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Patching it together: epicutaneous vaccination with heat-labile *Escherichia coli* toxin against birch pollen allergy

S. S. Killingbeck, M. Q. Ge, and A. Haczku

University of California, Davis, CA, USA haczku@ucdavis.edu

In immunotherapy, a person's immune system is utilized to induce or suppress specific immune responses that are needed for the prevention or treatment of disease. This requires the use of vaccines (biological preparations of disease-causing agents) that elicit a coordinated response by the adaptive and innate immune systems, with professional antigen-presenting cells such as dendritic cells playing a crucial role. Successful immunotherapy results in the induction of broad and specific immune responses involving B and/or T cells depending on vaccine design (1). Vaccine development is considered among the greatest achievements in the history of medicine (2); they can be applied in the treatment of allergic and autoimmune diseases, organ transplantation, chronic infection, and even cancer (3, 4). Allergen-specific immunotherapy (AIT) entails administration of increasing doses of allergens in an attempt to restore tolerance and reduce allergic symptoms, once a patient has identified the root cause(s) of their allergy. The allergen can be given intramuscularly, subcutaneously, sublingually, orally, or intralymphatically. The doses of allergen are increased as the patient's tolerance grows over time (5). AIT has been used for IgE-mediated allergic diseases since the early 1900s (3, 6–11), although it remains fraught with problems (3, 6–11). For example, it is difficult to produce a strictly regulated composition for the allergenic compounds and adjuvants used, or to establish consensus in regard to the doses, intervals, and length of application. The danger of anaphylaxis occurring during administration of AIT also remains high. When successful however, AIT treats and prevents development of allergic asthma, rhinitis, and venom-induced anaphylaxis and was demonstrated to prevent sensitization with new allergens for up to 12 years (3, 12). Therefore, the quest to develop safer and more effective AIT continues today.

Induction of allergen tolerance requires a decrease in IgE and increase in allergen-specific IgG levels (13). In this issue of *Allergy*, Cabauatan et al. describe birch pollen (rBet v 1)-specific AIT in a guinea pig model, using a patch delivery system (originally developed for epicutaneous vaccination against traveler's diarrhea). They chose guinea pigs to work with because their skin has similar features to human skin and the patch delivery system of the same size is used for humans (14). To enhance IgG levels, Cabauatan and colleagues applied heat-labile *Escherichia coli* (*E. coli*) toxin (LT) as adjuvant together with the rBet v 1 birch

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Sarah Killingbeck has provided the first draft and the illustration for the paper. Moyar Q. Ge commented and edited the manuscript. Angela Haczku edited and finalized the manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

allergen in the skin patch and compared the effects with a traditional alum-adsorbed rBet v 1 vaccine given subcutaneously. Patch vaccination with a high allergen dose induced relevant allergen-specific IgG responses (similar to that of the subcutaneous immunization) but only when the patch contained LT. These responses were detected on day 43 after only three vaccinations, even though outbred guinea pigs might be poor responders for the rBet v 1 allergen.

During successful allergen-specific immunotherapy, changes in IgG and in particular in IgG4 can result in a 10- to 100-fold reduction in the allergen-specific IgE/IgG4 ratio (15). IgG4 is a nonproinflammatory immunoglobulin that is uniquely capable to form bispecific and functionally monovalent antibodies through Fab arm exchange (16), has low affinity for activating Fc γ receptors, and cannot activate complement. Allergen-specific IgG4 protects against allergic responses by competitively sequestering the allergen from IgE binding (13, 17, 18). It is significant therefore that the LT adjuvant in this paper was essential for the induction of allergen-specific IgG. According to the authors, in an unpublished clinical study epicutaneous application of a high dose of rBet v 1 (without adjuvant) only induced T-cell responses but no relevant allergen-specific IgG. The authors here demonstrate that sera derived from the guinea pigs with robust *rBet v 1*-specific IgG responses inhibited the binding of allergic patients' IgE to the *rBet v 1* allergen. This elegant result indicated that patch vaccination using LT as adjuvant can induce allergen-specific blocking IgG.

By changing the ratio of allergen-specific IgE and IgG, effective AIT should desensitize the high-affinity IgE-receptor (Fc ϵ RI)-bearing mast cells and basophils, decrease the numbers and activity of eosinophils and basophils in the circulation and mast cells in mucosal allergic tissues, and induce regulatory T- and B-cell activation (reviewed in (4)). To assess the clinical significance of the findings of Cabauatan and colleagues, it would be important to verify whether allergen-specific patch vaccination with LT would induce these changes.

The heat-labile enterotoxigenic *E. coli* enterotoxin (LT) contains an 'A' and a pentameric 'B' subunit (AB₅). The highly enterotoxic A subunit has ADP-ribosyltransferase activity. It activates the Gs α component of adenylate cyclase leading to elevation of intracellular cAMP, activation of cAMP-dependent PKA, and inhibition of NF- κ B-dependent transcription of proinflammatory cytokines (such as TNF- α). The A subunit is delivered into the cell by the nontoxic B pentamer subunit. Given the toxic nature of the A subunit, for vaccination purposes only the engineered B subunit and various mutant derivatives are used. The main receptors for LT are the ubiquitous gangliosides, but it also binds TLR-2 on antigen-presenting cells (reviewed in (19–21)). While cholera toxin (another AB₅ member) has Th2-inducing and immunosuppressive activities, LT induces a Th1 mucosal immune response. Skin-patch vaccines containing LT have been recently tested in a large clinical trial for traveler's diarrhea. Although it did not prevent diarrhea, the skin-patch vaccine induced a strong IgG response in volunteers (22) indicating high immunogenicity. In addition to its proinflammatory and immunogenic properties, when coadministered with different antigens, LT also markedly enhances the antigen-specific immune response (against the coadministered antigens) indicating adjuvant effects (reviewed in (19–21)). However, neither the proinflammatory properties nor the high immunogenicity of this molecule can explain its remarkable 'adjuvanticity'. It is speculated that this effect is due to induction of MHC-class

II expression on antigen-presenting cells, cell clustering, and delay/arrest of T-cell proliferation (23).

A limitation of the study presented here is a lack of data on the structure and mechanism of action of the LT adjuvant used. Based on the literature showing that LT-treated dendritic cells had a slower rate of antigen endocytosis, the authors reasoned that prolonged retention of intact antigen on the cell surface may allow B cells to recognize the bound antigen by their immunoglobulin receptors. This was supported by their findings that IgG induced by patch vaccination only recognized the intact, folded, and complete rBet v 1 allergen, but not unfolded recombinant rBet v 1 fragments. By contrast, IgG antibodies induced by immunization with alum-adsorbed rBet v 1 also recognized unfolded rBet v 1 fragments and showed a broader cross-reactivity to rBet v 1-related pollen and food allergens. These data emphasize the importance of dendritic cells in mediating 'adjuvanticity'.

Acting as the crucial link, dendritic cell targeting is a major vaccination strategy against many diverse pathogens. In the skin, these cells are the prime initial mediators of protection. As they become activated upon exposure to allergen, they migrate to the draining lymph nodes (Fig. 1). This in turn activates the adaptive immune system, inducing a T-cell cytokine profile that favors the production of large amounts of IgG antibodies by plasma cells that are able to block allergy-causing IgE molecules specific for the same allergen (24). Skin-resident antigen-presenting cells therefore act as a reservoir of protection and are robustly induced upon vaccination with adjuvants.

In addition to the use of novel adjuvants, current immunotherapy research is focused on enhancing antigen-presenting cell function in a number of different ways. For example, in a design targeting dendritic cell subsets, recombinant proteins with the vaccine epitope in question were fused to GM-CSF (25). To investigate alternate routes of administration, allergen tolerance was recently induced after intralymph node injections of a molecular allergen translocation-Fel d 1 vaccine, mediated by increased cellular internalization of the allergen, activation of inflammasome, and generation of allergen-specific peripheral T-cell tolerance (26). Adoptive transfer of antigen-specific T cells to allogeneic hematopoietic transplant recipients is also being explored in human studies and holds the promise of vaccinating against aspergillosis (2). Other potential future strategies employ microspheres made of biodegradable polymers containing the epitope of the allergen in question, given to maximize delivery to antigen-presenting cells. This method has a specific advantage in that it is designed to induce both B- and T-cell responses and lasting memory to certain immunodominant epitopes (27). The novel patch delivery system presented here is a well-standardized technique and may be suitable to deliver epicutaneous AIT for various allergens in patients. Patch vaccination is an attractive needle-free alternative to allergy shots (offering the possibility of self-administration for patients), and it targets professional antigen-presenting cells (i.e., dendritic cells, Langerhans cells) residing in the skin (14).

These examples illustrate that the fields of vaccine design and allergen desensitization are constantly evolving. Recent advancements in AIT mechanisms and use in clinical practice were described in the practical allergy (PRACTALL) initiative report that was endorsed by both the European Academy of Allergy and Clinical Immunology and the American

Academy of Allergy, Asthma & Immunology (3, 12). In spite of all the advances made, safer and more effective AIT strategies are still needed, especially for patients with asthma, atopic dermatitis, or food allergy (3, 12).

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Figure 1.

E. coli enterotoxin (LT)-induced “adjuvanticity” for production of allergen specific IgG. 1. Naïve, epidermis-resident Langerhans cells become activated through LT:TLR-2 triggering that delays allergen+LT endocytosis. 2. CD11b⁺ and CD103⁺ (migratory) dendritic cells (DCs) are attracted and activated by Langerhans cells, upregulate MHC II, costimulatory molecules, and CCR7. 3. Activated DCs migrate to lymph nodes through CCR7:CCL19/21 interaction and carry the allergen and LT on their surface. 4. DCs activate naïve T cells through MHC-II and B cells through the B cell receptor. 5. Activated B cells form a germinal center. 6. The mature GC (~day 40) produces allergen-specific memory B cells and plasmablasts that release large amounts of allergen-specific IgG.