Epicutaneous sensitization with protein antigen

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Abstract

In the past few decades there has been a progressive understanding that epicutaneous sensitization with protein antigen is an important sensitization route in patients with atopic dermatitis. A murine protein-patch model has been established, and an abundance of data has been obtained from experiments using this model. This review discusses the characteristics of epicutaneous sensitization with protein antigen, the induced immune responses, the underlying mechanisms, and the therapeutic potential.

Introduction

It was long considered very difficult, if not impossible, for atopic allergens to penetrate normal skin because the skin was considered to be impermeable to high-molecular-weight, hydrophilic proteins. However, this notion has always been challenged by clinical observations. Contact urticaria occurs a few minutes after putting on latex gloves in a latex-sensitized patient. Protein contact dermatitis, observed in butchers, can be reproduced using meat proteins. Atopic patch tests to protein allergens are positive (clinical dermatitis), even when carried out on normal skin of atopic dermatitis (AD) patients. Moreover, proliferative responses of memory T cells to allergens are preferentially detected in cutaneous lymphocyte antigen (CLA) subset, whereas the percentage of type 1 cytokine-producing cells is decreased. The last two studies suggest that these cells were primed or reactivated in the cutaneous immune system. In recent years, the demonstration that mutation in the flaggrin gene is a predisposing factor for AD has convinced many investigators that epicutaneous (EC) sensitization with protein antigen (Ag) is one of the important routes of allergen sensitization for AD.

Methodology of murine models of EC sensitization with protein Ag

Our laboratory developed a murine protein-patch model to study EC sensitization with protein Ag approximately 20 years ago. In this model, ovalbumin (OVA) solution is first applied to a 1-cm² gauze on patches or discs in Finn chambers, which were applied to shaved backs without prior tape-stripping. The patches were renewed either every day for 5 successive days or on Day 4. Our method emphasizes mimicking physiologic conditions with repeated exposure, without disruption of the skin barrier and without the use of adjuvants. Subsequently, Spergel et al reported another EC sensitization model in 1998. Spergel et al also used a 1 cm patch of sterile gauze secured to the back skin, but with two modifications. First, they performed tape-stripping before application of the patch to disrupt the skin barrier. Second, one patch was placed for 1 week before being removed. Two weeks later, an identical patch was reapplied to the same skin site. Thus, one mouse had a total of three 1-week exposures to the patch separated by a 2-week interval. A number of researchers have since used the protein-patch model to study EC sensitization using different protein Ags, including allergens of atopic diseases, rubber latex Ag, autoantigens, parasite Ags, superantigens, toxins, and hapten-conjugated immunoglobulins.

The immune responses induced by EC sensitization with protein Ag

After establishment of the murine protein-patch model, immune responses induced by EC sensitization with protein Ag were...
explored. We first demonstrated that EC sensitization with OVA induced a predominant T helper 2 (Th2) and a marginal Th1 response with high IgE production in mice. EC sensitization with house dust mite Ag was also shown to elicit a Th2-dominant cytokine response. Strid et al. emphasized the importance of the route of immunization by comparing EC with subcutaneous immunization and showed that EC immunization with peanut protein generates a predominant Th2 response, whereas subcutaneous immunization elicits a predominant Th1 response. For Th17 cells, He et al. reported that EC sensitization with OVA induced a remarkable Th17 response. In contrast, we demonstrated that EC sensitization with OVA induced a modest increase in Th17 response. The discrepancy in the magnitude of the Th17 response might be explained by the use of tape-stripping before EC sensitization because in addition to removing the skin barrier, tape-stripping has been shown to induce epidermal inflammation, which might promote Th17 development. EC sensitization with protein Ag also generates regulatory T cells (Treg), which is evidence that EC immunization with an autoantigen induces Treg that prevents experimental allergic encephalomyelitis. EC immunization also induces T cell receptor αβ CD4+CD8+ double-positive Treg that inhibit contact hypersensitivity and experimental allergic encephalomyelitis. Finally, Th9, a new Th lineage, has been defined and we demonstrated that EC sensitization with OVA also induces a small number of Th9 cells. For CD8 T cells, surprisingly, cross-priming with an soluble protein antigen introduced epicutaneously generates cytotoxic T cell (Tc1), but not Tc2 cells.

Mechanisms of EC sensitization with protein Ag

The role of the skin barrier

Protein Ag sensitization via the EC route needs to first overcome the epidermal barrier. The barrier function of the skin has the following three elements: the stratum corneum (air-liquid barrier); the tight junction (liquid-liquid barrier); and the Langerhans cell (LC) network (immunologic barrier). Skin barriers face harsh challenges in modern lifestyles with regular use of soap in bathing and long-term exposure to air conditioned or heated environments. This might account, in part, for the progressive increase in atopic diseases in industrialized countries in the past few decades. For the stratum corneum, filaggrin mutations have been repeatedly demonstrated to be a predisposing factor for AD. An altered stratum corneum barrier, enhanced allergen sensitization, and spontaneous development of dermatitis have all been demonstrated in filaggrin-deficient mice. Filaggrin loss-of-function mutations have further been shown to be associated with enhanced IL-1 expression in the stratum corneum of patients with AD and in filaggrin-deficient mice. The contribution of a stratum corneum deficiency to EC sensitization with protein Ag is further supported by the clinical observation of an association of genes controlling desquamation, such as serine protease inhibitor and stratum corneum chymotryptic enzyme, with the development of AD. For tight junctions, a polymorphism in the claudin-1 gene, which is one of the major components of epidermal tight junctions, was recently reported to be associated with AD. Interestingly, cutaneous barrier perturbation can not only stimulate proinflammatory cytokine production in the epidermis, but also induce LC activation with the dendrites penetrating the tight junction barrier and facilitating capture of Ag by LCs. Thus, disruption of the skin barrier can enhance EC sensitization with protein Ag by allowing Ag penetration, inducing inflammation, and triggering LC activation. For the quality of induced immune responses under a skin barrier deficiency, it appears that all of the Th1/Th2/Th17 responses are increased and no polarization of Th1/Th2/Th17 responses occurs.

The role of cytokines

The gene knockout mouse system has been used to investigate the elements and the associated contributions in EC sensitization with protein Ag. Because the predominant immune response induced by EC is the Th2 response, it was first hypothesized that Th2 cytokines, especially interleukin (IL)-4, might be essential. However, Herrick et al. demonstrated that IL-13, but not IL-4 is necessary but not simply sufficient for epicutaneously-induced Th2 responses to soluble protein antigen. He et al. further showed an exaggerated Th17 response after EC sensitization with OVA in IL-4/IL-13 double knockout mice. Laouini et al. also demonstrated that IL-10-deficient mice have a decreased Th2 and increased Th1 response to EC sensitization, and suggested that dendritic cells (DCs) and T cells participate in IL-10 skewing of the Th2 response. IL-21R-deficient mice have been shown to have impaired Th1 and Th2 responses after EC sensitization, which is likely to be due to defective mobilization of skin DCs to draining lymph nodes. In contrast, SMA3-deficient mice exhibit higher levels of OVA-specific IgE, but not IgG2a after EC sensitization with OVA than wild-type controls, implying that transforming growth factor (TGF)-β-SMAD3 signaling has a suppressive effect on the induced Th2 response. Recently, we demonstrated that IL-9 can promote Th2 responses induced by EC sensitization with OVA. Taken together, the predominant Th2 response induced in EC sensitization with protein Ag is promoted by IL-13, IL-10, IL-21, and IL-9, but suppressed by TGF-β.

The role of Toll-like receptor ligands and other innate elements

The effects of various Toll-like receptor TLR ligands on the Th responses induced by EC sensitization with protein Ag have been investigated. TLR2 is important for the Th1 response, but not the Th2 response, because (interferon) IFN-γ production (Th1 response) by splenocytes after restimulation and anti-OVA IgG2a Ab levels are impaired in TLR2-deficient mice, whereas the Th2 cytokine production and anti-OVA IgE Ab level are comparable to wild-type controls. In contrast, the Th1 and Th2 responses induced by EC sensitization with protein Ag is TLR4-independent. Ptak et al. further showed that EC sensitization with protein antigen in the presence of TLR4 ligand induced constrast suppressor cells that can reverse skin-induced suppression of Th1-mediated contact sensitivity. For CD8 T cells, topical co-administration of TLR9 ligand with protein Ag promotes the generation of cytotoxic T cells in EC sensitization, whereas ligands for TLR2, TLR3, or TLR4 have no effect. Overall, the predominant Th2 response induced in EC sensitization with protein Ag is TLR4-independent; however, TLR9 ligand can promote cross-priming in EC sensitization to CD8 T cells.

Other mediators in innate immunity have been reported to modulate the immune responses induced by EC sensitization with protein Ag. Cyclooxygenase-2 suppresses the induced Th2 response, whereas agonizing prostaglandin D2 receptor (CRTH2) has no effect on the induced immune responses as evidenced by the observation that CRTH2-deficient mice showed comparable responses with wild-type mice. Macrophage migration inhibitory factor (MIF)-deficient mice have decreased Th2 and increased Treg production after EC sensitization when compared with wild-type controls. Galectin-3 deficiency results in a decreased Th2 response and a Th1-polarized response. C3-deficient mice exhibit an exaggerated Th2 response, whereas C3-deficient mice have impaired Th1 and Th2 responses. Moreover, skin
The role of DC subsets

The roles of various DC subsets play in EC sensitization with protein Ag. Ag have been studied using Langerin-diphtheria toxin receptor knock-in mice and Langerin-diphtheria toxin A transgenic mice. It has been shown that LCs initiate EC sensitization with OVA and induce Th2 immune responses via thymic stromal lymphopoietin signaling. LCs are also critical for induced Th2 responses when using exfoliative toxin as an antigen. In contrast, probably because EC sensitization induces a Th2-predominant response, there are still no langerin+ dermal DC-deficient mice available, the roles that langerin+ dermal DCs and langerin+ dermal DCs play in inducing Th responses in EC sensitization remain obscure. With respect to CD8 T cells, recent studies have demonstrated that langerin+ dermal DCs have an essential role in cross-presentation of EC sensitization and LCs are dispensable. Most importantly, Derp 2 has been shown to have structural and functional mimicry of the major dust mite allergen, Der p1, the major allergen in house dust mite, facilitates transepithelial allergen delivery by disruption of tight junctions, with occludin serving as a functional target of peptidase activity. The effects of natural allergens on DCs have been investigated. The proteolytic activity of the major dust mite allergen conditioned DCs to produce less IL-12, thus directing DCs to induce Th2 development. Aqueous birch pollen enhances the migratory capacity of DCs and prepare DCs for Th2 attraction by modulating the expression of chemokines and chemokine receptors. House dust mite extract stimulated a robust production of cysteinyl leukotrienes from pulmonary DCs via dectin-2 recognition. Natural allergens also have effects on epithelial cells. Natural allergens induce proinflammatory cytokines from respiratory epithelial cells and delayed epidermal permeability barrier recovery, both mediated by activation of PAR-2. Most importantly, Derp 2 has been shown to have structural and functional homology with MD-2 (a protein associated with TLR4 on the cell surface), facilitating signaling through direct interaction with the TLR4 complex, and reconstituting LPS-driven TLR4 signaling in the absence of MD-2. Thus, house dust mite extract has Th2 adjuvant and tolerogenic activities in intranasal sensitization. Because EC sensitization with protein Ag has been shown to be TLR4-independent, whether or not all or part of the above-mentioned mechanisms operate in EC sensitization needs further investigation and remains largely unknown at present.

Establishment of murine models of AD, asthma and food allergy by EC sensitization with protein Ag

Spergel et al first demonstrated that by thrice-repeating EC OVA patch application, an AD-like cutaneous inflammation could be induced. The cutaneous inflammation was characterized by infiltration of T cells, eosinophils, and neutrophils, and by local expression of mRNA for IL-4, IL-5, and IFN-γ. Later, by using IL-4, IL-5 and IFN-γ knockout mice, they showed that Th2 and Th1 cytokines play important roles in AD-like dermatitis. Spergel et al also extended the EC route as a sensitization method for asthma, showing a single exposure of EC-sensitized mice to aerosolized OVA induced eosinophils in the bronchoalveolar lavage fluid and airway hypersensitivity to intravenous methacholine. Later, He et al suggested that Th17 cells play an important role in driving airway inflammation after inhalation challenge. The investigation of underlying mechanisms of the progression from AD to asthma revealed that thymic stromal lymphopoietin overexpressed by skin keratinocytes is the systemic driver of this bronchial hyper-responsiveness. Akei et al established an experimental allergic rhinitis model by showing that EC aeroallergen exposure induces systemic Th2 immunity that predisposes to allergic nasal responses via a STAT6-dependent pathway. Akei et al also reported that EC antigen is a primer for experimental eosinophilic exposure esophagitis in mice. For food allergies, our group showed that food allergy (anaphylaxis) could be induced by EC. It has also been shown that EC exposure to peanut protein can prevent induction of oral tolerance and may even modify existing tolerance to peanuts. Thus, at least in the murine experimental system, primed T cells in EC sensitization with protein Ag can traffic to develop pathologic changes in other organ systems.

EC sensitization with protein Ag as a novel method of allergen-specific immunotherapy

Because EC sensitization with protein Ag induces Th2 and Treg cells, the effects on established immune responses or ongoing pathologic conditions have been explored. In 2006, Strid et al first reported that EC immunization converts subsequent and established antigen-specific Th1−to Th2-type responses in mice. Subsequently, they demonstrated that EC immunization with type II collagen inhibit the onset and progression of murine chronic collagen-induced arthritis. Senti et al first developed EC immunotherapy in humans. By applying four allergen-containing patches to the patient’s upper arm at weekly intervals, they reported that patients receiving EC immunotherapy showed significantly decreased scores in a nasal provocation test in 2009. Later, a French company (DBV Technologies) developed a new EC delivery system, called Viaskin, that could promote diffusion of allergens toward skin without any skin preparation or adjuvant. DBV Technologies developed a new regimen to apply the EC delivery system for 48 hours/week for eight times and showed that in mice sensitized to the four allergens tested, EC immunotherapy was as efficient as subcutaneous immunotherapy. Dupont et al reported that EC immunotherapy was safe, well tolerated, and exhibited a clear trend toward clinical efficacy for children with cow’s milk allergy. EC immunotherapy could also block the allergic esophago-gastro-enteropathy induced by sustained oral exposure to peanuts in sensitized mice. The underlying mechanisms might be that after protein Ag is applied repeatedly with the EC delivery system, specific local and systemic responses are downregulated in association with the induction of regulatory T cells.

Conclusions and future perspectives

At present, we have learned that EC sensitization with protein Ag is an important route for AD, which induces predominant Th2,
Epicutaneous sensitization with protein antigen

- **Protease activity of antigen ↑**
- **Skin barrier defect ↑**
- **IL-13 ↑ IL-10 ↑ IL-21 ↑ IL-9 ↑ TGF-β ↓**
- **MIF ↑ Gelectin-3 ↑ TSLP ↑ Cox-2 ↓ C3a ↓**

Figure 1 Summary of epicutaneous sensitization.

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Marginal Th1, and significant Th17 and Treg responses. Some modulating factors have been identified, including cytokines, T cell receptor signaling, complement, and lipid mediators (summarized in Figure 1). However, an understanding of the mechanisms of EC sensitization with protein Ag is just beginning. Some basic questions remain. For example, why does EC sensitization with protein and hapten induce such different responses? Why does EC sensitization with protein Ag induce differential responses in human atopy and nonatopy? EC sensitization with protein antigen and with hapten are major routes for atopic dermatitis and allergic contact dermatitis, respectively. Haptenes are small chemicals that penetrate skin easily and need to react covalently with carrier proteins before being recognized as immunogens by the immune system. Protein antigens are large hydrophilic molecules that penetrate skin with difficulty. Thus, knowledge obtained from murine contact hypersensitivity model and human allergic contact dermatitis could not be applied to EC sensitization with protein antigen directly. The clinical importance as well as its therapeutic potential emphasizes the demand for EC sensitization with protein Ag to be intensively investigated.

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