Title: Classification of inflammatory skin diseases: A proposal based on the disorders of the three-layered defense systems, barrier, innate immunity and acquired immunity.

Author(s): Dainichi, Teruki; Hanakawa, Sho; Kabashima, Kenji

Citation: Dainichi, Teruki ...[et al]. Classification of inflammatory skin diseases: A proposal based on the disorders of the three-layered defense systems, barrier, innate immunity and acquired immunity. Journal of dermatological science 2014, 76(2): 81-89

Issue Date: 2014-11

URL: http://hdl.handle.net/2433/191232

Rights: © 2014 Japanese Society for Investigative Dermatology. Published by Elsevier Ireland Ltd.; This is not the published version. Please cite only the published version.
Category of article:
Invited Review Article

Title:
Classification of inflammatory skin diseases: A proposal based on the disorders of the three-layered defense systems, barrier, innate immunity and acquired immunity.

Authors:
Teruki Dainichi, MD, PhD, Sho Hanakawa, MS, and Kenji Kabashima, MD, PhD

Affiliation:
Department of Dermatology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Corresponding Author:
Teruki Dainichi, MD, PhD
Department of Dermatology
Kyoto University Graduate School of Medicine
54 Shogoin Kawahara-cho, Sakyo, Kyoto 606-8507, Japan
Phone: +81-75-751-3310 / Fax: +81-75-761-3002
E-mail: dainichi@kuhp.kyoto-u.ac.jp
Abstract:

The host defense system of the skin is composed of 1) a barrier, 2) innate immunity, and 3) acquired immunity. Inflammatory skin diseases can be classified into one of the disorders of these layers of the defense system, unless there is an ordinary response to specific infectious agents or internal/external injury. Any inflammatory skin disease partly simulates the response to real infections or dangers. Disorders of acquired immunity can be classified into 1) immunodeficiency, 2) immunohyperactivity (allergy), and 3) qualitative disorder (autoimmunity). Disorders of innate immunity can be classified into 1) innate immunodeficiency, 2) innate immunohyperactivity (general or local autoinflammation), and 3) qualitative disorder (general or local innate autoimmunity). The barrier of the skin is composed of 1) the physical barrier and 2) the chemical barrier. Several diseases, such as atopic dermatitis, are attributed to the disorder of these components of the barrier. Here, we propose an algorithm to classify the pathology of inflammatory skin diseases by means of what disorder in the specific layer of the host defense system is truly responsible.

Keywords: dermatitis, innate immunity, acquired immunity, barrier
1. Layers of the host defense system

2. Disorder of acquired immunity in skin diseases
   2.1. Immunodeficiency
   2.2. Immunohyperactivity/Allergy
   2.3. Autoimmunity
      2.3.1. Systemic autoimmunity
      2.3.2. Organ-specific autoimmunity

3. Disorder of innate immunity in skin diseases
   3.1. Innate immunodeficiency
   3.2. Innate immunohyperactivity
      3.2.1. Systemic innate immunohyperactivity: Autoinflammatory diseases
      3.2.2. Organ-specific innate immunohyperactivity: Acne, pyoderma chronica, and rosacea
   3.3. Innate autoimmunity
      3.3.1. Systemic innate autoimmunity: “Classical” systemic autoimmune diseases
      3.3.2. Organ-specific innate autoimmunity: Psoriasis

4. Disorder of the barrier in skin diseases
   4.1. Defects in the physical barrier: Atopic dermatitis
   4.2. Defects in the chemical barrier
      4.2.1. Proteases and protease inhibitors
      4.2.2. Antimicrobial peptides
      4.2.3. Other mediators

5. Conclusion

6. Acknowledgements
We dedicate this manuscript to Dr. Yoshiki Miyachi, Professor and Chairman of the Department of Dermatology, Graduate School of Medicine, Kyoto University, from 1998 to 2014.

1. Layers of the host defense system

The host defense system of the skin is mainly composed of the following three layers: 1) barrier of the skin, 2) innate immunity, and 3) acquired immunity [1]. In general, these layers are aligned in this order chronologically, functionally, and phylogenetically. A disorder in a specific layer can arise from defects in the superior layer. Each layer has unique roles to protect the body against specific infectious agents and external/internal dangers. Inflammation is defined as a series of protective and regenerative responses of the body. Therefore, inflammatory skin diseases are originally a result of these protective and regenerative responses of the skin against infections and dangers. If primarily causative infections and dangers are ruled out, the dermatitis is due to hereditary or acquired disorder in a specific layer of the host defense system.

Descriptive dermatology of the morphological phenomena of skin has been developed for more than two thousand years. Despite recent and ongoing progress in immunology, innate immunity, and the skin barrier, inflammatory skin diseases have not yet been fully classified in terms of the defects in each layer of the host defense system.

In this review, we propose a new algorithm to understand the pathology of inflammatory skin diseases in terms of the specific roles of each three layer in host defense system of the skin (Box). We describe the disorder of these layers in reverse order from acquired immunity to barrier, for the better understanding (see Chapter 5). Physiological disorders of the body machine can be divided into quantitative disorders and qualitative disorders. We classified the disorders of this dichotomy, except for the disorder of barrier, whose understanding is insufficient for this classification (Table 1). This classification proposed here would be effective to clarify the current problems and the directions of research in this field.
2. Disorder of acquired immunity in skin diseases

2.1. Immunodeficiency

A number of hereditary diseases have been identified as a genetic lack of the specific molecules that are essential in acquired immunity. Many of them are associated with a variety of cutaneous infections and inflammation. For example, X-linked agammaglobulinemia (XLA) develops furunculosis, impetigo, and atopic-like eczematous eruptions. X-linked lymphoproliferative (XLP) disease is highly accompanied by infectious mononucleosis due to Epstein-Barr virus (EBV) infection. Chronic mucocutaneous candidiasis (CMCC) is composed of heterogeneous diseases, some of which have molecular defects in the acquired immunity, such as defects in the autoimmune regulator (AIRE) gene *(See Chapter 2.3.1)* [2]. Acquired immunodeficiency syndrome (AIDS) patients are highly susceptible to fungal and herpes virus infection, such as human herpes virus 8 (HHV8), and patients develop repetitive and severe herpes and Kaposi’s sarcoma in the skin.

2.2. Immunohyperactivity/Allergy

Another face of acquired immunity is an antitoxin, which neutralizes toxic agents such as venoms and poisons from insects, worms, and plants in nature [3]. However, hyperactivity of acquired immunity to a specific antigen results in allergic diseases. An allergy can be divided into two groups by the course of sensitization. One is an allergy with irritative and destructive sensitization, such as allergies to wasps and poison ivy. Another is an allergy without remarkable damages on the body at the sensitization phase, such as food allergies, and allergies to the cosmetic and industrial materials. The former allergy is originally beneficial and protective for species, whereas the latter is not.

As for the skin, allergic contact dermatitis is thought to have originated as a beneficial response in the skin, to eliminate toxic molecules and harmful insects and worms by oozing, acanthosis, hyperkeratosis, and exfoliation in the epidermis, accompanied by scratching behavior. However, a new material that has not exposed to organisms, as
well as a new exposure condition of the existing materials for an ethnic, such as manufactured metals and peanut oil in industrial materials, can accidentally elicit acquired immunity in limited responders. Most people commonly inherit the former allergy, which is usually associated with irritant dermatitis by toxic allergens at the sensitization phase [3]. However, the latter allergy has not yet been phylogenetically inherited or eliminated for thousands of years, regardless of whether it is beneficial or not. Thus, the latter allergies to cosmetic materials, industrial products, and unspecified materials suffer limited people only since specific events in the history of specific ethnic groups.

2.3. Autoimmunity

Autoimmunity is a qualitative disorder mainly due to trouble within the organization of immune tolerance, which is essential for acquired immunity. Both central tolerance in the thymus and peripheral tolerance in extrathymic tissues are indispensable to avoid severe systemic autoimmune diseases. Here, two basic questions are raised. 1) Is a breakdown of the tolerance a primary disorder of acquired immunity or a secondary disorder attributed to the preceding layers of the host defense system in ‘classical’ systemic autoimmune diseases? 2) Are there any antigen-specific breakdowns of tolerance or anergy involved in the pathogenesis of organ-specific autoimmune diseases?

2.3.1. Systemic autoimmunity

Autoimmune diseases are classically divided into systemic and organ-specific autoimmune diseases. Mice with lpr or gld mutation develop systemic autoimmune disease [4]. They possess loss-of-function mutation in Fas or FasL, respectively, which are necessary for the negative selection of autoreactive T and B-lymphocytes in thymus and extrathymic tissues. In humans, Fas mutation also develops autoimmune lymphoproliferative syndrome (ALPS) that presents similar clinical features as lpr mice. Defect in AIRE develops multiorgan disease in mice and humans as autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) [5]. The lack of Foxp3 causes severe systemic autoimmune disease in mice and humans
called immune dysregulation, polyendocrinopathy, enteropathy, and X-linked (IPEX) syndrome [6].

However, despite a number of regulatory molecules of acquired immunity, there is limited evidence showing that they are definitely involved in the pathogenesis of systemic autoimmune diseases. It is obscure whether most systemic autoimmune diseases are due to primary disorder of acquired immunity or secondary disorder caused by the primary disorder in the innate immune system (see chapter 3).

2.3.2. Organ-specific autoimmunity

There are quite a few hypotheses to explain the breakdown of antigen-specific tolerance to an autoantigen. Infections precede some organ-specific autoimmune diseases, and cross-reactivity and bystander activation of autoreactive T-lymphocytes are implicated in their pathogenicity. Viral infections appeared to precede the onset of type-I diabetes mellitus. In skin diseases, fogo selvagem, an endemic pemphigus foliaceus, anti-desmoglein 1 autoantibodies cross-react with sand fly salivary antigen [7]. However, most cases of autoimmune skin diseases lack apparent evidence of preceding infection or inflammation.

Organ-specific autoimmune diseases may not be due to a disorder of acquired immunity, but may instead be due to acquired changes in the antigenicity of self-antigens. For example: 1) Autoantibody in linear IgA bullous dermatosis (LAD) recognizes neoepitopes generated by ectodomain shedding of 180-kD bullous pemphigoid antigen (BP180)/type XVII collagen [8, 9]. 2) Laminin 332 from cancer cells possess unique laminin globular (LG) 4 and 5 domains, suggesting preferential association of anti-laminin 332 mucous membrane pemphigoid with malignancy [10]. 3) Laminin γ1 is a ubiquitous, highly glycosylated protein. Autoantibodies from anti-laminin γ1 pemphigoid do not react with blood vessel-derived, but do react with skin-derived laminin γ1, suggesting that the autoantibody recognizes unique glycosylation patterns [11]. Thus, post-translational modification of autoantigens may lead the normal response of acquired immunity to the newly arisen epitopes in individuals whose immune tolerance is accidentally abrogated in some autoimmune bullous diseases.
3. Disorder of innate immunity in skin diseases

Despite the large number of TLRs, RIG-I-like receptors (RLRs), and the other pattern recognition receptors (PRRs), only a few transcription factors drive the innate immune response of an organism (Figure 1). In other words, the immune system has evolutionally developed to interpret the activation patterns of a couple of essential transcription factors to elicit the most suitable type of immune response, and protected an organism against specific infectious agents [12-14]. Thus, one can imagine that the defective homeostatic balance of the activation state of these transcription factors is crucial in the development of inflammatory skin diseases simulating the real innate immune responses to infectious agents and dangers (Figure 2).

3.1. Innate immunodeficiency

Primary immunodeficiencies are partly defined as genetic deficiencies in the innate immune system [15, 16]. Innate immune receptors, such as TLRs, recognize conserved pathogen-associated molecules that are shared by different microorganisms, and subsequent engagement can trigger the production of inflammatory cytokines and chemokines through the nuclear factor-κB (NF-κB)-dependent and interferon (IFN)-regulatory factor (IRF)-dependent signaling pathways (Figure 1) [15]. Complete defects in each of two main TLR-dependent pathways have been reported so far [16]. The first is a defect in myeloid differentiation primary response 88 (MyD88) or interleukin (IL)-1 receptor-associated kinase 4 (IRAK4), which lacks NF-κB-dependent expression of pro-inflammatory cytokine, and leads to greater susceptibility to pyogenic bacteria in early infancy. The second is a defect in the TLR3-TRIF-TRAF3 pathway, which lacks expression of type 1 IFNs, and results in greater susceptibility to herpesviruses [16].

Dominant negative mutations in STAT3, another transcription factor regulating the expression of a number of cytokines and growth factors, causes unique primary immunodeficiency called hyper-IgE syndrome [17]. Newborn rash, atopic dermatitis-
like skin lesions, cold abscesses, and CMCC characterize the skin manifestations of this syndrome although precise mechanisms underlying this syndrome remain unclear.

Defects in myeloid cells or components of the complements in inflammatory skin diseases are described elsewhere [18, 19].

3.2. Innate immunohyperactivity

3.2.1. Systemic innate immunohyperactivity: Autoinflammatory diseases

“Excessive” innate immune responses characterize systemic autoinflammatory diseases. A number of autoinflammatory syndromes are caused by single gene mutations that are crucial in the regulation of interleukin (IL)-1 [20-22]. For example, a gain-of-function mutation in NLRP3, a component of inflammasomes, causes cryopyrin-associated periodic syndrome (CAPS). Deficiency of IL-1 receptor antagonist (DIRA), TNF receptor-associated periodic syndrome (TRAPS), and IL-10 receptor deficiency are included in monogenic, systemic autoinflammatory diseases. Anti-IL-1 therapy is the standard of care in treating CAPS and other autoinflammatory diseases [23].

Some polygenic, systemic autoinflammatory diseases partly share their clinical states, pathogenesis, and responsible genes with monogenic autoinflammatory diseases. [24]. Behçet’s disease has been classified as an autoinflammatory disorder [25, 26]. Two large genome-wide association studies (GWASs) reported the association between Behçet’s disease and the single nucleotide polymorphism (SNP) of IL-10, IL-23R/IL-12RB2, TRAF5, and TRAF3IP2 genes [27-29]. Pyoderma gangrenosum, characterized by sterile neutrophilic infiltration of the skin and other organs, is one of the clinical manifestations of a monogenic autoinflammatory disease, pyogenic arthritis, pyoderma gangrenosum, and cystic acne (PAPA) syndrome [22], in which CD2BP1 mutation results in the activation of the NLRP3 inflammasome [24]. A clinical study demonstrated that infliximab, a monoclonal antibody against tumor necrosis factor (TNF)-α, is effective for the treatment of pyoderma gangrenosum [30].
3.2.2. Organ-specific innate immunohyperactivity: Acne, pyoderma chronica, and rosacea

In some diseases, hyperactivity of the innate immune response is highly specific to skin appendages. Therefore, it is convenient to understand that unique and distinctive pathomechanisms are involved in the folliculosebaceous units-specific innate hyperactivity. Folliculosebaceous units are known to be immune privileged sites, which prevent sensitization and elicitation of an antigen-specific, acquired immune response. On the other hand, it would also be theoretically appropriate to say that the state of the defect in the ‘innate’ immune privilege in folliculosebaceous units elicits hyperactivity of the innate immunity in these organs.

The pathogenesis of acne is multifaceted, and it was originally thought that inflammation follows comedo formation. However, there is evidence that dermal inflammation may actually precede comedo formation [31]. Human follicular keratinocytes demonstrate hyperproliferation and microcomedone formation when IL-1α is added [32]. IL-1 receptor antagonists inhibit microcomedone formation [33]. *P. acnes* has been shown to stimulate the release of proinflammatory cytokines, such as IL-1α, IL-8, IL-12, and TNF-α by binding to TLR2 on monocytes and polymorphonuclear cells surrounding the sebaceous follicle [34].

As well as acne, chronic pyoderms, such as dermatitis papillaris capillitii, hidradenitis suppurativa, and pyoderma chronica glutealis, develop in several monogenic autoinflammatory disorders. Despite bacterial colonization and infection, they are not always a primary reason for these diseases. Defective Notch-induced organogenesis can also be associated with cyst formation in these diseases [35].

In rosacea, lesional keratinocytes show TLR2 upregulation and increased receptor sensitivity leading to a higher expression and activity of serine proteases of the kallikrein family. [36]. Kallikrein is involved in the processing of cathelicidin antimicrobial peptides (CAMPs) and other smaller fragments, which are involved in the pathogenesis of rosacea (*see chapter 4. 2. 2*). Although the trigger for TLR2 in rosacea remains unspecified, chitins and *Bacillus oleronius* from *Demodex folliculorum* infestation could serve as the trigger for TLR2 on keratinocytes. Thus,
hyperactivation of a specific TLR signaling pathway in folliculosebaceous units can be a primary disorder and precede the dysfunction of CAMPs in rosacea.

3.3. Innate autoimmunity

While autoinflammatory diseases are defined as a quantitative disorder of the innate immune response, many other diseases have been implicated as involving defects in the innate immune system: 1) they are not explained simply by an excessive activation of innate immunity and 2) activation of autoreactive lymphocytes is inevitable for pathogenesis. Here, we name this group “innate autoimmunity” as a qualitative disorder of the innate immune system.

3.3.1. Systemic innate autoimmunity: “Classical” systemic autoimmune diseases

Recent work highlighted the important role of innate immunity and IFN-α signaling in the pathogenesis of both systemic lupus erythematosus (SLE) and systemic sclerosis [12, 13]. The activation of plasmacytoid dendritic cells (pDCs) by circulating immune complexes following release of neutrophil extracellular traps (NETs) is an early trigger of autoimmunity in patients with SLE, by directing the chronic IFN-α production [37, 38]. NETs immobilize and kill invading microbes through a unique form of neutrophil cell death, NETosis. Antimicrobial peptide LL-37 antibody is expressed at high levels in SLE. NET-associated LL-37-DNA immune complex, together with anti-LL-37 or anti-DNA autoantibodies, appears to stimulate the production of IFN-α by pDCs, and may also prompt autoreactive B-cell activation [39]. Several microarray analyses have revealed a genomic “signature” of upregulated IFN-α-inducible genes from peripheral blood mononuclear cells of SLE and systemic sclerosis patients [40, 41]. IFN-α is induced by pDCs in systemic sclerosis presumably through RNA binding with TLR7 [42].

Thus, the disorder of the innate immune response can promote systemic autoimmunity and precede the expansion of autoreactive lymphocytes and tissue injuries in patients with SLE and systemic sclerosis. Antimalarials have recently been
shown to inhibit TLR signaling, and it may be through this mechanism of action that antimalarials display efficacy in lupus [43].

3.3.2. Organ-specific innate autoimmunity: Psoriasis

Next, one would easily come up with the idea of organ-specific innate autoimmunity among inflammatory skin disorders. The early events and players in the proposed pathogenesis of SLE described above are very similar to that which were recently proposed in the pathogenesis of psoriasis [44], although the functional character of the T effector lymphocytes and the resulting histological and clinical features are quite different. In psoriasis, it is suggested that the breakdown of tolerance to self-nucleic acids occurs when self-DNA and -RNA aggregate with the LL-37 [44]. By inducing pDC activation and type I IFN production, LL-37-self-DNA complexes initiate a pathogenic cross talk between stressed epidermal cells and recruited pDCs. pDC-derived IFN-α promotes the maturation and activation of bystander myeloid DCs, which are key to sustaining and amplifying T cell responses in skin inflammation.

Gain-of-function mutations in caspase recruitment domain (CARD) 14, potentially an upstream regulator of IκB kinase (IKK) β, have been identified in the familial type of psoriasis and pityriasis rubra pilaris (PRP) [45, 46]. On the contrary, the mice lacking IKKβ in the epidermis develop psoriasiform dermatitis, and a high amount of IL-24 produced from the keratinocytes precedes the infiltration of neutrophils and lymphocytes and disease development [47]. IKKβ is a master kinase at the bottleneck of the NF-κB, IRF3, IRF7 and MAPK signaling pathways downstream of PRRs. However, the transcriptional factors that are directly involved in the pathogenesis of the epidermis-specific IKKβ knockout, and the skin lesions in human psoriasis and PRP, have not been completely specified.

4. Disorders of the barrier in skin diseases

In this article, as described above, we consider a barrier to be fundamental structure and system that is not inducible, but is permanent and preventive against external dangers at the frontline of skin immune sentinels. A barrier is classified into two
elements: 1) A physical barrier, which is a visible structure covering the outside of the body to protect an organism against any external dangers. 2) A chemical barrier, which is invisible and composed of the biologically active molecules, is constitutively produced at the surface of the body to attack and neutralize the potential invaders. In other words, a physical barrier is a shield while a chemical barrier is a landmine at the interface of the external environment and the surface of an organism. Disorder in the physical barrier is usually accompanied by disorder of the chemical barrier. Although the disorder of a barrier cannot yet be clearly determined quantitatively or qualitatively, several diseases, such as atopic dermatitis, can be attributed to disorder of the specific components of the barrier.

4.1. Defects in the physical barrier: Atopic dermatitis

Atopic dermatitis is not a monogenic disorder. However, it has been well accepted that defects in the physical barrier are involved in the pathogenesis of, and continuous inflammation in, atopic dermatitis [48]. Filaggrin governs multiple components of the physical barrier in the skin. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Filaggrin gene mutations are detected in 20 to 50% of patients with atopic dermatitis in Europe and Japan [49, 50] and are also associated with bronchial asthma [51].

There is evidence that primary defects in the other components of the physical barrier, such as corneodesmosin [52] or desmoglein 1 [53], causes atopic dermatitis-like dermatitis with hyper IgE levels and eosinophilia. However, the common defect in the physical and chemical barrier in these diseases and atopic dermatitis remains undetermined. The lack of claudin-1 gene coding a tight junction protein in humans leads to ichthyosis not eczema [54] despite diminished expression of claudin-1 and claudin-23, and association of SNPs in claudin-1, in patients with atopic dermatitis [55].

A number of recent investigations suggest that a vicious cycle of the defects in the physical barrier and eczema is involved in the persistence of the atopic dermatitis [48]. Th2-cytokines produced in eczema lesions impair keratinocyte differentiation and
diminish the expression of the molecular components of the physical barrier, and the scratching behavior damages the physical barrier and accelerates skin inflammation.

Then why do not all patients with ichthyosis vulgaris develop atopic dermatitis? It is a natural idea that atopic dermatitis requires second and third cues for anything but barrier. In terms of the barrier, however, we propose three possibilities to explain this paradox: 1) Disorder in the normal flora of the skin may be a principal defect in atopic dermatitis. The skin topography of microbes elegantly proposes the interaction of the skin microbiome and the frequently affected sites in atopic dermatitis [56]. The bacterial flora on the skin can shape and affect the development of the acquired immune system, and the defects in the physical barrier may reduce the threshold of immune modulation. 2) Defects in the physical barrier are heterogeneous and only an unspecified defect is responsible for the development of atopic dermatitis. 3) Atopic dermatitis may not be a disease of the defective physical barrier, but rather a disease of the defective chemical barrier.

Certainly, only a defect of the barrier simulating damages on the surface of the skin by real invaders, such as scabies, hookworms, dermatophytes, and staphylococci, may provide molecular cues and elicit eczematous inflammation and an itchy sensation, which is reasonable for protecting the body from these agents. This process may involve defects in the chemical barrier, as discussed in the following section.

4.2. Defects in the chemical barrier

The interface of the external circumstance and the surface of the body is also protected by chemical materials, such as acids [57], reactive oxygen species (ROS) [58], lipid mediators like prostanoids [59], lysozymes and antimicrobial peptides (AMPs) [60, 61], and protease inhibitors [62], which are abundantly produced in the uppermost layer of the skin. The chemical barrier is the most basic and phylogenetically ancient system for an organism to protect oneself. Only effective systems have been phylogenetically selected and inherited among host-infection interaction, and innate immunity and adoptive immunity have effectively utilized this preceding mechanism for more selective protection in an elaborate manner. Therefore, each component of the chemical barrier has specific roles to protect the body against
specific dangers and infectious agents, and its defects can result in unique forms of skin inflammation.

4.2.1. Proteases and protease inhibitors

Endogenous proteases are important in 1) the keratinocyte differentiation and the desquamation process of the cornified layer [62], and 2) processing and regulation of the defense molecules in the epidermis [63]. On the other hand, there are numerous endogenous protease inhibitors, such as cystatins and lymphoepithelial Kazal-type-related inhibitor (LEKTI). They regulate the proteolytic activity of endogenous proteases in the epidermis [63]. They may also control the proteolytic activity of the exogenous proteases from microorganisms and hamper their adhesion and transmigration. For example, proteolytic cleavage of cathelicidin produces LL-37 and another bioactive fragment that has cystatin-like activity [64]. Thus, the delicate balance of proteases and protease inhibitors contributes to the physical and chemical barrier function of the skin.

Cysteine proteases are known to be a strong Th2 adjuvant [65], and are a major antigen from parasitic helminthes, the infection of which elicits a robust Th2 response [66]. Cysteine proteases are contained in a number of antigens and allergens, such as house dust mite antigens, staphylococcal antigens, and sweat, all of which are known to be complicating factors in atopic dermatitis. Indeed, papain, a cysteine protease from papaya, causes occupational asthma [67]. A cysteine protease, papain or Der p1, directly induces thymic stromal lymphopoietin (TSLP) and IL-33 secretion from lung epithelial and stromal cells [68-70], activation of dermal basophils [71], dendritic cells [72, 73], and innate lymphoid cells (ILCs) [68], and subsequent Th2-responses in animal experimental models. Cysteine proteases hold central roles in the regulation of cell apoptosis [74]. Various evidence suggests that cysteine proteases are involved in inflammasome activation [75] and processing of the IL-1 family cytokines [21, 76] and TLRs [77]. However, the precise mechanism of how their protease activity contributes to activating target cells for the induction of the Th2-type immune response remains unknown [65, 78]. Th2 induces excessive discharge from the gut and respiratory tract, as well as itchy and oozy dermatitis. These responses are quite reasonable to eliminate harmful agents from the body. Therefore, one would be
tempted to speculate that the disorder of the homeostasis in cysteine protease activities in the skin induces the Th2-type immune response and subsequent eczematous skin lesions.

The molecular mechanism by which serine proteases directly induce the Th2-type immune response has been clarified through extensive efforts [63]. Activation of a receptor for serine proteases, protease-activated receptor 2 (PAR2), is involved in the Th2 immune induction through TSLP expression. Loss-of-function mutations in the SPINK5 gene, encoding serine protease inhibitor LEKTI, result in Netherton syndrome, characterized by ichthyosis and persistent atopic eczema-like skin lesions in humans, and similar skin manifestations in mice.

4.2.2. Antimicrobial peptides

There are numerous AMPs identified in keratinocytes, including human β-defensins (HBD)-1, -2 and -3, cathelicidin (LL-37) and psoriasin (S100A7) [61]. In addition to their antimicrobial effects, they have roles in migration of granulocytes, dendritic cells, and T-lymphocytes and the activation of the innate and acquired immune responses.

The expression of AMPs is downregulated in atopic dermatitis, probably due to the effect of the Th2 cytokines IL-4 and IL-13 [61]. High levels of aberrantly processed forms of cathelicidin peptides contribute to the increased inflammation in the rosacea skin [61]. In psoriasis, as mentioned in the preceding section, cathelicidin self-DNA complexes promote activation of TLR9 on pDCs in the dermis, resulting in enhanced cutaneous inflammation that contributes to its pathogenesis [61].

Thus, the levels of AMPs expression are related to the specific states of cutaneous inflammation. However, expressions of a considerable part of AMPs can be modified by the innate and acquired immune response [61]. Therefore, disorder of the AMPs in several inflammatory skin diseases might not be a primary, but a part of the secondary responses while AMPs can be involved in the persistence of the diseases.

4.2.3. Other mediators
ROS are constantly produced in the skin, because skin is exposed to oxygen from outside and the oxygen is activated by light [58]. ROS have potent inflammation-inducing and immunomodulatory properties. ROS provide an important host defense mechanism against microbial invasion, and therefore, are probably involved in the pathogenesis of inflammatory skin diseases. Thus, ROS are a potential target of preventive and therapeutic drugs for inflammatory disorders as well as ischemic diseases and cancers. However, despite a great deal of descriptive information, the primary role and its defect in ROS regulation in cutaneous inflammation is poorly understood.

It is well known that prostanoids are abundantly produced and that the prostanoid receptors are highly expressed in the skin [79]. The balance of prostanoid production and receptor expression has been revealed to be important for immune homeostasis in the skin. Recently, we reported that prostaglandin (PG) D$_2$ is produced in the lesions of eosinophilic pustular folliculitis [80]. The metabolites of PGD$_2$ induce sebocytes to produce eotaxin-3, which leads to infiltration of eosinophils. Thus, the disorder in PGD$_2$ metabolism might be a primary defect in this disease and would explain the successful treatment of this disease by indomethacin, a cyclooxygenase inhibitor.

Some defects of barrier components lead to selective susceptibility to specific pathogens, such as in epidermodysplasia verruciformis caused by mutation in the $EVER1/TMC6$ or $EVER2/TMC8$ genes [81]. Patients with epidermodysplasia verruciformis have persistent infection with human papilloma viruses although the precise role of EVER proteins remains unknown.

5. Conclusion

The development of the host defense system is essential in the phylogeny of each organism. The components of the barrier, each of which has a unique role, are ready to protect an organism. The best-suited response of the innate and acquired immunity is elicited upon infections and dangers in order to survive them. On a crisis of the cell, the fundamental response at the transcriptional level in the cell is very limited (Figure
1). However, the innate immune system amplifies these cues and the acquired immune system translates the signals most effectively (Figure 2). A defect at the specific site among these three layers of the host defense system of the skin can induce an inflammatory skin disease with unique manifestation, which partly simulates the actual protective response against infections and dangers (Table 1).

One can classify the inflammatory diseases in the skin and other organs more comprehensively using an algorithm to understand the host defense system as three layers, and through consideration of the original purpose of the inflammation type and a primary defect among three protective layers in the disease (Figure 3). First, one should suppose infectious diseases in the perspective of inflammation induced by damages for the organs. Next one should consider primary defects in the acquired immunity, innate immunity and barrier in this order, which is opposite to the functional order, because of effectively differential diagnosis. Defective functions of the functionally preceding layer usually cause disorders in the following layers, although the acquired immune response affects innate immunity and barrier functions in some instances. If one cannot attribute the ongoing inflammation in the body to any primary defects in all the three layers, one could consider a proper response to artificial or iatrogenic disorders, such as drug adverse effects and graft-versus-host disease (GVHD), or endogenous disorders that simulate external dangers, such as gout.

This new algorithm would also be effective to investigate the function and kinetics of the specific molecule and cell subsets in a disease, by focusing on the interaction of each layer of the defense system.

6. Acknowledgements

This work was supported in part by the JSID’s Fellowship Shiseido Award and the Japan Society for the Promotion of Science (JSPS) KAKENHI.
References:


Figure legends:

**Figure 1:**
Representative signaling pathways in epidermis involved in the defense system. TLR signaling pathways are mainly composed of MyD88-TRAF6-IKK-NF-κB pathway that induces transcription of NF-κB response genes, such as TNF, IL-6, IL-1β and TSLP, and TRIF-TRAF3-TBK1-IRF3/7 pathway that induces transcription of type-I IFNs and IL-33. MyD88-dependent transcription is also regulated by a part of MAPK pathway. ASK1, apoptosis signal-regulating kinase 1; Erk, extracellular-regulated kinase; HIF1, hypoxia-inducible factor-1; IFN, interferon; IL, interleukin; IRF, interferon regulatory factor; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MKK, MAP kinase kinase; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa B; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; TAK1, transforming growth factor beta-activated kinase 1; TBK1, Tank-binding kinase-1; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRAF, TNF receptor-associated factor; TRIF, TIR-domain-containing adapter-inducing interferon-beta; TRX, thioredoxin; TSLP, thymic stromal lymphopoietin.

**Figure 2:**
Speculated relation among primary triggers from infectious agents and external dangers, transcription factors, type of immune responses, and resulting diseases in skin. TSLP is a NF-κB response gene and is involved in the induction of Th2 response, typically resulting spongiotic dermatitis. Both expression of NF-κB response genes and Type-I IFNs are involved in interface/necrotic dermatitis. Psoriasiform dermatitis induced by Th17 response seems to be NF-κB independent since epidermis-specific depletion of IKKβ presents psoriasiform dermatitis with IL-24 expression from epidermis although precise pathway has not been fully investigated. IFN, interferon; IL, interleukin; IRF, interferon regulatory factor; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; TSLP, thymic stromal lymphopoietin.

**Figure 3:**
Algorithmic method to classify the inflammatory skin diseases by means of the defects in the three layers of the defense system in skin. AMPs, antimicrobial peptides; GVHD, graft-versus-host disease; ROS, reactive oxygen species.

Table 1:
Proposed classification of inflammatory skin diseases. AIDS, acquired immunodeficiency syndrome; CAPS, cryopyrin-associated periodic syndrome; CMCC, chronic mucocutaneous candidiasis; DAMPs, damage-associated molecular patterns; GVHD, graft-versus-host disease, IRAK4, IL-1 receptor-associated kinase 4; MyD88, myeloid differentiation primary response 88; PAMPs, pathogen-associated molecular patterns; PRP, pityriasis rubra pilaris; PRR, pattern-recognition receptor; ROS, reactive oxygen species; SCID, severe combined immunodeficiency; SLE, systemic lupus erythematosus.

Box
Core of the new classification of inflammatory skin diseases.
Box: Core of the new classification of inflammatory skin diseases:

- Inflammation is defined as a series of protective and regenerative responses of the body.
- Host defense system of the skin is composed of a barrier, innate immunity and acquired immunity.
- Three layers of the protection drive the most suitable response against infectious agents and external dangers whereas the basic response at the transcriptional level in the cell is very limited.
- Disorder of the specific layer of the host defense system of the skin can induce an inflammatory skin disease, which partly simulates the actual protective response against infections and dangers.
Inflammation

IFN-α/β, IL-33

Protection/Survival

TNF, IL-6, IL-1β, TSLP

Apoptosis

Survival

IL-24?
Protection | Scratch | Regeneration | Exfoliation
---|---|---|---
Dermatitis | Eczema | Irritation | Ringworm/psoriasis
Histologic changes | Spongiotic dermatitis | Interface/necrotic dermatitis | Psoriasiform dermatitis
Immune response | Th2 | Th0/Th1 | Th17
Cytokines | TSLP | IL-33? | IFN-α/β | IL-24
Epidermis | NF-κB | IRF3/7 | MAPK
Transcription factors | Cysteine proteases? | LPS | Cell death/oxidative stress | Lectins?
Triggers | | | |
Dermatitis?

Causative infection or dangers?

Yes

Infectious diseases:
- Bacterial, viral, fungal, etc

Others:
- Trauma, irritants, ultraviolet, etc
- Foreign body reaction, etc

No or Not primary

Defects in Acquired Immunity?

Yes

Immunodeficiency
Immunohyperactivity/Allergy
- Phylogenetically protective or Not
Autoimmunity
- General or Local

No or Not primary

Defects in Innate Immunity?

Yes

Innate immunodeficiency
Innate immunohyperactivity
- General or local
Innate autoimmunity
- General or local

No or Not primary

Defects in physical barrier
Defects in chemical barrier
- Proteases and protease inhibitors
- AMPs
- Prostanoids, ROS, etc

No or Not primary

Defects in Barrier?

Yes

Proper reaction:
- GVHD, drug eruption, etc
- Gout, etc
- Organ-specific autoimmunity?
<table>
<thead>
<tr>
<th>Category</th>
<th>Acquired Immunity</th>
<th>Innate Immunity</th>
<th>Barrier</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dangers</strong></td>
<td>Venoms and poisons</td>
<td>Physical damages, foreign body, dead cells</td>
<td>Any dangers and infectious agents</td>
</tr>
<tr>
<td></td>
<td>Toxic haptens and allergens</td>
<td>Cellular oxidative stress</td>
<td></td>
</tr>
<tr>
<td><strong>Infectious agents</strong></td>
<td></td>
<td>Bacteria, viruses, fungus and parasites</td>
<td></td>
</tr>
<tr>
<td><strong>Ligands</strong></td>
<td>Protein antigens, etc</td>
<td>PAMPs, DAMPs</td>
<td></td>
</tr>
<tr>
<td><strong>Sensors</strong></td>
<td>Lymphocytes and antibodies</td>
<td>PRRs</td>
<td></td>
</tr>
<tr>
<td><strong>Effectors</strong></td>
<td>Lymphocytes, antibodies</td>
<td>All cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Monocytes and granulocites</td>
<td>Monocytes and granulocites</td>
<td>Physical Barrier</td>
</tr>
<tr>
<td><strong>Deficiency</strong></td>
<td><strong>Immunodeficiency:</strong> SCID, AIDS, etc CMCC?</td>
<td><strong>Innate Immunodeficiency:</strong> MyD88 deficiency, IRAK4 deficiency, etc</td>
<td>Trauma, burns Atopic dermatitis?</td>
</tr>
<tr>
<td><strong>Hyperactivity</strong></td>
<td>Allergy: Allergic contact dermatitis Drug eruptions, GVHD Chronic eczema? Papullo-erythroderma?</td>
<td><strong>Autoinflammatory Disease:</strong> CAPS, etc Behçet’s disease Pyodermia gengrenoum</td>
<td><strong>Autoinflammatory Folliculitis:</strong> Acne? Pyodermas? <strong>Autoinflammatory</strong></td>
</tr>
<tr>
<td><strong>Qualitative disorder</strong></td>
<td><strong>Organ-Specific Autoimmune Disease:</strong> Pemphigus, Pemphigoid Alopecia areata</td>
<td><strong>Systemic Autoimmune Disease:</strong> SLE? Scleroderma?</td>
<td><strong>Innate Skin Autoimmunity:</strong> Psoriasis? PRP?</td>
</tr>
</tbody>
</table>