Role of cellular immunity in the pathogenesis of autoimmune skin diseases

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ABSTRACT
The pathomechanism of most autoimmune skin diseases is still elusive; however, recent clinical and basic research is leading novel insights into the cellular and molecular biological underlying pathways. Several types of infectious skin diseases are infiltrated by significant number of γ/δ T cells and similar observations have been made in selected immune-mediated skin conditions. In particular, a role for γ/δ T cells has been suggested in discoid lupus erythematosus, contact dermatitis, herpetiformis dermatitis, necrotizing cutaneous vasculitis, and cutaneous lesions of systemic sclerosis. The pathogenesis of these diseases is different and this may suggest multiple potential functions of this subset of T cells in the immune system of the skin. Furthermore, most T cells infiltrating tissue and organs undergoing fibrosis have the potential to produce high levels of interleukin 4. This is particularly true for the CD8⁺ or CD4⁺CD8⁺ double positive T-cell subsets. Furthermore, leukocyte recruitment is a key event in immunity and a better understanding of the signals involved in autoimmune diseases constitutes a valuable basis for the development of new strategies, which control leukocyte migration and function under pathological conditions.

Mechanisms of leukocyte recruitment
Leukocyte recruitment is a key event in immunity and chemotactic agonists represent a crucial determinant of leukocyte trafficking. A better understanding of the signals involved in the migration of leukocytes in vivo constitutes a valuable basis for the development of new strategies for the control of leukocyte migration and function under pathological conditions.

The localization of leukocytes into tissues and the migration to lymphoid organs are essential steps in the immunobiology of dendritic cells (DC). DC are potent antigen presenting cells with a unique ability in inducing T- and B-cell responses as well as immune tolerance (1, 2). In the past few years, the role of DC in the induction of certain pathological conditions, including autoimmune diseases has become evident. DC reside in an immature state in peripheral tissues where they exert a sentinel function for incoming antigens. Upon uptaking antigens, DC migrate via the afferent lymphatics into the T-cell area of the draining lymph nodes to initiate immune responses.

DC are a heterogeneous population that possesses unique homing properties (1, 3). Among human peripheral blood DC at least two distinct subsets have been defined: myeloid DC and plasmacytoid DC. Myeloid DC express myeloid markers and produce high levels of type I interferon. Plasmacytoid DC are a rare subset of cells present in circulation and in secondary lymphoid organs. Chemokines are small-secreted chemotactic cytokines that regulate the migration of leukocytes under steady state and inflammatory conditions (4). Immature DC express a unique repertoire of chemokine receptors that bind a pattern of “inflammatory” chemokines (5). A dramatic change in the repertoire of chemokine receptors is responsible for the migration of DC from the periphery to the draining lymph nodes. Activation of DC is associated with down-regulation of chemokine inflammatory receptors and the de novo expression of CCR7, the receptor for CCL19 and CCL21, two chemokines that are expressed at the luminal side of high endothelial cells and in the T-cell rich areas of secondary lymphoid organs.
The expression of chemokine receptors on sorted blood myeloid DC and plasmacytoid DCs is, in general, fairly similar (3). In contrast with the overall similar pattern of chemokine receptor expression, circulating myeloid DC and plasmacytoid DC exhibit a profound difference in their capacity to migrate in response to chemokines, with CXCL12 being the only chemokine active on plasmacytoid DC in a classic chemotaxis assay or in transmigration assays across an endothelial cell monolayer. Plasmacytoid DC are normally absent from peripheral tissues and they are believed to migrate constitutively from the blood into lymph nodes through high endothelial venules (HEV). A decrease of circulating plasmacytoid DC and their recruitment into non-lymphoid tissues is observed in some pathological conditions, such as autoimmune diseases (i.e. SLE, psoriasis and rheumatoid arthritis), allergic diseases (i.e. contact dermatitis and in nasal mucosa polyps) and in tumors. The mechanisms leading to the recruitment of plasmacytoid DC to inflammatory sites are not fully understood and might be dependent on the local production of chemerin, a non-chemokine chemotactic agonist of recent characterization (7). Chemerin, a chemotactic protein that belongs to the cathelicidine family, was recently identified as the natural ligand of ChemR23, a previously orphan G protein-coupled receptor expressed by immature monocyte-derived dendritic cells and macrophages (8). Chemerin was purified from ovarian cancer ascites and found to correspond to the product of the Tig-2 gene. Chemerin is expressed by many tissues, including spleen and lymph nodes, and is secreted as prochemerin, a poorly active precursor protein. Extracellular proteases involved in the coagulation cascade or released by leukocytes convert prochemerin into a full agonist of ChemR23 by proteolytic removal of the last six amino acids (9). Elevated chemerin production was found in the synovial fluids of patients suffering from rheumatoid arthritis, and prochemerin transcripts were also found in the skin of pre-psoriatic subjects.

Recent work has documented that ChemR23, in addition to being a chemotactic receptor for myeloid DC, represents the only inflammatory chemotactic receptor that is functional in immature plasmacytoid DC in vitro. Chemerin immunoreactivity was observed in secondary lymphoid tissues, whereas normal non-lymphoid tissues, like skin and lung, were negative. In secondary lymphoid organs, the strongest reactivity for chemerin was detected at the laminal side of high endothelium venules. The presence of plasmacytoid DC positive for ChemR23 in close proximity expressing chemerin, suggests that chemerin represents an alternative/additional signal to CXCL12 for the recruitment of blood plasmacytoid DC into lymph nodes. Plasmacytoid DC are directly implicated in the pathogenesis of lupus erythematosus (6). In patients with cutaneous forms of this disease, the number of circulating plasmacytoid DC is reduced and these cells are present in the skin lesions, suggesting that plasmacytoid DC are selectively recruited to the skin (10). Chemerin was found to be expressed in skin lesions obtained from 6 patients by dermal vascular cells and by sparse stromal-like cells. Notably, a high degree of plasmacytoid DC infiltration was present in the same sections. On the other hand, chemerin was not detectable in normal skin, suggesting that the ChemR23/chemerin axis is likely to play a key role in regulating the trafficking of plasmacytoid DC to lymph nodes and to pathological tissues.

γ/δ T cells in immuno-mediated skin diseases
Two T-cell lineages are defined by their expression of T-cell receptors, α/β or γ/δ heterodimers. The majority of T lymphocytes express a CD3-associated α/β heterodimer. These cells are CD4- or CD8-positive and recognize foreign peptides presented by classical, highly polymorphic MHC class I or class II determinants. T lymphocytes bearing the γ/δ heterodimer represent a minor population of human lymphocytes (1-5%), the majority of them expressing the CD3-CD4-CD8- phenotype. In contrast to α/β T cells that recognize peptide/MHC complexes, γ/δ T cells appear to recognize unprocessed protein antigens, γ/δ T cells share some features with CD8+ α/β T lymphocytes and with natural killer (NK) cells. The exact function of γ/δ T lymphocytes has not been fully elucidated, but some studies suggest a role for these cells in early immune response toward mycobacterial and parasitic antigens, autoimmune disorders, and tumor immune surveillance. The δ gene of the T-cell receptor (TCR) γ/δ consists of at least six V gene segments but over 95% of the γ/δ T cells express either Vδ1 or Vδ2 gene segments. Interestingly, normal γ/δ T lymphocytes present in spleen, thymus, and intestinal epithelium predominantly express the Vδ1 gene, whereas the majority of γ/δ T cells in peripheral blood, tonsils, and skin express the Vδ2 gene (11, 12).

In man and mice, only a small proportion of T cells in the peripheral lymphoid compartment express the γ/δ TCR. In mice, however, γ/δ T cells comprise the predominant population in the epithelium and epidermis of intestine, lung, reproductive tract, tongue, and mammary glands. A unique population of γ/δ T lymphocytes, designated as dendritic epidermal T cells (DETC), resides in murine skin. These cells are characterized by a TCR with no junctional diversity suggesting that they have a limited ligand recognition capacity. The distribution of γ/δ T cells in human epithelial layers has demonstrated that γ/δ T cells constituted less than 5% of total T cells. In particular, despite intensive investigations, no counterpart of the murine TCR γ/δ-bearing dendritic T-lymphocyte population has been identified and α/β TCR-bearing T cells appear to predominate in human epidermis (13).
Substantial information exists about the distribution of γδ T cells in pathologi-
cal skin conditions, e.g., several types of skin lesions in leprosy are infiltrated by
significant number of γδ T cells. Similar observations have been made in cut-
aneous leishmaniasis (14). A role for γδ T cells in immune-mediated skin condi-
tions has been suggested on the basis of the findings in discoid lupus erythematosus (DLE), contact derma-
titis, herpetiformis dermatitis, necrotiz-
ing cutaneous vasculitis, and cutane-
ous lesions of systemic sclerosis (SSc).
In DLE, γδ T cells have been observed in close proximity to the damaged basal keratinocyte layer expressing the Vδ2 chain of the γδ TCR. γδ T cells recogn-
ize stress protein, a family of conserved
production of stress proteins which are specifi-
cally recognized by γδ T cells. These cells may contribute to further epider-
mal damage by their cytotoxic capacity or by eliciting a delayed-type hypersens-
itivity reaction (15).

The idea that γδ T cells are involved in a delayed type of reaction is supported by investigations which have analyzed the occurrence of γδ TCR+ cells during skin reactions of allergic contact derma-
titis. In allergic contact dermatitis from DNCB, increased γδ TCR+ cells were observed both in the epidermis and in the dermis 48 h after the chal-
lenge. Most of the γδ TCR+ cells were TCRδ2+ T cells expressed the Vδ2 gene segment indicating that they had the same phenotype as γδ T lympho-
ocytes in the peripheral blood. Moreover, they were “memory” T cells indic-
ating that the γδ TCR+ cells in skin lesions of allergic contact dermatitis may not be involved in the initiation of delayed-type hypersensitivity but may have a role in the effector phase of contact hypersensitivity (16).

In lesional skin of leukocytoclastic necrotizing vasculitis with documented infective origin, a high number of γδ T cells has been observed in the late phase of the condition together with an abnormal expression of the heat shock
protein (HSP) 72. These observations are consistent with the idea that raised
tissue levels of HSPs, which are prefer-
entially recognized by γδ T cells, may induce the skin migration of this sub-
population of T lymphocytes. The pre-
ence of γδ T cells in the late phase of findings suggest a role of these cells in
leukocytoclastic necrotizing vasculitis and indicates that skin lesions of leuko-
cytoclastic necrotizing vasculitis with a high number of γδ T cells should speci-
cifically be investigated to exclude a
possible infective etiology (17).

An unusually high percentage of T lympho-
ocytes with γδ TCR has been described in cutaneous lesions from pa-
tients with dermatitis herpetiformis, implicating a role of the γδ T cell subset in the pathogenesis of this disease. γδ T cells were also found in increased num-
bers in gastrointestinal biopsies from patients with dermatitis herpetiformis
further supporting the role of these cells in the pathogenesis of this auto-
immune condition (18,19).

In the skin of patients with SSc, γδ T cells were significantly increased and
were found in perivascular areas, par-
ticularly in the early phase of SSc and the majority of these cells were Vδ1+. In
the advanced phase of SSc, Vδ1+ T cells were also increased compared with controls. These results indicate a
role of γδ T cells in this condition whereas the increase in the Vδ1 subset is consistent with a restricted Vδ gene
usage (20).

In conclusion, there are several data suggesting that TCR ϵδ T cells represent
an active component of the skin imm-
une system and not a minor redu-
dant T cell subset, although the results concerning the accumulation of these cells in immunomediately skin disorders are often controversial.

T cell-fibroblast interactions in
systemic sclerosis
SSc is a disease of unknown origin characterized by fibrosis of the skin and internal organs associated with dif-
use fibroproliferative microangiopa-
thy and the presence of autoantibodies. Fibrosis is a complex tissue response to various pathological events predomin-
antly characterized by excessive de-
position of extracellular matrix (ECM), especially collagen. Excessive ECM deposition results then in altered tissue and organ architecture leading to dys-
function and ultimately pathology. His-

tologic examination of the early skin
lesions of SSc has demonstrated that an
inflammatory infiltrate, in which CD4+ T cells and CD8+ T cells are numerically
important, precedes fibrosis and the development of the vasculopathy, in-
cluding ultrastructural changes affect-
ing endothelial cells (21). Of interest,
collagen synthesis determined by in situ localization of procollagen I alpha appears to be higher in fibroblasts adjacent to inflammatory cells (22). These findings have led to the hypothesis that inflammatory cells and in particular T cells provide important stimuli that
drive collagen synthesis in fibroblasts (23).

In addition, it has been shown that T cells recruited at sites undergoing fi-
brosis express a restricted T cell recep-
tor repertoire, thus hinting to an (auto-
toantigen-dependent response (24).

Recently, we explored the characteris-
tics of T cells infiltrating the skin of patients with SSc and found heterogeneous
CD4+, CD8+, CD4+CD8+ double positive T-cell subsets with a sizable propor-
tion of T cells producing high levels of IL-4 therefore belonging to the Th2-like subset (25). The potential for T cells infiltrating the skin or lung of SSc patients to produce high levels of IL-4 has been previously described (26, 27). From a polarized T-cell point of view, it is interesting to note that IFN-γ inhibits, while IL-4 and IL-13 enhance collagen synthesis. Thus, based on the effect on ECM deposition of the
prototypic cytokines they produce, Th1-like T cells have been predicted to
decrease while Th2-like T cells to in-
crease ECM deposition (28). We per-
formed experiments aimed to directly assess T helper cell effects on collagen production. We found that cloned Th2 cells inhibited type I collagen produc-
tion by normal fibroblasts. Th2 cell-
dependent inhibition was, at least in part, contact-dependent, essentially mediated by TNF-α, and dominant over proinflammatory IL-4 and TGF-β cyto-
kines. Simultaneously to collagen in-
hibition, Th2 cells induced MMP-1

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production at protein and mRNA levels. These findings indicate that Th2 cells, despite their production of IL-4, depress type I collagen synthesis by normal dermal fibroblasts because the dominant effect of TNF-α and cast doubts on the current interpretation which equal Th2-like T cells (as opposed to IL-4) with fibrosis development. However, it should be stressed that SSC fibroblasts were not inhibited by Th2 cells and that Th1 cells were more powerful inhibitors of collagen synthesis than Th2 cells (25, 29).

An additional and intriguing observation while characterizing T cells infiltrating the skin of early SSC was that some of the α/β TCR+ T cells expressed simultaneously the lineage-specific markers CD4 and CD8, usually mutually exclusive in peripheral compartments. Double positive (DP) T-cells were present in 17 of 20 parental skin-derived cell lines from 6 distinct biopsies. In DP, T-cell clones generated by limiting dilution the CD8 molecule was preferentially induced the production of IFN-γ and Th2 cells of IL-8, while MCP-1 was equally induced by both subsets at mRNA and protein level. Neutralization experiments indicated that membrane-associated TNF-α and IL-1 played a major role in the induction of IL-8 and MCP-1 by Th1 and Th2 cells, while membrane-associated IFN-γ - present only in Th1 cells - was responsible, at least in part, for the lower IL-8 and higher IP-10 production induced by Th1 cells. The contribution of TNF-alpha, IL-1, and IFN-γ was confirmed when fibroblasts were cultured separated by a semi-permeable membrane from living T cells activated by CD3-crosslinking. No distinct differences in chemokine production were observed when the responses to T cell contact or to prototypic Th1 and Th2 cytokines were examined in systemic sclerosis compared to normal fibroblasts. These results indicate that fibroblasts have the potential to participate in shaping the inflammatory response through the activation of flexible programs of chemokine production that depend on the T helper subset eliciting their response (31-33).

In conclusion, in tissues and organs undergoing fibrosis the inflammatory infiltrate includes T cells poised to high IL-4 production. They may therefore participate to modifications of fibroblasts metabolism and function by enhancing ECM deposition over degradation thus favoring fibrosis development. However, not all the features of IL-4 producing T cells are consistent with a pro-fibrotic phenotype and the mechanisms involved in polarization of T cells toward a Th2-like phenotype in these settings are unknown. Further, the antigen-specificity of the infiltrating T cells remains to be elucidated. Thus, the issue whether T cells are responsible for initiating fibroblast modifications that then result in fibrosis or whether the recruitment of T cells is a secondary event in response to previous pathological hits is an issue that awaits further investigations.

### Apoptosis in drug-induced immune-mediated skin diseases

Toxic epidermal necrolysis (TEN) and Stevens-Johnson syndrome (SJS), rare blistering skin diseases, are nearly always associated with drug intake; however, the pathogenesis, although regarded as immune-mediated, is not fully elucidated (34, 35). TEN is a potentially fatal disorder whose clinical findings consist of painful bullous or erosive lesions that rapidly spread over large areas of the skin; epithelial layers of several mucous membranes may also be involved and this contributes to the severity of the process. SJS is now regarded as the same, although less severe, disease (34, 35). Erythema multiforme (EM) is a much less rare and severe disease, clinically characterized by typical target lesions or raised atypical target lesions mainly located on the extremities; blisters usually present in so-called EM majus and rarely in EM minor, are scattered and involve much less than 10% of the body surface. Mucous membrane involvement occurs uncommonly in EM minor, whereas is usual and sometimes severe in EM majus (34, 35). From a pathogenetic point of view, an immunological reaction to viral antigens, notably herpes simplex virus-associated, or, more rarely, to drugs seems to initiate the process. Until recently, most textbooks of dermatology considered EM to be a spectrum of disorders that included EM majus, SJS, and TEN.

A few years ago, some authors suggested that EM majus is different from SJS and TEN, not only in severity but also in the pattern and distribution of the skin lesions (34, 35). The cutaneous pattern in the SJS/TEN spectrum is due to widespread death of epidermal cell through the full thickness of the epidermis. Reports that used immunolabeling to characterize the effector cells of the epidermal injury in SJS/TEN obtained discordant results. While most authors reported that the necrotic epidermis contained nearly exclusively CD8+ T lymphocytes, others suggested that
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cells of monocyte-macrophage lineage were predominant. It is conceivable that CD8+ T lymphocytes are more important in initiating the death of keratinocytes at the early stage of SJS/TEN, while CD68+ monocyte-macrophages have a prominent role at the late stage of SJS/TEN (36). Keratinocyte apoptosis has been shown to play an important part in the pathogenesis of SJS and TEN (37-40).

Apoptosis is a form of cell death occurring in several physiological conditions, most notably normal development and tissue homeostasis as well as preventing pathological processes such as cancer, immunodeficiency and autoimmunity. Furthermore, programmed cell death plays an important role during many inflammatory and malignant pathological processes, including skin diseases (41-43). Apoptosis is a sequence of events based on cellular metabolism that lead to cell shrinkage, nuclear and cytoplasmic condensation, chromatin fragmentation and phagocytosis. Cell death by apoptosis can be triggered by several stimuli, including intracellular stress and receptor-mediated signaling. The intracellular transmission of these signals is mainly mediated by the members of the so-called caspase family, which are critical executioners of the apoptotic pathway. Indeed, the caspase-activated Dnase enters the nucleus and cleaves DNA to produce DNA laddering.

Two major pathways can initiate apoptosis in mammalian cells: one involving engagement of death receptors, the other the release of cytochrome c from mitochondria. An endoplasmic reticulum-specific apoptosis has also been identified (44). The mitochondrial pathway is triggered both by external and internal cues, such as DNA damage. Pro- and anti-apoptotic members of the Bcl-2 family gather at the surface of mitochondria, where they compete to regulate cytochrome C release. In the event of apoptosis, cytochrome c is released from mitochondria, leading to caspase activation. Members of Bcl-2 family have been classified into three functional groups. The most important are members of group I, such as Bcl-2, possessing anti-apoptotic activity, and members of group II, such as Bax, which are pro-apoptotic (41, 42). Death receptors are a subfamily of transmembrane proteins which belong to the TNF-family of receptors, including, among others, Fas and the receptor for TNF-alpha, TNFR1 (40). Apoptosis upon binding of a death ligand to its specific receptor is also depending on the activation of caspases. The best known death-receptor signaling pathway to date is that triggered by the binding of Fas ligand (FasL) to Fas. Fas is expressed almost ubiquitously on a variety of cells, including keratinocytes, whereas FasL is mainly expressed on activated T cells and natural killer cells. Although some authors reported that FasL is also expressed on keratinocytes of TEN lesions (38), it remains controversial whether keratinocytes express biofunctional forms of FasL. Recent data suggested that the activation of Fas through FasL is an important initial step leading to diffuse apoptotic cell death of epidermal cells in SJS/TEN spectrum. Soluble FasL, sFasL, has also been investigated concerning its potential to mediate apoptosis. Significantly increased amounts of sFasL secreted by peripheral blood mononuclear cells have been demonstrated in TEN and SJS, but not in drug-induced EM, suggesting that the serum sFasL level may be a good indicator for the early diagnosis of these disorders (39). In EM, CD4+, CD45Ro+ T lymphocytes co-operating with monocyte-macrophages are regarded as the effector cells responsible for the interface damage, with CD8+ T lymphocytes playing a secondary role. Interestingly, we found CD8+ T lymphocytes being the major cell population within the inflammatory infiltrate both in EM major and a subset of EM minor patients (unpublished data), suggesting that a cytotoxic reaction may contribute to the pathogenesis of EM. The role of apoptosis in EM remains controversial. We recently demonstrated on immunohistochemistry an overexpression of Fas in the epidermis of EM, both major and minor, as compared with normal skin (unpublished data). Based on these findings, Fas-mediated apoptosis may be involved in the pathogenesis of EM in concert with other pathogenetic mechanisms.

References


