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EXPERT REVIEWS

Therapeutic vaccines against IgE-mediated allergies

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Lars Hellman
Department of Cell and Molecular Biology, BMC, Box 596, 751 24 Uppsala, Sweden
Tel.: +46 184 714 532
lars.hellman@icm.uu.se

Therapeutic vaccines targeting various self-molecules is an emerging field of vaccine development that is studied extensively in areas such as birth control, cancer, allergy and autoimmunity. Promising results have come from a number of animal studies and several vaccines are in advanced clinical trials. However, no vaccine is currently on the market. This review will focus on the progress in the development of vaccines against IgE-mediated allergies. Targets under investigation are the IgE molecule itself and several Th2 cytokines, that is, IL-4, -5, -13, -33, -18 and thymic stromal lymphopoietin. This review will also discuss new methods to enhance the immunogenicity of the vaccines and how this can contribute to more rapid progress in the field.

KEYWORDS: atopic allergies • cytokine • dermatitis • IgE • therapeutic vaccine

During the past few decades, allergic diseases have increased to almost epidemic proportions and estimates suggest that 20–30% of the total population in many Western countries is affected. The dominant forms are IgE-mediated allergies, which include hay fever, fur, dust mite and insect-venom allergies, extrinsic asthma and many types of food allergy.

Current treatment regimens are based primarily on the use of antihistamines, antileukotrienes, cromoglycates, β-agonists and on general anti-inflammatory compounds such as corticosteroids. Many of the affected patients have their disease under relatively good control with these drugs [1]. However, often they must be taken daily, or even several times a day, and patient compliance is a major problem [2]. The lack of compliance, especially for children, may lead to deterioration of the disease and, in the worst cases, after years of under medication, the disease may not be treatable. In addition, many patients do not have sufficient control of their disease. This is particularly relevant for patients with severe asthma and severe atopic dermatitis where new treatments are urgently needed [1].

Present strategies primarily focus on relieving symptoms and not treating the disease. New strategies should, therefore, also preferably act at a more early stage of the disease, before the release of histamine and other inflammatory mediators.

Several lines of research are presently ongoing to address these problems. The aims of this research are to target the early steps in the disease process and to have relatively infrequent administrations. The latter of which is primarily to increase compliance.

One interesting area of development is the use of humanized monoclonal antibodies (mAbs). Several such antibodies are already important tools in the clinic. A humanized anti-IgE antibody sold under the name Xolair® is the first antibody of this kind used for the treatment of allergies [3,4]. This antibody is primarily used in the treatment of severe asthma and rhinitis. The prime-efficacy parameters have been the reduction in corticosteroid use and the reduction in clinically significant asthma exacerbations. A 26–38% reduction in such exacerbations has been observed in several recent clinical trials [5]. However, the cost per patient and year has been estimated at US$15,000–44,000 [6], which makes it difficult for Xolair to serve as a general strategy for treatment of allergies. It will most likely be used primarily for patients with very severe symptoms and where other strategies have been shown to give little relief.

To overcome the problems of high cost and frequent administrations, an alternative is to trigger our own immune systems to produce the therapeutic antibodies by vaccination. This strategy has been named therapeutic vaccination. Such vaccines may only need to be administrated a few times a year, and owing to the low amount of recombinant protein in each dose, they are also relatively cost effective. For more than 100 years, vaccination has

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been the preferred and the most successful method of controlling most viral and many bacterial diseases. Successful vaccination programs have resulted in tight control or even the eradication of many of the major human infectious diseases. The years of experience in manufacturing, storing and administrating vaccines is also an important factor in the development process of new types of vaccines.

Therapeutic vaccines use the same basic concept as classical vaccines but differ in several important aspects. One important difference is that they are primarily aimed at therapy and only infrequently at prevention. By contrast, almost all classical vaccines are used for prevention.

Another important difference is that most therapeutic vaccines target self-molecules and not foreign antigens, such as viral, bacterial or parasite proteins. This adds several complex problems. Immune responses against self-antigens are normally heavily suppressed by tolerance-inducing mechanisms [7]. Therefore, in the development of vaccines against self-molecules, it is critical to use potent adjuvants and to try to mimic structural characteristics of viral and bacterial antigens to obtain as high antibody titers as possible in order to achieve good therapeutic effects.

Currently, no therapeutic vaccine is on the market. However, active research is ongoing and clinical trials have been conducted in several areas such as allergy, cancer, birth control and autoimmunity. Target molecules in allergic diseases are primarily IgE, IL-5, -4 and -13 (Figure 1) [7–12]. In cancer, the prime focus is on the breast cancer gene, *HER-2*, but several other antigens are also of interest [13]. In birth control, vaccines targeting sperm, oozyte zona pellucida, lutenizing hormone-releasing hormone (LHRH/GnRH, FSH) and human chorionic gonadotropin (HCG) are under development [14]. The HCG vaccine is the first of these vaccines that has undergone clinical Phase I and II trials in humans [15]. The prime target for vaccine development in the area of autoimmunity has been TNF-α [16]. The successes with the treatment regimens based on mAbs or soluble receptors is certainly a positive factor in the anti-TNF-α vaccine development process.

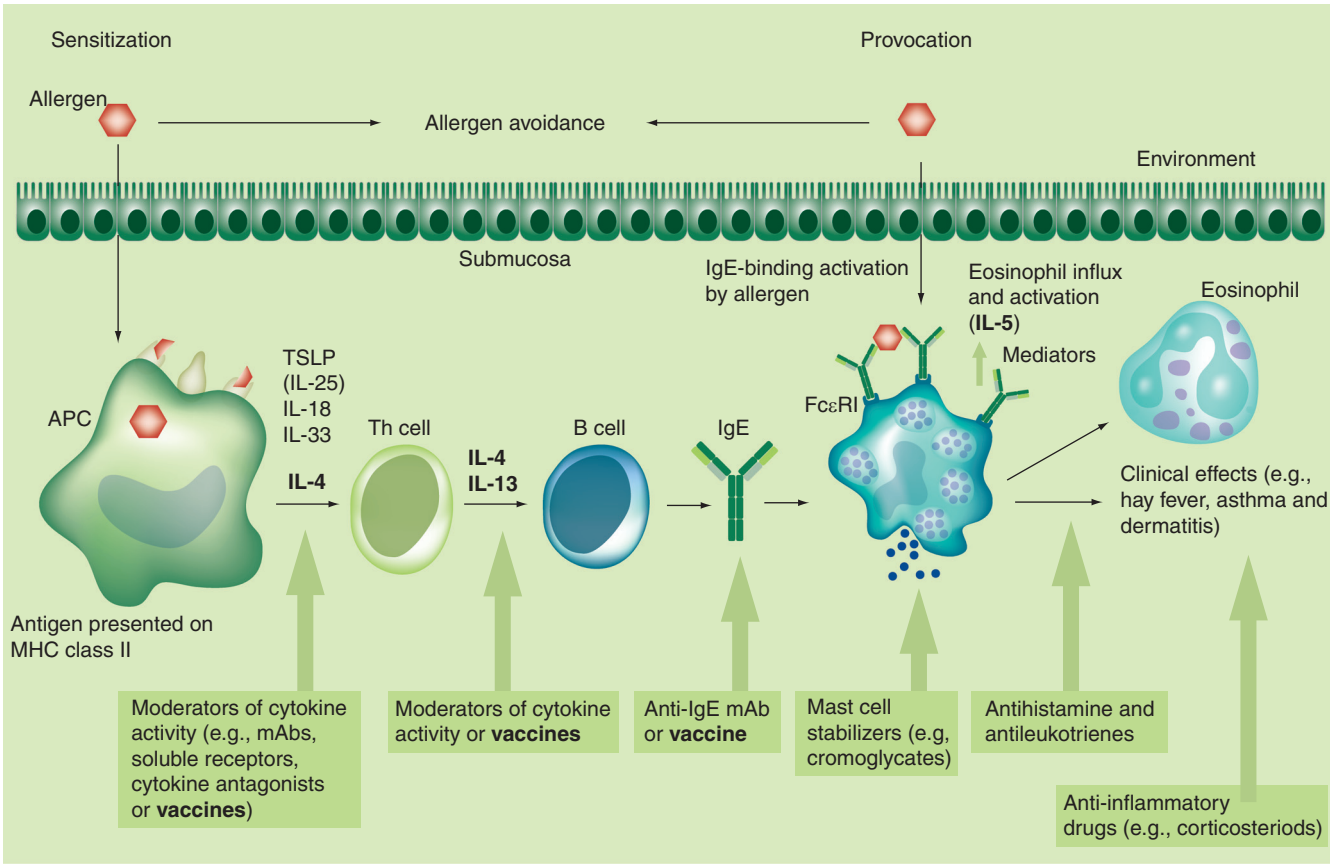

Figure 1. Allergic immune response – potential targets for intervention and intervention strategies. The different cell types and how they actively participate in the sensitization and activation phase of an allergic reaction is depicted. The different cytokines and other regulatory and effector molecules are also listed and they are listed at the site in this process where they are primarily involved. At the bottom of the figure there are listings of potential intervention strategies, where vaccines are marked in bold.
APC: Antigen-presenting cell; FcεRI: High-affinity receptor for IgE; mAb: Monoclonal antibody; TSLP: Thymic stromal lymphopoietin.
Modified from [74].

The following sections will primarily describe results from vaccines in the area of IgE-mediated allergies. Results from other areas will be discussed mainly when there are implications for the entire area of therapeutic vaccines. In the area of IgE, almost all data originate from this author's own laboratory and; owing to our own direct experience in the field, the description will be slightly more detailed.

Therapeutic vaccines: mechanism of action

As mentioned previously, the majority of therapeutic vaccines under development use the immune system to modulate the levels of various self-molecules. However, we are normally tolerant to all self-proteins and it should, at least in theory, be very difficult to vaccinate against a self-protein. In general, this is true and vaccination with unmodified self-proteins almost never results in an anti-self response. However, there are ways to circumvent or break tolerance.

Autoreactive B cells are present in the circulation at low levels. Generally these B cells do not expand or cause any harm, primarily due to the lack of T-cell help. By contrast, self-reactive T cells are either clonally deleted in the thymus or anergized (inactivated) in the periphery. If, however, a self-antigen is covalently coupled to a foreign protein of any kind – fungal, bacterial, viral, plant or nonself animal species – T cells specific for carrier epitopes (the non-self-protein) are recruited and activated (Figure 2). These T cells have the capacity to activate autoreactive B cells directed against the same antigen. Thus, the injection of a self-antigen coupled to a foreign carrier triggers an antibody response against both the carrier and the self-protein (Figure 2). If the immune response is strong enough, these antibodies have the capacity to reduce the level of the target protein. This phenomenon has been documented in several model systems, such as the vaccination against Her-2 (a tumor antigen), TNF-α (involved in inflammatory disease), IgE, IL-5, IL-4 and IL-13 (allergic disease) [7–13,16–18].

However, it is much more difficult to vaccinate against self- than non-self-proteins and we need to use all possible methods to enhance the immunogenicity. This can be achieved by the use of strong adjuvants and the optimization of the antigen structure.

The selection of target molecules is also of utmost importance. Targets with high *in vivo* levels are difficult to vaccinate against, owing to the strong tolerizing effect induced by the antigen. Antigens that are only present in low quantities or those that appear very transiently, such as cytokines or other regulatory molecules are, therefore, the most suitable targets.

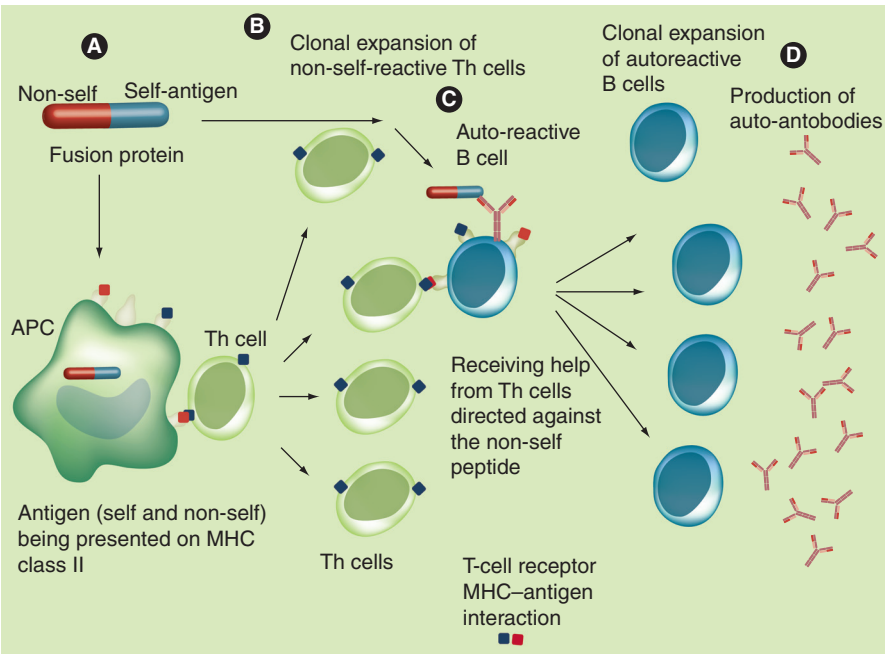

Figure 2. Therapeutic vaccination. (A) The fusion protein is taken up by APCs, and peptides from both the self-antigen and fusion partner are presented on the cell surface by MHC class II. **(B)** Non-self peptides are recognized by T cells, leading to expansion of the activated T cells. **(C)** A small number of auto-reactive B cells are always present in the circulation. These B cells selectively take up the fusion protein and hence present both the self-antigen and the foreign peptides on MHC class II. **(D)** The non-self peptides presented by the autoreactive B cells are recognized by the activated T cells, which stimulate the autoreactive B cells to expand and initiate an immune response against the self-antigen. APC: Antigen-presenting cell; Th: T helper.

Potential target molecules for allergy vaccines

The first step in the development of a therapeutic vaccine is to select a suitable target molecule. What then are the most important criteria for such a target? The vaccine antigen should, of course, be a molecule of major importance for the actual disease, preferably either a regulator or a mediator (Figure 1). Second, the molecule should be present in relatively low concentrations *in vivo* and preferably also be an extracellular non-cell-surface-bound protein. Low levels of the target molecule reduces the problem of B-cell tolerance and a soluble molecule can often be removed without the problem of tissue damage. It may also be favorable if the target molecule is found primarily in areas where antibodies can easily access the target to form immune complexes that are taken up by the phagocytes and removed from the circulation.

Molecules that fulfill all, or most, of these criteria are the IgE molecule and several Th2 cytokines, such as IL-5, IL-4, IL-13, IL-33, thymic stromal lymphopoietin (TSLP) and IL-18.

Vaccines against IgE

As a central mediator of IgE-mediated allergies, the IgE molecule itself is one of the most interesting targets for vaccine development. IgE is normally present at very low concentrations in plasma, in the range of 10–400 ng/ml. This is in stark contrast to IgG, where plasma levels are in the range of 8–16 mg/ml. The difference in concentration is, thereby, 10,000- to 1 million-times. As previously mentioned, this is a key factor for the success of a therapeutic vaccine. High concentrations of the target molecule give a strong tolerizing effect on the immune response. A target present at lower levels is also easier to remove from the circulation.

IgE is the target where most studies currently have been performed and where we have relatively detailed information concerning potency and mechanisms of action. Detailed analysis of differences between various adjuvants in their capacity to induce strong antiself responses have also been performed with this antigen. A more detailed description will, therefore, be given on IgE as a target. This section is also primarily based on this author's own work.

To make an active vaccine against IgE, as for all other self-targets, one has to bypass the T-cell tolerance to this self-protein. This is done by covalently coupling the entire self-protein or part of it, to a foreign peptide sequence, as previously described. However, when targeting IgE, the selection of target region is of particular importance. The vaccine has to elicit a response that results in antibodies that only bind circulating IgE and IgE on B cells, and not IgE sitting on high-affinity receptors on mast cells and basophils. Antibodies that bind IgE on mast cells and basophils may cause a massive histamine release and there is a potential risk of anaphylactic shock which, of course, has to be avoided (Figure 3).

When IgE sits on the surface of mast cells and basophils, specific regions of the IgE molecule are blocked from interaction with other ligands. These regions are preferential targets of an allergy vaccine. Experimental data have shown that regions on both CH3 domains of the IgE molecule interact with the second extracellular domain of the IgE receptor α-chain (Figure 4) [19,20]. Preferably, only these regions should be part of a vaccine antigen. However, the proper 3D structure of these regions is formed by interactions involving the entire CH3 domain. Therefore, it is almost impossible to remove these peptide regions and put them in the same conformation in another protein context. The same linear epitopes may be preserved but it is very difficult to recreate the more complex epitopes.

In order to have most epitopes in their native conformation, entire domains were used in the first prototype vaccine. At that time, the exact location of the receptor-interacting region had not been fully clarified and there were indications that both CH2 and CH3 domains were involved. Both of these domains were, therefore, used in the initial vaccine construct. This was also done to maximize the efficacy. It was judged that a larger target region with more surface epitopes would increase the therapeutic effect. The entire CH2–CH3 region was, therefore, used as a target molecule. A vector construct for the production of a fusion protein between these two domains of rat IgE and the glutathione-*S*-transferase (GST) of the parasitic worm, *Schistosoma japonicum,* was produced and transfected into a bacterial host (*Escherichia coli*) [8]. However, upon production in bacteria, this fusion protein, named GST–C2C3, was not soluble and formed inclusion bodies, which had to be dissolved in 8M urea and dialyzed against phosphate buffered saline before being used as the active component in the vaccine. Although, in a denatured form, this antigen gave very encouraging results in vaccination experiments. High anti-IgE titers and a marked effect on skin reactivity in response to an allergen challenge was observed [8]. In addition, attempts to trigger mast-cell histamine release by rabbit anti-IgE antibodies in the vaccinated animals did not succeed in generating a skin reaction, indicating that almost no IgE was present on mast cells in the skin of these animals [8].

These initial results were very encouraging and led to a screening process that was initiated to obtain a vaccine component

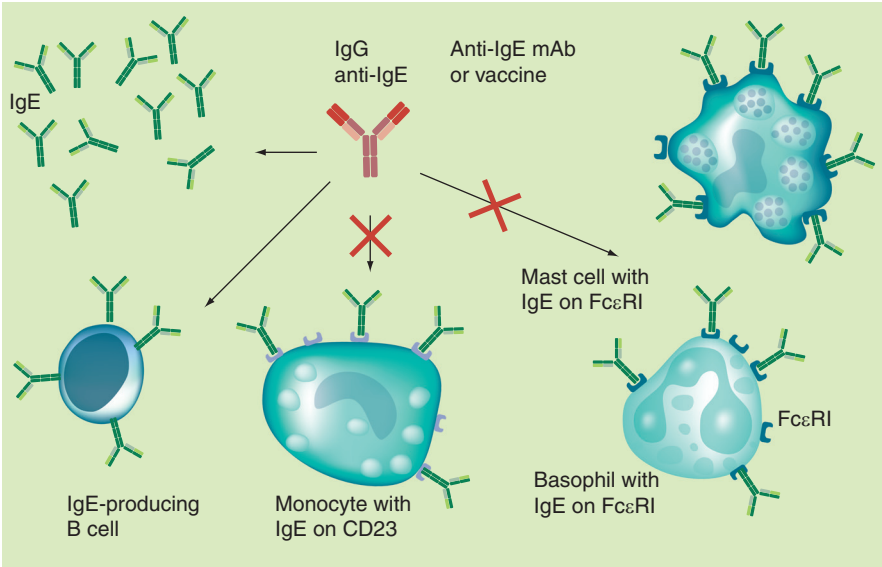

Figure 3. Targeting of IgE. To avoid receptor crosslinking, activation and mediator release, the anti-IgE response has to be targeted to regions of the IgE molecule not exposed when IgE binds to high- or low-affinity receptors on mast cells, basophils and monocytes. The crosses indicate unacceptable reactivity and the black arrows show the molecules that the vaccine or mAb should bind.
CD23: Low-affinity receptor for IgE; FcεRI: High-affinity receptor for IgE; mAb: Monoclonal antibody.
Modified from [20].

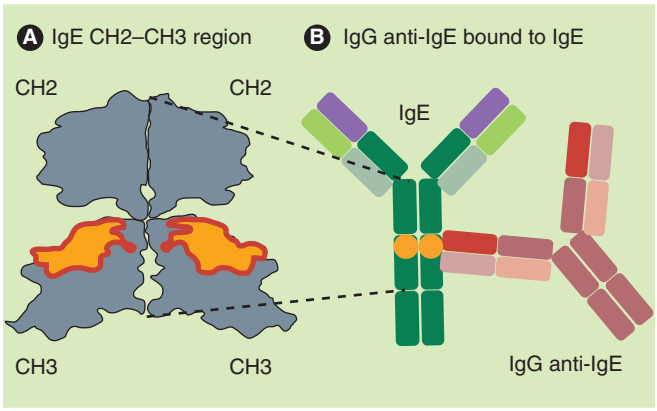

Figure 4. Receptor interacting region of human IgE.
(A) A schematic representation of the crystal structure of the IgE CH2–CH3 region, where the regions of the IgE molecule involved in direct interactions with the IgE high affinity receptor are marked in orange. **(B)** Interaction between IgG anti-IgE antibodies directed against this region of the IgE molecule. This part of the figure illustrates how large regions of the IgE molecule will be blocked for interaction with other ligands when a therapeutic antibody is bound to the target site in IgE.
Modified from [20].

that could be produced in a fully soluble form. Such a protein would enable production under good manufacturing practice (GMP) conditions for potential future clinical trials. This turned out to be very difficult and several attempts were made to produce the fusion protein in different expression systems: such as bacterial, fungal and insect cells, and with different fusion partners: including GST, maltose-binding protein or thioredoxin, all without much success [Hellman *et al.* Unpublished results] [8]. In the bacterial expression systems, close to 100% of the proteins formed insoluble inclusion bodies and in a fungal (*Picha pastoris*) expression system, no protein could be recovered, indicating aberrant folding and lysozomal degradation.

However, parallel studies on evolutionary aspects on IgE and mast cells [21–23] led to the emergence of an idea on how to solve the difficult folding and solubility problem of the CH3 domain. This involved producing the vaccine antigen as a hybrid IgE molecule. The IgE CH3 domain was taken from the species to be vaccinated, in this case, the rat, dog or human, and the carrier domains were taken from a nonplacental mammal. The chimeric IgE molecule produced for initial tests in a rat model consisted of the CH2 and CH4 domains of the American opossum (O) and the CH3 domain of the domestic rat (Cε2Cε3Cε4) [7]. The molecule was designated opossum–rat–opossum (ORO) (Figure 5). This molecule folded properly and soluble dimers were exported from the producer cell line, the human embryonic kidney cell line 293-EBNA (Figure 5) [7]. The ORO molecule was also found to bind the IgE receptor, which shows that the molecule folded in a native conformation [7].

The opossum sequences of the ORO molecule have dual functions. They act both as structural support for the CH3 domain and as carrier molecules by supplying a large number of nontolerized T-cell epitopes. The sequence identity between rat and opossum is only approximately 40%, which explains why almost all 10–20-amino acid long opossum peptides serve as potent T-cell-activating non-self-epitopes.

The hybrid IgE molecule (ORO) was used as the active component in several preclinical studies in rats. Compared with the GST–C2C3 vaccine antigen, this new antigen was found to increase the anti-IgE antibody titer by a factor of approximately 30 [7]. The larger number of epitopes in their native conformation in the new antigen is the most likely reason for this enhancing effect.

Results from these experiments also showed that the vaccination is reversible and that only a few percent of the anti-IgE antibodies remain 11 months after discontinuing the treatment [7]. From a safety perspective, this was a very important finding; to be able to enter clinical trials, the effect of a single or a few injections has to be reversible and not permanent. It should also be noted that the self-molecule, in this case IgE, did not boost the anti-IgE response because it does not contain any nontolerized T-cell epitopes (Figure 2). Reversibility has also been well documented for therapeutic vaccines in other systems, such as anticytokine vaccination and in contraceptive vaccines [14,18].

It is also very important that the opossum CH2 and CH4 carrier domains do not induce the production of antibodies that cross-react with the corresponding domains of the host. Potentially, this could lead to the induction of antibodies that could result in crosslinking IgE sitting on the mast-cell surface and to the release of histamine. To study the potential cross-linking activity of antibodies to the carrier, both the opossum (OOO) and the platypus (PPP) CH2–CH3–CH4 regions were therefore produced as recombinant proteins (Figure 6) [7]. Upon injection into rats, together with adjuvant, none or only very low levels of cross-reactivity were observed between the anti-opossum or anti-platypus response and rat IgE, showing that it is safe to use these distantly related IgE domains as carrier sequences in the vaccine antigen [7].

For questions concerning safety, a protein containing only half the CH3 domain of rat in an opossum background (OROO) was also produced (Figure 6) [7]. The idea behind this protein was to reduce the number of self-epitopes and, thereby, limit the target region to only the minimal receptor-binding region of IgE. After injection into rats, the OROO vaccine resulted in much lower anti-IgE titers, indicating that the number of self-epitopes is also very important. This finding has important implications for the use of alternative strategies to produce the vaccine antigen. A peptide vaccine is often presented as an alternative to fusion proteins, primarily because they are more easily produced. However, these often relatively short peptides only contain, at most, a few epitopes and these are often not in native conformation but strictly linear. Most peptide vaccines are, therefore, likely to be much less efficient than the corresponding fusion protein

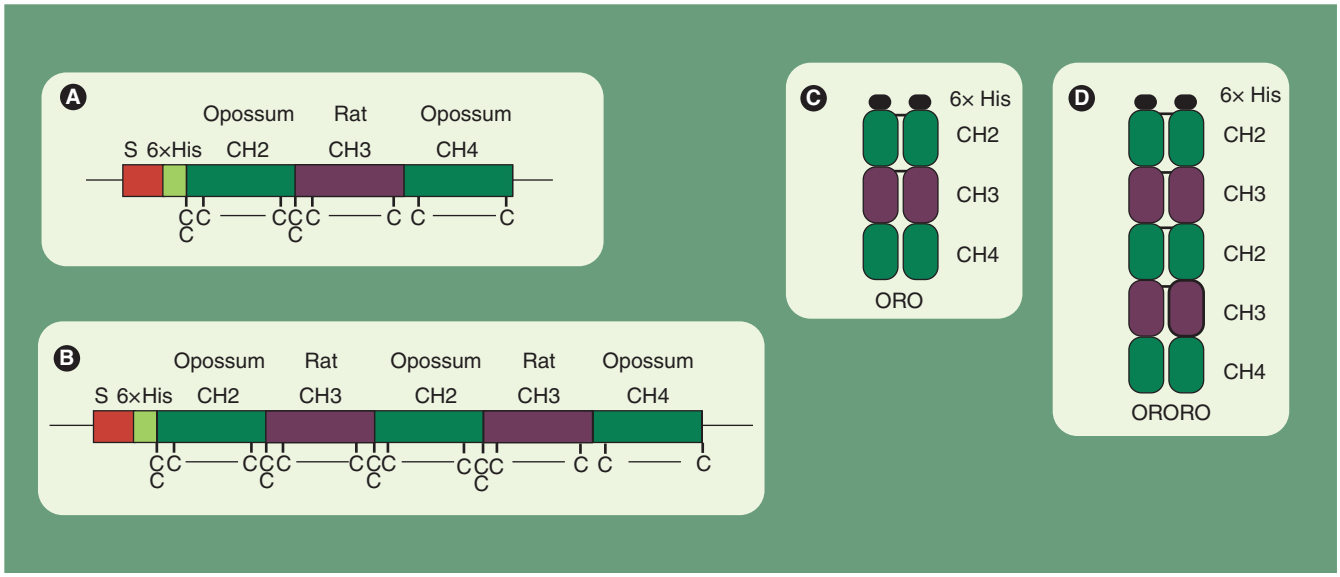

Figure 5. IgE vaccine constructs. (A) The ORO construct used in the first experiments with the soluble IgE vaccine described in [7]. **(B)** A variant of the soluble IgE vaccine containing two rat CH3 domains. This latter antigen was later used to study enhancing effects on anti-IgE titers by antigens containing repeated self-epitopes [73]. **(C & D)** shows the properly folded cysteine-bridged protein products. ORO: Rat vaccine (opossum–rat–opossum).

vaccine. In contrast to the other proteins, OROO was also very difficult to produce. It gave very low yields, most probably due to difficulties during folding [7], a result that clearly illustrates the difficulties in extracting epitopes out of their normal setting and presenting them in another context.

To study potential crosslinking activity of autoantibodies elicited against regions in the CH3 domain, outside of the regions directly involved in receptor binding, serum from vaccinated rats with high anti-IgE titers was injected into the skin of naive Brown Norway rats. No crosslinking (mast-cell-activating) activity was observed in these rats, indicating that no, or only very low levels, of crosslinking antibodies were present in the vaccinated animals [7]. This is very encouraging from a safety perspective.

To study the effect of point mutations in the target region, the corresponding mouse vaccine antigen (opossum–mouse–opossum [OMO]) was also produced (Figure 6). Mouse and rat are 81% identical in the IgE CH3 domain. Injection of the mouse vaccine into rats made it possible to compare effects of 19% amino acid differences in the target region on the antiself response. The question was, do the mutations increase the response due to lower inhibition by tolerance or does the decrease in number of self-epitopes lead to a decrease in antiself response? Compared with the rat vaccine (ORO), the mouse vaccine (OMO) was much less active in inducing antirat IgE responses in the rat, a finding which clearly shows the importance of the self-epitopes being in a native and non-mutated form [7]. This result also clearly shows that it is important to use species-specific vaccines to obtain a good therapeutic effect (i.e., human in human, dog in dog and pig in pig sequences).

To study the effect of different concentrations of the self-antigen on the anti-IgE response, the vaccine was also tested in several different strains of rat. To obtain maximal effect, the strongest adjuvants known were used in these experiments, the combination of Freunds-complete and -incomplete adjuvant. In three out of four of these rat strains, the vaccine was able to reduce IgE levels to baseline [7]. However, very little effect was seen in the fourth strain. The effect correlated with total IgE levels. Low IgE levels resulted in good anti-IgE responses and a good effect on lowering IgE, whereas in strains with high IgE, the vaccine had a poor effect or no effect at all. Based on the information collected from several studies, using both the early GST–C2C3 vaccine antigen and the new soluble hybrid IgE molecule (ORO), it was observed that it is very difficult to reduce IgE to baseline levels in rats when total IgE levels exceed 100–200 ng/ml [7,8].

It was very encouraging that IgE levels could be reduced to baseline and that a good effect on allergen- and anti-IgE-induced skin reactivity was obtained in several strains of rats. However, the effect of high IgE levels in the patient to interfere or even block the anti-IgE response may become a major problem in humans. Possibly, vaccination against IgE may only work in patients with 200 ng/ml of IgE or less, even when using strong adjuvants. This corresponds to a relatively small fraction of all allergy patients. The majority of severely allergic patients have IgE levels above 400 ng/ml.

To summarize the results from the preclinical studies in rats, it was concluded that the new vaccine antigen (ORO) can be produced in its native form, it is possible to purify it to almost homogeneity (minimum 98–99% pure), that the

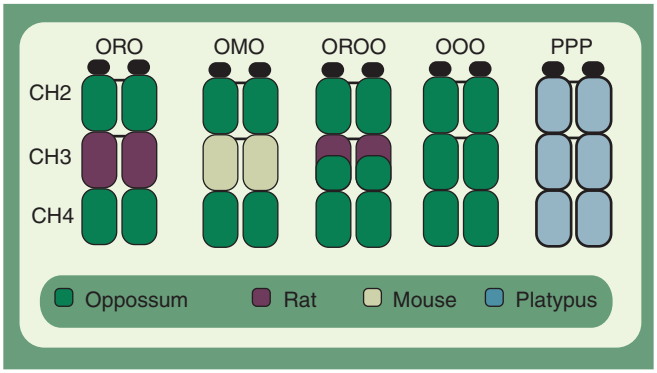

Figure 6. Five different protein antigens used in the above described studies. Design of vector constructs and production is described in detail in [7].
OMO: Mouse vaccine; OOO: Oppossum IgECH2–CH3–CH4; ORO: Rat vaccine; OROO: Rat half-CH3 in an opossum background vaccine; PPP: Platypus IgECH2–CH3–CH4.

carrier domains do not induce cross-reactive antibody responses, that the response to the vaccine is reversible and is effective in individuals with IgE levels below 200 ng/ml.

The next step in the IgE-vaccine development process was to study efficacy and safety parameters in naturally allergic species. Mice and rats are not naturally allergic and do not normally suffer from allergic disease whereas a large percentage of humans, dogs, horses, cats and possibly pigs, do. To obtain more clinically relevant information on the vaccine, studies in mice or rats should be complemented by studies in one or several naturally allergic species.

Based on the results described previously, where the mouse vaccine was injected into rats and observed lower activity, it is clear that for every new species we want to study, we have to construct and produce a new species-specific vaccine. Therefore, species-specific vaccines for dogs (ODO), pigs (OPiO), humans (OHO) and cynomolgus monkeys (OCO) were produced. The cynomolgus vaccine antigen was intended for studies in a primate model, which we considered to be of major interest for studies of efficacy and safety parameters, preceding clinical trials in humans. The dog vaccine was selected because allergic diseases are common in dogs and the involvement of IgE in the pathogenesis of canine atopic dermatitis and flea allergy has been documented. Many dogs suffer from allergic diseases, and there is a great need for new treatment strategies. Of these four new vaccines, the dog vaccine (ODO) was, therefore, the first to be tested *in vivo*. Relatively high anti-IgE titers were induced upon vaccination of dogs, and IgE levels were reduced by approximately 65% [24]. However, dogs turned out to have exceptionally high total-IgE levels, making it very difficult to reduce IgE to baseline levels, and a reduction of IgE levels to concentrations below 10–50 ng/ml will probably be needed to obtain good clinical effects. The median value of the total IgE levels in a panel of approximately 100 dogs was around 30 µg/ml [24]. This value is almost 100-times higher than what is observed in nonatopic humans and most laboratory strains of rodents. Therefore, it is questionable if we will be able to obtain good clinical effects with anti-IgE vaccines in dogs, even after optimizing adjuvant and antigen structure for maximal effect [24]. As mentioned previously, atopic allergies are a major veterinary issue and a vaccine for dogs would be extremely valuable in the clinic. Allergies are also a very serious medical issue in horses. However, horses have even higher total-IgE levels than dogs, making an IgE vaccine even less likely to work in this species [25].

Concerning other species, the pig vaccine has not yet been tested and no data on the cynomolgus vaccine is publicly available yet. The biotech company, Resistentia Pharmaceuticals AB (Uppsala, Sweden), continues the development of the IgE-vaccine concept. The human vaccine component has been produced under GMP and clinical trials have been initiated. Alum has been selected as the adjuvant for the clinical trials and none, or only mild, side effects have been reported from the initial Phase I clinical trials conducted by the company. However, no information concerning efficacy has been reported. A Phase II clinical trial has also been initiated where the same adjuvant (Alum) is being used, but no information concerning this study has been publicly released yet [101].

Structural features of the IgE CH3 domain

During the past 5–10 years, a number of interesting findings concerning the structure of the IgE CH3 domain have been published. This information may have important implications for the design and evaluation of various treatment strategies targeting this domain of IgE. As previously mentioned, a detailed analysis of the regions within the two CH3 domains of the IgE molecule engaged in direct interaction with the membrane proximal domain of the IgE receptor α-chain has been published by Garman *et al.* in 2000 [19]. Interestingly, additional studies of IgE–FcεRI complexes have shown that IgE molecules also bend asymmetrically, which may have direct impact on the accessibility and conformation of these regions of the IgE molecule [26]. In addition, the CH3 domain of IgE seems to have a relatively unusual structural feature, a 'molten globule' structure in an isolated form, which only assumes a well-structured form upon binding to FcεRI [27]. All of this information has to be taken into account when evaluating results of various treatment strategies targeting this domain of IgE, and may also reveal new methods to block the interaction between IgE and its high-affinity receptor [27].

Targeting other regions of IgE

A few years ago, a new membrane-bound form of IgE was identified. This variant of IgE has a short 52-amino acid long insertion in the extracellular region, positioned just outside the membrane of the membrane-bound form [28]. To study the effects of targeting IgE-expressing B lymphoblasts and memory cells, a set of mouse mAbs was developed against this 52-amino acid region [29]. Five monoclonals were obtained and all of them were found

to bind the same epitope, RADWPGPP, located at the C-terminal end of the peptide [29]. Binding of one of these monoclonals to membrane IgE on cells of the IgE producing B-cell line SKO-007 induced crosslinking of surface IgE. These monoclonals were also found to cause complement-mediated cytotoxicity *in vitro* on transfectants expressing this membrane-IgE variant [29]. Targeting this 52-amino acid region by monoclonals or a vaccine could potentially be a very interesting strategy to deplete IgE-producing B cells and, thereby give a longer-lasting reduction in plasma IgE levels [4]. However, one factor that complicates studies of the therapeutic effect of this potential treatment strategy in animal models is that the splice variant only exists in humans and possibly in closely related primates [30]. The expression level of this splice variant is also very low compared with the classical membrane-bound form in some IgE-producing cell lines (e.g., U-266), which can make it difficult to achieve good therapeutic effects [31]. Future studies in human or nonhuman primates and with normal human IgE-producing B cells will be needed to obtain information concerning the potential of this strategy to reduce IgE levels *in vivo* and also to give more information on safety parameters [4].

Other vaccine targets

Although the IgE molecule is certainly one of the most intensively studied targets in the area of allergy vaccines, there are also other targets of major interest. Several of these are already in advanced stages of development (e.g., the classical Th2 cytokines IL-4, -13 and -5 [17]). In addition, a new class of early Th2-regulating cytokines has recently been identified that may become promising targets in this field. At least four such cytokines have been identified; IL-25, -18, -33 and TSLP. All four have profound effects on the overall Th1–Th2 balance. The following section will attempt to give an updated summary of the progress in these emerging areas of therapeutic vaccine development.

Interleukin-5

Interleukin-5 is one of the key cytokines during an allergic response. Its primary roles in allergic inflammation are probably its effects on eosinophil numbers and the activation and recruitment of eosinophils to the area of inflammation. IL-5 is the most extensively studied of the classical Th2 cytokines and substantial information is available on the safety and efficacy of different treatment strategies. A humanized mAb directed against this cytokine has been developed by GlaxoSmithKline Pharmaceuticals. In clinical trials, this antibody had a remarkable effect on eosinophil numbers in the blood of asthma patients, but only partially reduced eosinophils in airways. Almost no effect on asthma exacerbations was detected, which indicates that it may not be effective in asthma treatment [32–34]. Several additional studies have been performed with this mAb, including studies of patients with hypereosinophilic syndrome and eosinophilic esophagitis [35,36]. In these latter studies, marked reductions in tissue eosinophil numbers and improvement in symptom scores were observed, indicating good effects in these patient groups [35,36]. A therapeutic vaccine against IL-5 is also being developed by Pharmexa AS in Denmark. They started by inserting a promiscuous foreign T-cell epitope from the tetanus toxoid into the mouse IL-5 sequence. Using a DNA vaccine encoding this antigen, they were able to induce neutralizing polyclonal antibodies against IL-5 in a mouse model. The vaccine also resulted in a marked reduction in eosinophil numbers, a 50% reduction in IL-4 and IL-5 expression levels and a reduction in airway inflammation [10]. The anti-IL-5 DNA vaccination also restored normal bronchial hyper-responsiveness to β-methacholine [10]. These results are clearly encouraging in light of the relatively poor effects of the monoclonal on symptom scores in asthma patients. It will certainly be interesting to follow this development process. No data on human trials with the corresponding human vaccine has been reported yet.

Interleukin-4

Interleukin-4 has been shown to act as a potent inducer of Th2-mediated immunity, even in the absence of IL-5, -9 and -13 [37]. IL-4 has, therefore, attracted a lot of attention as a potential target for therapeutic intervention.

The first attempt to modulate the effect of IL-4 was done by administrating an antagonistic cytokine to limit excessive IL-4-induced Th2-mediated immunity. By introducing mutations in the cytokine, a human IL-4 analog was generated that allowed receptor binding but hampered signaling [38]. However, to perform preclinical conceptual studies in an animal model, the corresponding mouse analog had first to be constructed. A mouse IL-4 variant (Q116D, Y119D) was produced and tested for its therapeutic potential in allergic sensitization and in established disease in a murine asthma model. Inhibition of the IL4/IL-13 system in the allergic sensitization phase resulted in a dose-dependent reduction in ovalbumin-specific IgE, and an inhibition of airway eosinophilia. However, if the IL-4/IL-13 system was inhibited at different time points after allergy was established, little effect on all measured allergic parameters was observed in this short-term study [39]. Although, it is possible that more long-term administration may reduce already established sensitization.

Soluble IL-4 receptors have been developed for both the mouse and human. In the mouse, this strategy was effective in suppressing airway allergic responses [40]. The human counterpart has also been well-tolerated and showed a detectable effect on forced expiratory volume and asthma scores in clinical trials [41].

A humanized monoclonal anti-IL-4 antibody, which binds both human and cynomolgus monkey but not mouse, rat, cow, horse or goat IL-4, has also been developed by GlaxoSmithKline [42]. The effect was analyzed *in vitro* where the antibody could block the effect of IL-4 on several human IL-4-responsive cell lines. In cynomolgus monkeys, the antibody was well-tolerated and no adverse clinical responses occurred after 9 months of treatment. However, three monkeys developed anti-idiotypic responses to the mAb that

resulted in rapid clearance [42]. This is a phenomenon that is seen with a majority of currently studied humanized monoclonals. A recent review noted that the majority of mAbs, even those that are fully humanized, suffer from problems with the induction of antibody responses to the injected mAb [43]. This situation, which may become a major problem for many of the mAbs in clinical use, speaks in favor of the development of therapeutic vaccines against the targets where substantial problems with antibody reactivity are observed. No more data on the anti-IL-4 mAb have been published, which may indicate problems with anti-idiotypic responses.

Two different therapeutic vaccines have also been developed against murine IL-4. One is based on native IL-4 that is chemically coupled to key limpet hemocyanine (KLH) and the second is a 14-amino acid peptide from murine IL-4 that is inserted in a truncated 149-amino acid long variant of the hepatitis B core antigen [11,17,18]. Both vaccines were effective in inducing high (above 10^5) antibody titers against IL-4 in mice. A potent mineral oil-based adjuvant was used with the KLH-coupled IL-4 vaccine, whereas no adjuvant at all was used with the hepatitis B core antigen-based vaccine. Both vaccines had effects on blocking ovalbumin sensitization after being vaccinated. Marked reduction in airway inflammation following ovalbumin challenge was also observed for both vaccines. In addition, reversibility was clearly seen with the KLH-coupled antigen, where anti-IL-4 titers were back to baseline levels after 20 weeks of treatment [18]. However, with the hepatitis B core-protein construct, very minor reductions in titers were observed after 11 weeks of treatment, indicating a more pronounced memory response when using repeated antigens [11]. The remarkable efficiency of the hepatitis B construct also clearly shows the importance of the antigen structure. This vaccine antigen forms capsid-like particles with a diameter of 25 nm that contain 180–240 hepatitis B surface antigen molecules, which result in a very effective display of the peptides in a highly ordered and optimally spaced repeats [11]. However, the peptide presented represents only one, or a maximum of a few, epitopes and it is questionable if it has the same structure as the native IL-4 protein. This may affect antibody affinity and, hence, clearance of the antibody–antigen complexes, as they may be very small and of low affinity. In spite of these potential drawbacks, the titers and the persistence of the response is clearly remarkable. Future clinical trials should clarify these issues and tell which of these two alternatives gives the most favorable response concerning affinity, immune complex clearance and safety.

Interleukin-13

Interleukin-13 is also one of the key Th2 cytokines. This cytokine, and IL-4, have been shown to be very potent inducers of IgE-isotype switching. These two cytokines are actually the only ones known to have this activity [44,45]. In addition, IL-13 is also an important regulator of tissue fibrosis, a characteristic feature of the inflammatory response in asthmatic airways. These two characteristics make this cytokine an attractive target for vaccine development [46].

A humanized anti-IL-13 mAb and a soluble IL-13 receptor have been produced by Wyeth Research. These two IL-13 blockers were tested in a sheep model of experimental asthma, and the mAb was also tested in cynomolgus monkeys [47,48]. Results from the sheep model show that both the antibody and the soluble receptor are safe and that they reduce, in a dose-dependent manner, an antigen-induced late-phase response and airway hyper-responsiveness [47]. In cynomolgus monkeys, the neutralizing antibody to human IL-13 was shown to markedly reduce both total cell numbers and eosinophil numbers, in particular, in broncho alveolar lavage (BAL) upon lung challenge with ascaris antigen [48].

A peptide-based therapeutic vaccine directed against IL-13 has also recently been developed by the same research group that produced the IL-4 vaccine described previosuly [12]. This vaccine, similar to the IL-4 vaccine, is based on a short (15-amino acid long) peptide inserted in the hepatitis B core antigen [12]. The IL-13 vaccine resulted in remarkably high antibody titers in the absence of adjuvant when injected into mice. Titers of up to 256,000 were observed, which further strengthens the notion that antigens presented as evenly spaced multimeric structures are very potent in inducing autoimmune responses. This vaccine was studied over a period of 8 months and reversibility and the possibility of reactivating the response were analyzed in more detail [12]. The antibody titers decreased by a factor of four to six over a period of 5.5 months, which shows that this vaccine is reversible [12]. The antibody titers also returned to maximal levels within a few weeks after a booster dose of the vaccine was administered to these animals [12].

The IL-13 vaccine was found to markedly suppress ovalbumin-induced cell numbers and IL-13, -5 and -12 levels in BAL fluid, but did not affect IFN-γ and IL-4 levels [12]. Moreover, allergen-induced goblet cell hyperplasia, lung-tissue inflammatory cell infiltration and pulmonary hyperresponsiveness to methacholine were significantly suppressed in vaccinated animals [12].

The results from both mAbs and the vaccine are very promising, and IL-13 is one of the most interesting new targets owing to its dual role, both in IgE switching and in the characteristic tissue remodeling observed in severe asthma. Clinical trials in patients with moderate-to-severe asthma would be very informative concerning the potential of this target.

TSLP, IL-33 & IL-18

During the last 10 years, four additional, potent, Th2-inducing cytokines have emerged as possible targets for the development of both mAb treatments and therapeutic vaccines. IL-25, -33, -18 and TSLP were all mentioned previously as involved in regulating the overall Th2 environment and are potent inducers of the more classical Th2 cytokines, IL-4, -13 and -5. They may, thereby, act as Th2 master switches. Proteins with these

characteristics may serve as good targets for allergy vaccines as they are present in relatively small amounts and appear transiently. Even partial reductions may significantly affect the disease process. To develop vaccines that have the potential to treat a relatively large fraction of all allergy patients, we recently initiated a project to produce recombinant vaccines for IL-18, IL-33 and TSLP in the rat, human and dog. We did not target IL-25 as this cytokine has been shown to be of major importance for parasite immunity and, therefore, downregulation may cause serious effects on parasite immunity in treated patients [49]. We are currently producing these recombinant cytokine fusion proteins and initiating animal testing in various models. However, except for positive anti-IL-18 levels, no efficacy data are available yet. A more detailed description of these new targets for therapeutic vaccine development is presented here.

IL-18 has, together with IL-12, been shown to be a very important inducer of cell-mediated immunity. This effect, to a large extent is mediated by the induction of INF-γ production by natural killer cells and several other cell types. However, IL-18 was also recently shown to be an inducer of IgE production and to induce symptoms similar to two cytokines involved in the early events in the induction of allergy, IL-4 and IL-13. Overproduction of IL-18 in keratinocytes by introducing a transgenic copy of the gene with a keratinocyte specific gene promoter resulted in severe atopic dermatitis in transgenic mice [50]. IL-18 has also been shown to induce asthma-like symptoms, indicating that it may be involved in several Th2-mediated inflammatory conditions [50]. This makes IL-18 an interesting target for novel treatment strategies, particularly against atopic dermatitis and asthma. In addition, owing to its potent INF-γ-inducing effect, IL-18 may also have effects in many autoimmune disorders, also making it a potential target for these types of inflammatory conditions.

IL-33 is an IL-1-like cytokine that shows strong Th2 potentiating effects. *In vivo* administration of this cytokine induces the expression of IL-4, -5 and -13, and leads to severe pathological changes in mucosal organs, such as the lung and digestive tract [51]. Intraperitoneal administration of IL-33 also results in increased numbers of eosinophils and lymphocytes and elevated levels of IgE and IgA [51]. This cytokine mediates its effects through the IL-1 orphan receptor ST2, a receptor that has been shown to act as a negative regulator of Toll-like receptors (TLRs) and IL-1-receptor signaling [52]. The ST2 receptor is expressed primarily on mast and Th2 cells and, in earlier studies, has also been linked to Th2-type effector functions [53–58]. Administration of antagonistic antibodies against ST2, or a soluble ST2 receptor, has inhibitory effects on Th2-associated allergic airway inflammation and promotes Th1 responses [55,58]. All of these characteristics of IL-33 make it a potential target for therapeutic intervention.

The third cytokine we selected for the development of a new family of therapeutic vaccines is TSLP. This cytokine has been described as the 'master switch' of allergic inflammation [59]. TSLP is an IL-7-like cytokine, that in humans, is produced predominantly by epithelial and mast cells, which in turn, stimulates myeloid dendritic cells to promote the differentiation of naive $CD4^+$ T cells into Th2 type effector cells [59]. These Th2 cells produce the allergy-promoting cytokines IL-4, -5, -13 and TNF-α, but not IL-10 and IFN-γ [60]. Recent findings also indicate that TSLP-activated dendritic cells play important roles, not only in Th2 priming, but also in the maintenance and further polarization of Th2 central memory cells in allergic diseases [61]. Keratinocytes in atopic dermatitis lesions have been shown to express high levels of TSLP, which may, therefore, be a key cytokine in the development of atopic dermatitis [60]. Mice engineered to overexpress TSLP in the skin also develop atopic dermatitis and a dramatic increase in circulating Th2 cells and elevated serum IgE [62]. TSLP appears to be one of the key regulators of Th2-mediated inflammatory conditions and is, therefore, one of the most interesting targets for therapeutic intervention [63].

Optimizing adjuvants & antigen structure

Two of the most important issues in the area of therapeutic vaccines, and in particular in vaccines that are aimed at modulating *in vivo* levels of various self-proteins, are the adjuvant and antigen structure. Very few, if any, potent adjuvants are presently available for human use and the most frequently used adjuvant in human vaccines is Alum, an aluminum salt-based adjuvant with very low adjuvant activity. Recent clinical trials with therapeutic vaccines against TNF-α and Her-2, where Alum has been used as adjuvant, have resulted in very low or undetectable antibody titers against the target antigen [64] (Pharmexa [102]). These data, and many studies conducted by us and others in animal models, clearly show that more potent adjuvants, and also possibly modifying vaccine antigens, are needed to obtain sufficient antibody titers against self-antigens. Therefore, data will also be summarized here from several studies aimed at enhancing the immunogenicity of a therapeutic vaccine, and suggest how this may contribute to an increased progress in the field.

Classical vaccines directed against viral and bacterial infections are, to a large extent, based on crude preparations of attenuated or killed viruses and bacteria. However, due to safety considerations, almost all new vaccines are based on subunits or modified recombinant antigens of very high purity. These antigens are usually much less potent in inducing strong immune responses. In the development of therapeutic vaccines, this phenomenon is even more pronounced. Immune responses against self-antigens are normally heavily suppressed by tolerance-inducing mechanisms. Previously, we observed 30–50-fold lower antibody titers to the self-region of the antigen compared with the response against the foreign carrier epitopes [7]. In the development of therapeutic vaccines against various self-molecules it is therefore of major interest to mimic viral and bacterial antigens to obtain as high antibody titers as possible to be able to achieve good therapeutic effects.

What then are the reasons for the superior efficacy of crude-virus or bacterial-vaccine antigens? One important factor is probably that bacterial whole-cell preparations often contain one or several molecules interacting with pattern-recognition receptors of the TLR family [65]. Activation of these receptors then triggers innate immune responses, which are a prerequisite for efficient induction of adaptive immune mechanisms. Examples of molecules that bind and activate Toll like receptors are bacterial lipopolysaccarides, different fungal or bacterial carbohydrates ds- or ss-RNA, and DNA fragments containing unmethylated CpG motifs [65,66]. Such molecules can be used as additives to the vaccine formulation to activate the immune system.

Recently, we have shown that the addition of TLR agonists, such as the lipid A portion of lipopolysaccharide from *E. coli* or the muramyl dipeptide of bacterial cell walls, can markedly enhance the immune response when added to a relatively weak plant oil-based adjuvant (Montanide ISA 720) (Figure 7) [67]. However, no additive effect of these TLR agonists was observed when added to a strong mineral oil-based adjuvant (Montanide ISA 51), indicating that this adjuvant had already reached maximal activity (Figure 7) [67]. One of the major findings from this study was also that Alum had almost no enhancing effect on the antiself response. This clearly indicates that more potent adjuvants are needed for therapeutic vaccines that target self-antigens (Figure 7A, arrow) [67]. This suggests that Alum should not be used as adjuvant in therapeutic vaccines because it has almost no enhancing activity on anti-self responses, and that the addition of TLR agonists to relatively weak biodegradable adjuvants, such as Montanide ISA 720, can serve as a very good alternative to the strong mineral oil-based adjuvants. This latter finding was very encouraging for the possible development of new adjuvants, which can be both potent and safe to use for injections three- to four-times per year for the rest of the patient's life. Compared with the very strong Freunds adjuvant, an activity of approximately 60% activity was observed with the Montanide ISA 720 and monophosphoryl lipid A (MPL) (Figure 7D, arrow) [67].

Based on the results described previously, and results from many other laboratories, TLR-interacting substances should be considered as very promising candidates for more broad usage as additives to vaccine formulations [66,67]. However, for therapeutic vaccines, the use of potent adjuvants alone may not be sufficient to obtain a good clinical effect. To further enhance the antibody response, one has to look to other potential differences between most recombinant antigens and viral and bacterial antigens. One example of such a difference is the fact that viral and bacterial antigens are frequently present in repeats. Repetitive epitopes on the surface of both bacteria and viruses have been observed to effectively stimulate B cells [68]. One of the enhancing effects on B-cell responses is probably caused by the very efficient cross-linking effect of highly repetitive epitopes on a solid surface, which may lead to the activation of B cells in the absence of, or with, moderate to low T-cell help [69,70]. The use of virus coat proteins to present antigens is, therefore, a very attractive idea of how to enhance immunogenicity of a vaccine antigen. Both hepatitis B and papillomavirus coat proteins have been used successfully to present short peptide epitopes of various vaccine antigens and good antibody responses have also been obtained, as previously described for IL-4 and -13 [11,12,71,72]. However, it has frequently been observed that it is a problem to obtain sufficient yield and stability of more complex antigens during production of the virus particles [71].

To study the potential of using repetitive epitopes of larger, more complexly folded proteins, we have analyzed the immunostimulatory effects of dimeric variants of a hybrid IgE molecule (Figure 5). This molecule (ORORO), in animal experiments, gave an increase in the anti-IgE titer in three independent experiments of four- to nine-times compared with the monomeric variant (ORO). These results show that only a moderate repetitive structure is still potent enough to serve as a very efficient way to increase the clinical effect of therapeutic vaccines (Figure 5) [73]. In addition, new ways to present larger epitopes on viral coat proteins are also being developed, which may be an alternative for at least some more complex antigens.

The take-home lesson is that repetitive structures are a strong enhancing factor that could be used to increase the therapeutic effect of various target antigens.

Expert commentary

Therapeutic vaccines targeting various self-molecules is an exciting field in rapid progress. Initial studies in various animal models have been promising. For example, vaccination against IgE has resulted in decreased IgE levels and reduced sensitivity to allergen exposure and vaccination against IL-5 has lowered the levels of IL-5 and inhibited eosinophil infiltration and proliferation. Both of these are clinically significant in allergic conditions. Viral coat protein based peptide vaccines against IL-4 and -13, or chemically coupled cytokines, have resulted in high anticytokine titers and have not shown any negative side effects. However, currently there is no vaccine on the market. One major reason for this is the lack of potent adjuvants for human use. Recent clinical trials with Alum, the relatively weak adjuvant presently available for human use, have not met the high expectations from the field. Only low or no detectable antibody responses to the vaccine antigen have been detected in the vaccinated subjects [64] (Pharmexa [102]). This clearly indicates that more potent adjuvants, and possibly also efficacy-increasing modifications of the vaccine antigen are needed to obtain sufficient antibody titers against the self-antigen, for a good clinical effect.

Preclinical studies of the IgE vaccine have also shown that, even with a strong adjuvant like Freunds, it is difficult to reduce IgE levels significantly in individuals with IgE levels above 200 ng/ml. Even with both optimized antigen structure (dimer or multimer) and more potent adjuvants, the IgE vaccine may not benefit more than a few percent of all allergy patients. Additional targets are, therefore, of major interest.

The various Th2 cytokines are the most promising of these targets today. However, questions have been raised concerning the targeting of cytokines. What will happen to the normal functions

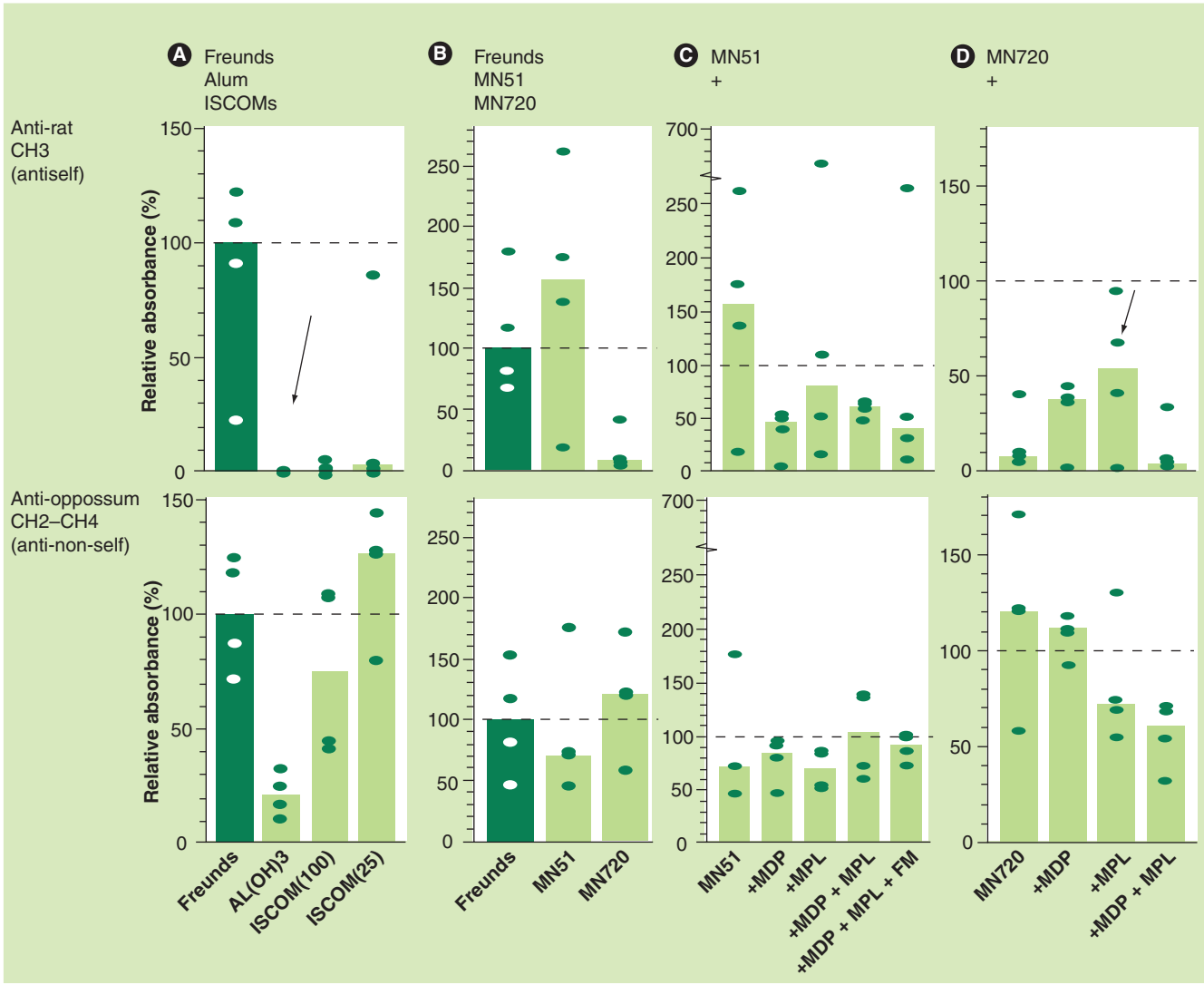

Figure 7. Screening for adjuvants and Toll-like receptor (TLR) agonists to be used as additives to adjuvants in therapeutic vaccines. The rat IgE vaccine (ORO) was injected in rats together with different adjuvants and adjuvant plus TLR agonist combinations, and the anti-rat IgE titers were assessed. Anti-opossum IgE titers were also measured to study differences in the activity of the adjuvant to enhance anti-self and anti-non-self responses. The upper panel shows the antiself responses (anti-rat IgE) and the lower panel the anti-non-self responses (anti-opossum IgE). All values presented in the figure are in percent of the response elicited by Freunds. A black dotted line shows the Freunds reference level in each experiment. **(A)** shows an initial screening of three commonly used adjuvants; Freunds, Alum and ISCOMs (two different protein densities were used on the ISCOMs). **(B)** shows a comparative study of three oil-based adjuvants; Freunds (complete and incomplete), Montanide ISA 51 (MN51; also a mineral oil-based adjuvant) and Montanide ISA720 (MN720), which is a plant oil-based and biodegradable adjuvant. **(C)** shows a study of the effect on anti-IgE titers by addition of various immunostimulatory compounds to a potent mineral oil-based adjuvant Montanide ISA5. The additives were MPL (lipid A portion of lipopolysaccharide), MDP (muramyldipeptide from bacteria cell walls) and formyl 2-methionine tripeptides MLP. **(D)** is the same experiment as in **(C)** but with another adjuvant, the plant oil-based MN720.
ISCOM: Immune stimulating complex; MDP: Muramyldipeptide; MPL: Monophosphoryl lipid A.

of these cytokines in regulating normal immune responses? Will cytokine-targeting lead to immunodeficiency? Apparently, this is not the case as no side effects have been observed in the various animal models and in the human clinical trials conducted [10–12,16–18]. Most cytokines can also act at short distances and, in normal immune responses, these short-distance interactions do not seem to be affected by antibodies directed against cytokines. The mAbs or the antibodies produced after vaccination primarily seem to bind excessive cytokines that leak out from the area of inflammation. The anticytokine antibodies, therefore, primarily limit excessive activation and appear to leave most normal signaling unaffected [17]. This has to be formally proved in clinical trials. However, based on the available preclinical data, it appears likely that there will be no, or very minor, side effects.

It appears relatively easy to induce high antibody titers against cytokines owing to their low levels of expression and transient expression patterns. However, here too, clinical trials will have to give the final answer on important efficacy and safety parameters. For some of the cytokines, the use of naturally allergic animal species may be an economical and a more rapid way to obtain such data. These new anticytokine vaccines may also become important tools in veterinary medicine, for example, for the treatment of allergies and atopic dermatitis in dogs and horses.

It is important that upcoming clinical trials are conducted properly taking all the available preclinical data into account. Through the use of more potent biodegradable adjuvants and with optimized antigens, such as low repetitive structures or viral coat protein-tagged multimers, the risk of negative results can be reduced. The use of peptide-based or domain-based vaccines presented on empty virus particles, as described previously for the IL-4 and -13 vaccines, may be an interesting possibility here. The question of enhanced immunogenicity is certainly a key issue in the field. However, the very encouraging preclinical data obtained so far and the large number of new targets identified, makes this area of therapeutic vaccine development very promising.

Five-year view

In my opinion, therapeutic vaccines have good prospects to become new and efficient tools for the treatment of atopic allergies. However, before taking these vaccines into the clinic, the adjuvant issue is clearly one of the most urgent questions to be addressed. The decision to use Alum in several of the first clinical trials has clearly shown that more potent adjuvants are needed. The good effects with both Montanide ISA 51, a mineral oil of high purity, and the biodegradable adjuvant Montanide ISA 720 with the addition of MPL, has clearly shown that it is possible to achieve good effects with adjuvants that have the potential to be used in the clinic. Using variants of these or other potent adjuvants, and to redo the clinical trials for IgE, will probably result in a vaccine that can be used to treat allergy patients with IgE levels below 200 ng/ml. However, for patients with higher IgE levels, anticytokine vaccines will most probably have to be used. The IL-13 vaccine is certainly one of the most interesting of such vaccines under development. This vaccine, as well as the IL-4 and IL-5 vaccines, seems to have the potential of being tested in the clinic within the coming 5-year period. However, issues of GMP production and adjuvant optimization have to be addressed first. Vaccines against the different members of this new family of Th2-inducing cytokines, TSLP, IL-18 and IL-33 also have great potential. However, these vaccines have to be tested in several animal models and GMP protocols, for their production has to be worked out before they can enter clinical trials. Based on the experience from other therapeutic vaccines, we can probably not expect that this will happen within the coming 5-year period. However, one possibility is that the great need for new treatment methods against atopic dermatitis in dogs may open opportunities for clinical trials with vaccines for developed canine use within the coming 5-year period.

Financial & competing interests disclosure

Lars Hellman has a small number of shares and a royalty agreement with Resistentia Pharmaceuticals AB. Patent applications on the vaccination against IL-18, IL-33 and TSLP have been submitted by a small biotechology company owned by Lars Hellman, Theravac Pharmaceuticals AB. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Key issues

- Based on experiments from several model systems, it is now well documented that it is possible to vaccinate against self-proteins by using modified self-proteins. The resulting antibody response can also reduce the levels of the self-protein to such an extent that it gives good effects on symptom scores in animal models.
- Therapeutic vaccines against IgE can markedly reduce IgE levels in animal models and also reduce or even block allergen-provoked skin reactions. However, this effect is highly dependent on the level of IgE in the vaccinated animal and it appears difficult to obtain good effects when IgE levels in the blood exceed 200 ng/ml.
- Vaccines against the classical Th2 cytokines IL-4, -5 and -13 are in a relatively advanced development stage. Efficacy has been shown in animal models and in the near future, clinical trials with these new targets will, hopefully, show their clinical potential.
- A new class of potent Th2-regulating cytokines has recently been identified (thymic stromal lymphopoietin, IL-25, -33 and -18). Vaccines based on these molecules are currently in a very early stage of development. However, these vaccines may have the potential to treat patients with high IgE levels and patients with severe asthma and atopic dermatitis.
- Adjuvant and antigen structure are two key issues in the field of therapeutic vaccines. New potent adjuvants have been identified. However, additional work needs to be done to identify adjuvants that are both potent and safe and that can be produced under good manufacturing practice.

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Websites

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Affiliation

- Lars Hellman
Department of Cell and Molecular Biology, BMC, Box 596, 751 24 Uppsala, Sweden
Tel.: +46 184 714 532
lars.hellman@icm.uu.se