

Blistering disorders

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Pathology of acquired and inherited blistering skin diseases is a nice example how technology transfers data obtained from immunology and molecular biology to dermatopathology. This session will overview the current knowledge on cell adhesion proteins and try to unravel the complexity of molecular pathology in selected bullous diseases. What is common in the pathomechanism of pemphigus foliaceus, impetigo and staphylococcal scalded skin syndrome? When the pathology indicates an IgA pemphigus or a paraneoplastic pemphigus? What is common and what is different in acquired and inherited epidermolysis bullosa caused by damages of collagen type VII? What is the role of bullous pemphigoid antigens in the pathomechanism of acquired and inherited blister formation? What should we do for the quick and correct diagnosis of a newborn baby with skin blistering?

In this talk we will overview 1, What studies and in what order improve our diagnostic accuracy? 2, The role of immunolabeling and molecular investigations. 3, The complexity of blistering dermatopathology focusing to fine but important differences for the appropriate diagnosis.

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AUTOIMMUNE CONDITIONS (A PERSPECTIVE FROM DERMATOPATHOLOGY)

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Introduction

Inflammatory dermatoses encompass an enormous area of dermatopathology. Our understanding of the subject comes from combination of histopathological observations and relevant clinical information. Diagnoses are generally reached at the H&E level by using various pattern recognition approaches including one devised by Dr. Ackerman (*1*). Recent advances in cell biology and immunology especially the field of T-cell regulation shed light to the intricate cellular interactions, associations and connect to inflammatory dermatopathology. This review attempts to identify and put into context the most significant advances in immunology relevant to the dermatopathology of autoimmune conditions.

Recent advances in the understanding of T-cell regulation

Many if not all inflammatory dermatoses are reactions of the cellular immune system. We can also carve out the autoimmune inflammatory subgroup with conditions such as lupus erythematosus, dermatomyositis, and psoriasis. T helper cells (Th cells) are traditionally thought to differentiate into Th1 and Th2 subsets based on their cytokine profiles. Th1 cells produce large quantities of interferon-gamma (IFN- γ), and Th2 lymphocytes generate interleukin 4 (IL-4), IL-5 and IL-13 (Mosmann, 1989 #8). The Th1 response is linked to delayed-type hypersensitivity reactions, anti-tumor defense system, it also activates macrophages and is effective in clearing intracellular pathogens. Th2 lymphocytes are essential for the production of immunoglobulin E, eosinophilic inflammation and may suppress cell-mediated immunity. Th1 cells are highly proinflammatory and have been implicated in the pathogenesis of many autoimmune diseases (*2*). Interestingly, loss of IFN- γ signaling in mice deficient in IFN- γ or the IFN- γ receptor makes the animals even more susceptible for autoimmunity. These findings suggested an additional cell type and led to the discovery of the IL-17 producing “Th17” cells (*3, 4*). These cells develop and function in a distinct way from Th1 or Th2 cells and have been shown to play a crucial role in the induction of autoimmune tissue injuries, inflammation and infection. Initial studies suggested that IL-23 is responsible for the differentiation of Th17 cells (*4*). However, recent data only supports a role for the population expansion and survival of these cells (*5*).

Both Th1 and Th17 cells seem to be capable of inducing autoimmunity. During the inflammatory process Th17 cells appear to be generated earlier, whereas Th1 cells function in prolonging and enhancing the inflammatory process (6). Th17 cells responsible for inflammatory reactions including autoimmune conditions can be suppressed by some CD4 (+), regulatory T cells (Tregs).

These CD4 (+) regulatory T-cells also constitutively express the CD25 molecule and the fork head box P3 (FoxP3) molecule, which belongs to the family of transcriptional regulators. FoxP3 is critical for regulation of T-cell development and function. Genetic anomaly of Foxp3 causes autoimmune and inflammatory disease in rodents and humans. Mouse scurfy is an X-linked disorder characterized by scaly skin, infections, gastrointestinal bleeding, anemia, thrombocytopenia, hypogonadism, leukocytosis, lymphadenopathy and cachexia and die a few weeks of age (7). Wildin et al., hypothesized and proved that the fatal human disease: immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is the human equivalent of scurfy. Naturally occurring mutations of the human gene FOXP3, -the ortholog of the gene mutated in scurfy mice (Foxp3)- lead to the generation of autoaggressive lymphocyte clones, which drive the inflammatory and autoimmune processes (8), (9, 10).

Functional FoxP3 (+) T cells, suppress the immune system to prevent overactive responses and inflammation induced by T-cells. (11, 12). These findings at the cellular and molecular levels altogether provide firm evidence for Foxp3(+)/CD25(+)/CD4(+) regulatory T cells as an indispensable cellular constituent of the normal immune system and for their crucial roles in establishing and maintaining immunologic self-tolerance and immune homeostasis. An intense research is focusing on the exploitation of regulatory T-cells for clinical use to treat autoimmune diseases and control pathological immune responses and treat cancer.(13)

Th1, Th17 cells and the FoxP3 expressing Tregs can be conceptualized as the “yin and yang” of the immune system. It is suggested the both Tregs and Th17 cells are differentiated from the same T-cell subset under the influence of TGF- β , IL-6 and probably due to other cytokines (Figure 1.). During the resting state of the immune system the production of TGF- β favors the induction of Tregs, which suppresses autoimmunity. However, activation of the innate immune system will lead to the generation of IL-6 and pushes the balance towards the generation and differentiation of proinflammatory Th17 cells (6).

The further characterization of Th1, Th17 and Treg cells can potentially hold the key for the treatment of patients suffering from autoimmune diseases, cancer, infection or those receiving organ transplants and have significant impacts in the prevention and/or treatment of immunity-related diseases in clinics.

T cell regulation appears to play a significant role in the etiopathogenesis of the following skin conditions and discussed in detail:

- 1. Lupus Erythematosus and Dermatomyositis**
- 2. Psoriasis**
- 3. Scleroderma/Systemic Sclerosis**
- 4. Graft-Versus-Host Disease (GVHD)**
- 5. Adoptive Immunotherapy**

Cutaneous Lupus Erythematosus (CLE)

Cutaneous lupus erythematosus remains an elusive disease despite of significant efforts trying to characterize this entity. Recent advances in the understanding of the effects of ultraviolet irradiation on the skin of patients with CLE resulted in a more comprehensive model for the

pathogenesis of the disease. Exposure to UV light induces apoptosis of keratinocytes and the release of pro-inflammatory cytokines. Apoptotic cells appear to be responsible for breaking the self-tolerance of the immune system and inducing the characteristic inflammatory skin lesions. Many other factors, in part genetically determined, are involved in CLE resulting in a very heterogeneous clinical manifestation. Among these factors, presence of autoantibodies, a decreased number of regulatory T cells at the site of inflammation and increased expression of pro-inflammatory cytokines like TNF- α and IFN-inducible protein myxovirus protein A have been shown to play a role in the pathogenesis of CLE (14, 15). In addition to Th1 cells, Th17 proinflammatory lymphocytes appears to play an important role in lupus erythematosus (16, 17). As CD4⁺,CD25^{high} regulatory T (Treg) cells play a crucial role in the maintenance of self tolerance and prevention of organ-specific autoimmunity, these cells are in the focus point of CLE related research. Franz et al. tried to define the phenotype and function of CD4⁺,CD25⁺ regulatory T cells (Treg) in patients with CLE. Immunohistochemical analysis of skin biopsies from CLE patients and controls using anti-Foxp3 and anti-CD4 monoclonal antibodies together with characterization of peripheral blood CD4⁺,CD25⁺ Treg from normal healthy donors and patients with CLE was carried out by flow cytometry. Quantitative analysis of CD4⁺ T cells in skin lesions from patients with CLE revealed that the number was similar to that in lesions from patients with other chronic inflammatory diseases, but the number of Foxp3⁺ Treg in CLE was significantly reduced. (18).

Contrary to these authors Yan and colleagues found significantly increased Treg counts in peripheral blood of CLE patients compared to normal controls. There was no difference in FoxP3 expression at either the mRNA or protein level in any CD4⁺,CD25⁺ T cell subset from SLE patients as compared with controls. According to their data antigen-presenting cells (APCs) from SLE patients were responsible for the decreased Treg cell activity and could also render dysfunctional Treg cells from healthy control subjects. CD4⁺,CD25⁺ Treg cells from SLE patients exhibited normal suppressive activity when cultured with antigen presenting cells (APCs) from healthy controls. The observed partial Treg cell blockade appeared to be induced by the high levels of IFN- α derived from SLE patient APCs and is responsible for the loss of peripheral tolerance (19).

A potential morphological correlate of this study is the one by McNiff et al., on the histological distribution of plasmacytoid dendritic cells in skin biopsies of CLE and dermatomyositis (DM). It is a challenge to discriminate CLE from DM on the histological characteristics alone, since they show greatly overlapping features. The distribution of the plasmacytoid dendritic cells depicted by the CD123 antibody, however, appears to offer some help. Plasmacytoid dendritic cells (PDCs) are antigen presenting cells and are capable of producing IFN- α . In skin biopsies of DM PDCs were distributed mainly in the epidermis, whereas in CLE these cells were primarily located in the dermis. These findings may suggest different pathogenetic mechanisms, as well as can be used as differential diagnostic tools on difficult skin biopsy specimens (20). Another potentially helpful tool is the use of direct immunofluorescence for the membrane attack complex C5b-9 to differentiate between lupus erythematosus and dermatomyositis. There is a stronger granular epidermal basement zone labeling with C5b-9 compared to C3 (C5b-9>C3) in dermatomyositis, which ratio appears to be reversed in lupus erythematosus (C3>C5b-9) (21).

Psoriasis

Psoriasis is a common skin disease affecting about 2% of the world's population with even higher numbers in North America (22). Currently psoriasis is viewed as a T-cell mediated inflammatory or autoimmune disease in which the interaction of dendritic cells and T-cells create a cytokine microenvironment proliferative to keratinocytes. Up until recently, the dominant pathway was thought to be Th1 driven based on gene expression profiles and the presence of tumor necrosis factor-alpha (TNF- α) and IF- γ (23). Recent body of evidence, however, emphasizes the

importance of Th17 cells in psoriatic lesions. (24-26). The histologically observed abnormal keratinocyte differentiation with regular epidermal hyperplasia, the diminished granular cell layer, prominent parakeratosis, and thinning of the suprapapillary epidermal plates appears to be the result of the prominent inflammatory cell infiltrate present in the dermis and the epidermis including Th17 cells localizing mainly to the dermal compartment of psoriatic lesions (24). According to the model proposed by Zheng et al., dendritic cells produce excess amount of IL-23, which then lead to the proliferation of Th17 cells. IL-22 produced by Th17 cells then induces hyperproliferation of keratinocytes and seems to be responsible for the crosstalk between immune cells and keratinocytes in psoriasis (27).

Scleroderma/Systemic Sclerosis

Scleroderma is a debilitating autoimmune condition with unknown etiology. The pathogenesis involves problems with microcirculation immune activation with antibody production and proliferation of fibroblasts associated with deposition of collagen rich tissues (22). Early stages of the disease appear to be linked with high levels of IL-6, TGF- β , IL-17 and IL-22 (28, 29). The high-affinity IL22 receptor is expressed on various cell types including keratinocytes and fibroblasts. It is conceivable that IL-22 produced by Th17 cells contribute to the inflammatory process and is responsible for the sclerosis observed clinically. Th17 cells also link systemic sclerosis with the chronic phase of graft-versus-host disease (30).

Graft-Versus-Host Disease (GVHD)

Acute and chronic graft-versus-host diseases (GVHD) are debilitating conditions and major complication of allogeneic bone marrow transplantation (BMT). T-cells within the graft are essential for the engraftment of hematopoietic stem cells involved in the reconstitution of host immune defense and exhibit anti-tumor activity. The distinction of acute and chronic phases is somewhat arbitrary and accomplished by using 100 days after the transplantation. The acute phase typically affects the gut, liver and the skin, however, during chronic stages a less discriminative involvement of tissues -similar to systemic autoimmune diseases- is evident. This transitioning characteristic is attributable to the progressive loss of CD4(+)CD25(+)Foxp3(+) regulatory T cells during the course of acute GVHD. This leads to the expansion of donor-derived CD4(+) T cells with T(H)1 and T(H)17 cytokine phenotypes that release proinflammatory cytokines and cause autoimmune-mediated pathological damage. Th17 cells, however, appears not to be involved in the acute phase of GVHD and seems to have a protective role in GVHD by decreasing the severity of the Th1 reaction (31) (30, 32). Recent studies support the above notion by underscoring the effectiveness of extracorporeal photochemotherapy (ECP) in refractory/resistant GVHD. ECP in this condition increase the number of CD4+CD25+ Tregs. (33). Similarly, bone marrow-derived facilitating cells -inducers of Tregs- appears to have beneficial role in the inhibition of GVHD after allogeneic bone marrow transplantation (34, 35). Rezvani et al. suggest that GVHD is preventable by using grafts containing high donor FOXP3-positive Tregs (36).

Mature donor T cells cause graft-versus-host disease (GVHD), but they are also the main mediators of the beneficial graft-versus-tumor (GVT) activity of allogeneic bone marrow transplantation. Suppression of GVHD with maintenance of GVT activity is a desirable outcome for clinical transplantation. CD4+CD25+ regulatory T cells suppress the early expansion of alloreactive donor T cells, their interleukin-2-receptor (IL-2R) alpha-chain expression and their capacity to induce GVHD without abrogating their GVT effector function, mediated primarily by the perforin lysis pathway (37). The above results are encouraging however need confirmation on human subjects.

Adoptive immunotherapy associated dermatitis

Adoptive immunotherapy is an evolving innovative approach to treat malignancies, including metastatic malignant melanoma (38). It utilizes the patients' natural T cell-based cytotoxic responses to attack cancer. Adoptive immunotherapy harnesses this natural force to eliminate metastatic malignant disease. T cells with a naturally occurring reactivity to a patient's melanoma can be found in the infiltrate surrounding the tumor cells (tumor infiltrating lymphocytes (TILs)). The tumor is harvested, and TILs are expanded in vitro using high concentrations of interleukin-2 (IL-2), anti-CD3 and allo-reactive feeders. These T cells are then transferred back into the patient along with exogenous administration of IL-2. Recent advances in the understanding of immune interaction helped greatly to modify immunotherapy approaches, namely combining adoptively transferred cells with lymphodepletion (39). The immunosuppression is achieved by the combination of chemotherapy and total body irradiation as Tregs are exquisitely sensitive to irradiation (40). The intensity of lymphodepletion has been dramatically increased in current research protocols to a level that requires hematopoietic stem cell (HSC) transplantation. Surprisingly, the HSC transplant and not the increased lymphodepletion causes a robust expansion of adoptively transferred tumor-specific CD8(+) T cells and leads to long standing anti-tumor responses in both experimental animals and human patients. (39, 41-43). Several patients responding to adoptive immunotherapy also exhibits a diffusely erythematous, edematous/vesicular and purpuric rash. The rash spares skin rendered vitiliginous by previous immunotherapy. Histology shows spongiosis, a sparse superficial, perivascular lymphocytic infiltrate with moderate interface dermatitis, vacuolar degeneration of the dermoepidermal junction and edema. Immunohistochemical studies reveal a predominantly CD3, CD8 positive T-cell infiltrate with activated cytotoxic T-cell phenotype and the expression of perforin and Granzyme B. The cytotoxic response in the skin biopsy specimens is directed against resident epidermal melanocytes (44). It appears that the lack or greatly diminished numbers of Tregs allows uninhibited proliferation of cytotoxic T-cells in these patients. This is culminating in an impressive anti-tumor activity and also as a collateral effect damage of resident melanocytes and clinically as skin rash.

CONCLUSIONS

Th1, Th17 and T regulatory cells bear unique immunological properties that make them potential therapeutic targets. Understanding the fine cellular interactions allows us to enhance self-tolerance in conditions such as graft-versus-host disease, erythema multiforme/toxic epidermal necrolysis, lupus erythematosus, and allergic conditions. On the contrary reducing self-tolerance can help to establish better immunity, faster clearance of infectious agents, and better antitumor responses. Recent advances on the field of immunity will help us to treat these conditions effectively.

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