and TNF-α showed an enhanced induction of ICAM-1. We have shown that IFN-γ and TNF-α induced an exaggerated NF-κB and STAT-1 binding activity in psoriatic keratinocytes (21). Indeed, perturbation in signal transduction pathways and in the activation of transcription factors has been implicated in the dysregulated functions of psoriatic keratinocytes (22).

Epidermal keratinocytes can activate a variety of molecular mechanisms implicated in the control of the inflammatory event. Indeed, they are the richest source in the mammalian body of an intracellular variant of IL-1 receptor antagonist (IL-1ra). IL-1ra is structurally related to IL-1α and IL-1β and binds to IL-1 receptors on various target cells including keratinocytes without inducing any biological response. Not only IL-1 bio-activity is regulated by specific receptor antagonism, but it is also modulated by the expression of two species of surface receptors both expressed by keratinocytes, IL-1R type I and type II, with the type II not being able to mediate a signal transduction, but rather acting as an IL-1 scavenger (23). The epidermal growth factor receptor (EGFR)-ligand system plays a fundamental role in self-protection and repair to injury in epithelial tissues, and its activation has been associated to accelerated cell regeneration and reduced inflammatory infiltrate following mechanical, chemical or ischemic tissue damage. A deeper investigation into the effects of EGFR activation unveiled its complex role in the control of chemokine expression in skin keratinocytes, where activation of EGFR-driven signaling down-regulated the expression of a cluster of chemokines, including CCL5, CCL2 and CXCL10 (24). Accordingly, in the mouse models of irritant and hapten-specific skin inflammation, the blockade of EGFR or ERK1/2 activation similarly led to an aggravation of the disease, characterized by a massive macrophage-monocyte infiltration (25). In the course of skin inflammation, keratinocytes are abundant producers of a variety of metalloproteinases (MMPs), including MMP1, MMP9 and MMP10, for a long time simply conceived as the major effectors of extracellular matrix degrada-

tion and remodelling. In the very last years, however, a multitude of non-traditional MMP substrates have been discovered, which hint at a much more complex role for MMPs in skin inflammation. In particular, MMPs control the availability of fibroblast-produced growth factors into the epidermis, and display a strong proteolytic activity on numerous keratinocyte-derived chemokines (26). Hence, MMPs appear to exert a protective, regulatory role on the epidermis during skin inflammation.
In conclusion, keratinocytes are relevant players of cutaneous immune responses. A better understanding of the regulatory mechanisms active in keratinocytes could provide clues for novel approaches to the management of chronic inflammatory skin diseases.

Autoantibody-mediated skin involvement in systemic autoimmune diseases

Systemic autoimmune disorders are characterized by the occurrence of autoantibodies: most of them display an association with a specific disease (i.e. anti-dsDNA and anti-Sm as specific markers of SLE) or with a subset of the same disease (i.e. anti-centromere in limited Systemic Sclerosis [SSc] and anti-topoisomerase I in diffuse SSc). Moreover, some others have been associated with specific features of a given autoimmune disease (i.e. anti-Ro antibodies in photosensitive skin rash of different forms of SLE). In some circumstances, rather than being diagnostic markers only, autoantibodies play a direct pathogenic role by inducing specific tissue damage or by modifying the phenotype of specific key cells in a way that might explain the disease manifestations. Three autoantibodies have been proposed as having a direct role in inducing skin inflammation in SSc and in SLE: anti-fibroblast, anti-endothelial cell and anti-Ro/SSA antibodies.

Anti-fibroblast antibodies (AFA)

Some authors have reported a specific association between SSc and AFA, detected in 46-100% of SSc sera (27). AFA IgG antibodies react with cell membrane antigens of normal and SSc fibroblasts *in vitro*; they recognize constitutive antigens, and are actively internalized via an Fcg receptor-independent way (28). AFA are able to activate fibroblasts, up-regulating the expression of ICAM-1 and of pro-inflammatory cytokines and chemokines in a dose-dependent way. Such a pro-inflammatory phenotype was shown to enhance the adhesion of mononuclear cells to fibroblast monolayers *in vitro* (27). Although the ability of AFA to induce collagen synthesis is still debated, the above mentioned fibroblast activation might play a key role in triggering and/or maintaining skin inflammation. It has been suggested that skin inflammation might be the *primum movens* for the development of dermal fibrosis. Recent findings demonstrate a close association between AFA and anti-topoisomerase I antibodies in SSc sera; in fact, anti-topoisomerase-I antibodies seem to react with specific antigens on fibroblast surface, displaying an AFA activity (28). This finding is in clash with previous reports that didn't find any correlation between anti-nuclear antibodies and AFA (29). Anyway, it is still unproven whether anti-topoisomerase-I antibody binding might have any role in fibroblast activation.

Anti-endothelial cell antibodies (AEAC)

AECA have been described in about 40% of SSc sera. These autoantibodies react with endothelial cells and induce a pro-inflammatory and pro-adhesive phenotype. In addition, several reports showed the capacity of SSc-AECA positive sera to mediate antibody-dependent cellular cytotoxicity (ADCC) on human endothelial cells *in vitro*. However, additional mechanisms have been described: i) AECA might induce endothelial apoptosis by themselves or through natural killer cells (30); ii) AECA can induce tissue damage through complement activation and cellular lysis (31). As a whole, these findings speak in favour of a direct AECA pathogenic role in inducing the endothelial dysfunction characteristic of the early phases of SSc. Endothelial perturbation might trigger platelet and leukocyte activation/adhesion with dysregulation of vascular tone that represents the hall-mark of the scleroderma microvasculopathy. These new SSc autoantibodies selectively inhibit the activity of different human matrix MMP collagenases, actively contributing to the development of fibrosis.

Anti-Ro/SSA

Anti-Ro/SSA antibodies were originally described as precipitating immunoglobulins reacting with different tissue extracts of patients affected by Sjögren's syndrome (SS) and SLE. Ro antigen is a ribonuclear complex, constituted by two proteins of 52 kD and 60 kD, encoded by different and unrelated genes. Ro 60 binds to small cytoplasmic RNAs (namely hYRNA), while Ro 52 shows a direct binding to three peptides of 60 kD Ro (32). Anti-Ro antibodies carry the most prevalent ANA specificity detected in different autoimmune diseases, but they are frequently associated with specific subset of SLE or SS (33): subacute cutaneous LE (60-80%), neonatal lupus, SLE (40%), homozygous complement deficiency SLE (C2 and C4) (75%), late onset SLE (92%) and hypergammaglobulinemic purpura (100%). Most of these cutaneous features are strictly correlated to photosensitivity, that is a decreased threshold of tolerance to natural or artificial light, inducing specific skin lesions after ultraviolet (UV) exposure. Several reports, in fact, suggest an association between photosensitivity and anti-Ro antibodies, showing a direct role of this autoantibody in the pathogenesis of photosensitive skin lesions. *In vitro* experiments suggest that anti-Ro antibodies selectively bind to human basal keratinocytes and this binding could be markedly increased by ultraviolet B light exposure (34). In fact, UV irradiation can induce apoptosis in keratinocytes and the subsequent exposure of Ro antigen by apoptotic blebs on the cell surface. This event leads to antigen presentation to the immune cells with the eventual production of anti-Ro autoantibodies (35). In addition, Ro antigen exposure might enhance the anti-Ro antibody binding on keratinocytes; these *in situ*-formed immune-complexes might activate

complement and the inflammatory cascade, resulting in erythema and the other clinical manifestations of photodermatitis (35, 36). However, skin damage might be mediated also by other mechanisms. *In vitro* experiments demonstrate that anti-Ro antibodies could be internalised by UV- exposed cells and interact with cytoplasmic structures. Moreover, keratinocytes sensitised by autoantibodies can be easily lysed by ADCC when mixed with peripheral blood mononuclear cells. According to the above mentioned findings, it has been suggested that skin involvement in anti-Ro positive SLE patients might be the consequence of the following events (37): (i) UV susceptibility would promote keratinocyte apoptosis and the expression of surface nucleoproteins (such as Ro); (ii) UV susceptibility would induce the production of high levels of inflammatory cytokines and adhesion molecule expression on dermis and epidermis, without an effective counterbalance by anti-inflammatory cytokines (such as IL10); (iii) Ro expression on the keratinocyte surface would facilitate the production of specific autoantibodies; (iv) keratinocyte toxicity and tissue damage would be eventually mediated by the in situ formation of antigen-antibody complexes, complement activation and cell lysis by ADCC.

Concluding remarks

The skin is continuously exposed to a tremendous diversity of antigenic stimuli, and a bewildering array of pathological immune responses can take place in the dermal and/or the epidermal compartments. These immunopathological reactions may occur in response to an inappropriate and innocuous stimulus, or proceed in an exaggerated fashion because of defective regulatory mechanisms. In some instances (eg,. psoriasis and AD) genetic predisposition can affect also the capacity of keratinocytes to initiate or perpetuate inflammatory responses. A deeper understanding of the specific molecular and cellular mechanisms underlying each disorder may allow the identification of novel targets for more effective therapeutic intervention.

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