Generation of therapeutic antibody responses against IgE through vaccination[1]

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SPECIFIC AIMS

Our aim is to develop an allergen-independent active immunization strategy against one of the major challenges for modern day medicine, atopic allergy. We describe the design and production of a soluble, properly folded vaccine component and demonstrate the effect of this novel vaccine component focusing on efficacy, reversibility, and safety in a sensitized rat model.

PRINCIPAL FINDINGS

1. Development of a soluble vaccine component

The active component in the vaccine is a chimeric immunoglobulin E (IgE) -like molecule consisting of the constant domains 2, 3, and 4 of the ε-heavy chain (Cε2-Cε3-Cε4). The target domain in the vaccine, Cε3, is derived from the recipient species. The amino acid regions involved in binding to the high-affinity receptor for IgE, FcεRI, are spread over most of the Cε3 domain. Therefore, antibodies directed against the Cε3 domain are likely to have the ability to clear IgE from the circulation without causing cross-linking of the receptor and mast cell degranulation. The flanking domains (Cε2 and Cε4) in the vaccine are derived from an evolutionarily distant mammal. Mature T cells are normally tolerant toward self-antigens. B cells, however, may be self-reactive, but remain inactive in the absence of T cell help. The presence of a foreign carrier that contains foreign T cell epitopes enables activation of self-reactive B cells by carrier-specific T helper cells. Hence, the flanking domains in the vaccine have dual functions, acting both as structural support for proper folding of the Cε3 domain and to break T cell tolerance by providing foreign T cell epitopes.

A vaccine component consisting of rat Cε3 flanked by the Cε2 and Cε4 domains of a distantly related mammal—a marsupial, the American opossum—was expressed in mammalian cells and purified from the conditioned media on Ni^{2+}-chelating columns. SDS-PAGE analysis revealed that under reducing conditions, the recombinant vaccine component, named ORO (opossum-rat-opossum), migrates as a protein with a molecular mass of ~48 kDa, whereas under nonreducing conditions most of the protein migrates at ~97 kDa (**Fig. 1*B***). Like IgE, recombinant ORO appears to be produced as a disulfide-linked homodimer. Preliminary Biacore measurements revealed that the ORO vaccine component and its human counterpart (replacing rat Cε3 with human Cε3) bind to FcεRI, suggesting that the hybrid proteins fold properly and are nearby native in the target region. Since B cells recognize epitopes of native antigens, a nearby native conformation of the target region is probably required to evoke an antibody response with sufficiently high affinity for native IgE.

2. Modifications defining the requirements of the vaccine

Another objective of the present study was to define requirements of the self-derived target region with regard to size and sequence alterations. With this aim, two additional vaccine components were designed and expressed, OROO containing a part rat and a part opossum Cε3 domain and OMO containing a Cε3 domain derived from the heavy chain of mouse IgE. The purpose of OROO was to investigate whether the self-component in the vaccine could be reduced even further without loss in efficacy, whereas the aim of OMO was to study the influence of alterations in the self-sequence on the response to vaccination. The mouse Cε3 domain differs from the rat Cε3 domain in 21 positions, corresponding to 19% difference in sequence, and thus can be considered an altered or mutated target region.

Opossum IgE (the carrier) and rat IgE (the target self-protein) are distantly related, with a sequence identity of ~43%. Most of the sequence identity is concentrated around the cysteine residues involved in disulfide bridge formation and therefore may not be easily available as B cell epitopes. However, the possibility of generating cross-reactive, cross-linking responses remains. To study the response to the carrier and the possibility of cross-reactivity between distantly related species, recombinant proteins consisting of either the entire Cε2-Cε3-Cε4 region of opossum,

[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.01–0879fje; to cite this article, use *FASEB J.* (April 10, 2002) 10.1096/fj.01–0879fje.

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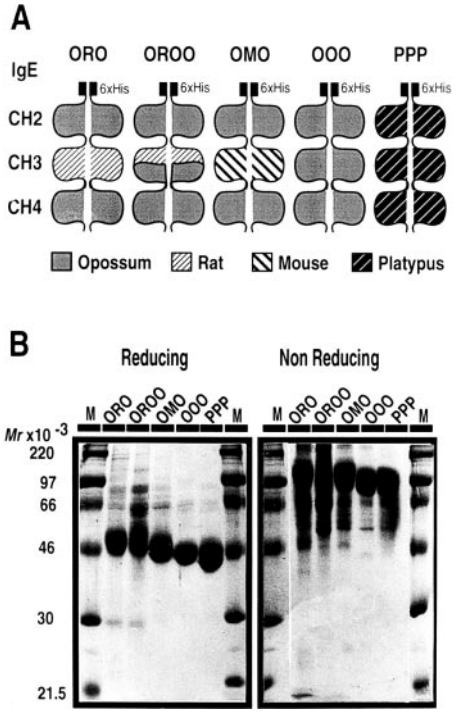

Figure 1. *A)* Schematic figures describing the structure of the various recombinant proteins. *B)* SDS-PAGE analysis of ORO, OROO, OMO, OOO, and PPP under reducing and nonreducing conditions. Aliquots of ~4 μg reduced protein or 8 μg nonreduced protein were loaded. Each band in the size marker, M, corresponds to ~0.75 μg protein.

OOO, or platypus, PPP, were designed. A schematic figure describing the various recombinant proteins and SDS-PAGE analysis under reducing and nonreducing conditions is shown in Fig. 1*A, B*.

3. Effects of vaccination

The most powerful antibody response against IgE was generated in ORO-vaccinated animals, and its effect on total IgE levels was striking (**Fig. 2*B, C***). In wk 14 of the treatment program, the geometrical mean of the IgE levels in ORO-vaccinated animals was 2.3 ng/ml vs. 24.0 ng/ml in the BSA-treated control animals, representing a 90% decrease in serum IgE levels in ORO-vaccinated animals. Hence, the antibody response generated against ORO can reduce serum IgE levels to a clinically significant extent. A visible effect of ORO vaccination was detected in the skin of ovalbumin-sensitized Wistar rats challenged with the allergen (data not shown). The apparent mast cell activity was significantly lower in ORO-vaccinated animals than BSA-treated control animals, indicating that the antibody response generated against ORO has a clinical effect on mast cell activity in the periphery.

Compared with ORO-vaccinated animals, the average anti-IgE antibody responses generated in the OROO- and OMO-vaccinated animals were considerably lower and no significant effects on total serum IgE levels were observed (Fig. 2*B, C*). These preliminary studies suggest that to obtain optimal efficacy the target region should be as large as possible without generating cross-linking responses and the species-specific sequence should not be altered.

4. Reversibility of vaccination

In modern societies, which provide environments almost devoid of severe parasitic infections, the IgE isotype might be redundant. Consequently, IgE depletion probably will not be accompanied by any adverse side effects. This assumption has already received support in studies of IgE-deficient humans and rodents. However, given our limited understanding of what future challenges our immune systems may face and the role IgE may play in these challenges, we thought it

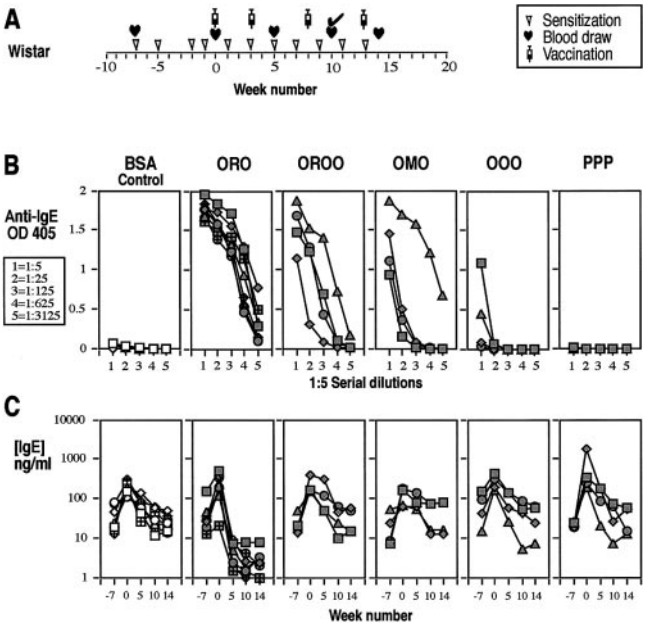

Figure 2. *A)* A time line describing the treatment program. To compare the efficacy of the various vaccines and to evaluate potential cross-linking activity in the immune response against the carrier, groups of ovalbumin-sensitized Wistar rats were vaccinated with ORO, OROO, or OMO or treated with OOO, PPP, or BSA (control). To obtain high titers of total and antigen-specific IgE before vaccination, all animals were sensitized by repeated injections of 3 μg ovalbumin over 7 wk. Sensitization resulted in an ~eightfold increase in serum IgE before vaccination. To mimic the deregulated control of IgE expression in atopic patients, sensitization was continued throughout the treatment program. All animals received an initial i.p. injection of 250 μg of recombinant protein or BSA in FCA, followed by three subsequent booster injections with the corresponding protein in IFA (100 μg in wk 3 and 25 μg in wk 8 and 13). ✔ The blood draw that was used to obtain the anti-IgE titration data presented in panel *B*. *B)* Therapeutic anti-IgE antibody titers. Comparative analyses among Wistar rats vaccinated with either ORO, OROO, or OMO or treated with OOO, PPP, or BSA presented in the form of titrations (1:5 serial dilutions) of sera from the blood draw in wk 10 of treatment. *C)* Total serum IgE concentrations in sera from the Wistar rats collected throughout the treatment program. The same symbol is used for the same animal in the anti-IgE (*B*) and total IgE (*C*) assays.

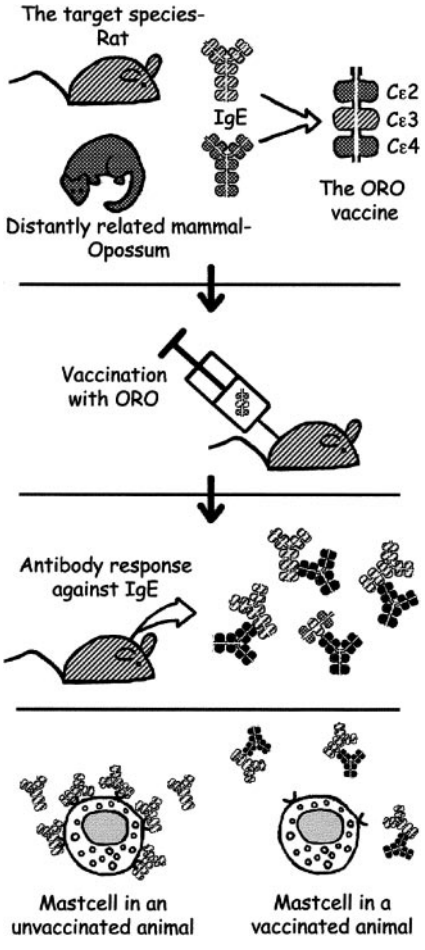

Figure 3. Schematic diagram illustrating the development of a novel vaccine component and the generation of therapeutic antibody responses against IgE on vaccination.

was important to demonstrate a return to the prevaccination state after discontinued treatment.

We found that by wk 45 of our study, ~11 months after the booster injection, anti-IgE titers were reduced by 89–99% (data not shown), suggesting that anti-IgE titers return to baseline after discontinuation of treatment. A similar reversibility has been shown for other modified self-proteins. Taken together, this suggests that therapeutic antibody responses against a self-protein are reversible with time and that the antibody response is not potentiated by the presence of the unmodified self-protein.

5. Potential cross-linking activity and safety of the vaccine

The risk of generating cross-linking antibody responses against either the self-component, Cε3, of the vaccine or cross-reactive and cross-linking responses against the carrier, opossum Cε2 and Cε4, was evaluated using a skin reactivity assay. No skin reactivity was detected on provocation with sera from Wistar rats vaccinated with OOO, ORO, or OROO whereas the positive control, a cross-linking mouse anti-rat IgE mAb caused extensive mast cell cross-linking and degranulation (data not shown). Thus, there is little or no mast cell activation potential in the sera from the vaccinated rats.

The fact that no cross-linking activity was detected in the anti-OOO sera suggests that the weak anti-OOO response detected in the ELISA is of limited biological importance (Fig. 2*B*), possibly due to low titers and/or low affinity for the antigen, native IgE. In conclusion, the cross-linking activity in response to ORO is minimal, which allows a vaccine with the entire Cε3 domain from self-IgE as the target region and the Cε2 and Cε4 domains from the distantly related IgE to be used safely in rats. The sequence identity between human and opossum IgE is 42% and 39% over the Cε2 and Cε4 domains, respectively. Hence, the sequence identity to opossum IgE is similar for rat and human IgE, which strongly suggests that opossum IgE will be safe to use as a carrier protein in humans as well.

CONCLUSIONS

IgE is a prime target in the development of preventive treatments for atopic allergy due to its central role in atopy and limited involvement in other biological reactions. In the present study, we demonstrate that active immunization against IgE can break tolerance and induce therapeutic antibody responses that can reduce circulating IgE to a clinically significant extent. Vaccination resulted in an 90% decrease in serum IgE and was accompanied by a clear reduction in mast cell reactivity in the skin of ovalbumin-sensitized rats.

Another approach to IgE depletion is passive immunization through repeated injections of a humanized monoclonal antibody against IgE. Several clinical trials have demonstrated that a substantial reduction in free serum IgE on passive immunization can result in beneficial effects in patients with allergic rhinitis or asthma. However, treatment with monoclonal antibodies may require infusions every 2–3 wk with a total annual dose 10,000- to 20,000-fold that required for active immunization. By reducing the amount of administered protein, the risk of antigen-induced immune complex disease and the cost of treatment should both be significantly reduced. Active immunization induces a polyclonal response, and the risk of dangerous immune complex formation is thereby minimal compared to that of long-term treatment with monoclonal antibodies.

We have successfully produced a soluble vaccine component that holds the receptor binding target region, Cε3, in a native or nearby native conformation. Variants of the vaccine containing partial or mutated self-components demonstrated significantly reduced efficacy. However, a vaccine containing the entire Cε3 domain from self-IgE appears to be safe to use as an antigen. No cross-linking activity was observed in the sera of vaccinated animals and the elicited response was reversible with time. Taken together with the additional clinical benefits of active immunization over passive immunization with a monoclonal, this suggests that the vaccine strategy has a potential to become a therapeutic method for humans. FJ