

Improvement of specific immunotherapy by human IgG and modified allergens

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Allergen avoidance, pharmacologically symptomatic treatment and immunotherapy (IT) are the treatments of choice for allergic patients. By IT is usually understood administration of small doses of allergen extract which are gradually increased as the patients develop a relative tolerance towards the allergen. Such terms as desensitization or hyposensitization have been used, but the latter should be preferred, as a total disappearance of symptoms seldom occurs. Rather, the patient's sensitivity towards a specific allergen is reduced and when symptoms develop, they are diminished compared to the situation prior to immunotherapy (54, 92).

Immunotherapy has been practised for almost 80 years. The efficacy has been documented. Nevertheless, we still do not know the exact mechanism by which symptom alleviation is produced. During the past 10-15 years, characterization, purification, standardization and even modifications of allergen extracts have evolved rapidly. Even though a vast amount of literature has appeared, no clearcut picture exists. This review considers various modifications of allergen extracts giving them properties clearly different from their native counterparts. These measures have been carried out along several lines and therefore a brief outline is given on the strategies for improvement of the treatment and on some of the immunological key events induced by IT. Finally, some pos-

sibilities of allergen-specific immunopharmacology are discussed.

History

Treatment of Type I allergic conditions - particularly hay fever - with IT is not of recent origin. Inspired by infection medicine Noon suggested in 1911 that active immunization with the "toxin" responsible for the symptoms might cure the disease. Noon managed to demonstrate that repeated subcutaneous injections of grass pollen extracts in varying concentrations led to decreased sensitivity as determined by end-point titration of the conjunctival sensitivity (72). Doses were increased during the treatment, but it was noted that large increments caused relapse, or even acute onset of symptoms for up to 24 h. Noon's investigations were continued by Freeman, who recorded objective (conjunctival reactivity) and subjective improvement in the majority of 20 patients (21).

In 1921 it was demonstrated by Prausnitz & Küstner that serum from allergic patients contains a factor that may transfer allergen reactivity to the skin of an unsensitized subject (97). By means of Prausnitz-Küstner's test Cooke demonstrated that in spite of clinical improvement on IT, no reduction of sensitizing antibodies in serum occurred. However, serum from treated patients contained another factor that upon preincubation would inhibit the Prausnitz-Küstner-reaction (13). For this block-

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ing factor – shown to be antigen-specific – the term “blocking” or “inhibiting” antibodies was suggested. It was further demonstrated that the blocking factor was a pseudo-globulin and could be produced by immunization of non-allergic subjects (14).

The development of an assay for histamine release from leucocytes produced a new *in vitro* model with capabilities of investigating allergic reactions (49). The combination of this assay with chromatographic and immunochemical methods made it possible to firmly establish the blocking antibodies as IgG immunoglobulin (50).

Recent strategies for improvement of IT

Although not completely curative, controlled investigations have demonstrated efficacy of IT with insect venoms and such aeroallergens as grass, tree or herb pollens, animal danders and house dust mite, reviewed in (54, 75, 92, 100). When side effects occur, they are nearly always associated with injection of allergen extract, and manifest themselves as immediate reactions of local or systemic character. Correctly administered, IT is considered safe and the risk of fatal side effects is very small (52, 54).

Many strategies have been followed in order to improve IT. These may be grouped accordingly and the last two will be discussed in detail:

- 1) Better knowledge of indications and contraindications.
- 2) Administration: dosage, regimen, and route of administration.
- 3) Refined extracts.
- 4) Addition of adjuvant or modification of allergen.
- 5) Allergen-specific immunopharmacology.

1. *Better knowledge of indications and contraindications* includes specification of symptoms, for which IT is indicated (54). Attempts have been made to predict the clinical outcome of IT based on an early determination of the specific IgG, but no clear guidelines can be given as yet (16, 18).

2. *Administration: dosage, regimen, and route of administration.* Numerous investigations have been carried out with variations of dose, injection interval, rate of dose increment, etc. (reviewed in 54, 92, 100). To avoid injection, oral (69, 94, 113), intranasal (22), and sublingual (24) administration has been examined. For potentially allergic offspring even intra-uterine administration has been suggested (23).

3. *Refined extracts.* Allergen chemistry has developed towards standardizing allergen extracts with respect to major allergens (122). Nordic Guidelines for allergen extracts are now in progress and international standards are being developed (77, 103). Several studies have shown that treatment of patients with only the major allergens is inferior to treatment which includes the minor allergens as well (1, 99, 131), although this has not been confirmed by others (78). To “fit” all patients, an extract should contain all allergens that can possibly be eluted from a raw allergenic material. However, this conflicts with the sane concept that no antigen should be given towards which the patient has no IgE antibody. Furthermore, it is not practicable, at present, to standardize an extract for more than 2–4 major allergens, and thus allergen extracts are designed to meet the needs of the “average allergy patient”. This discrepancy is further discussed in the paragraph dealing with immunological changes during IT.

4. *Addition of adjuvant or modification of allergen* includes simple mixtures and actual physiochemical modifications of the native allergenic molecules. This is reviewed in detail below.

5. *Allergen-specific immunopharmacology* comprises selective measures aiming at modulation or suppression of the immune system in an allergen-specific manner. Passive immunization has been suggested by several authors, and is discussed below, as it is based on the observation that an IgG response develops during IT. Another example from this field is the concept (borrowed from oncology) of an “allergic bullet”, a strongly radiolabelled allergen molecule that destroys the immunocompetent cells by

which it is recognized (20). More delicate procedures involve *in vitro* boosting of T-suppressor lymphocytes (98) or administration of allergen in conjugation with IgE-restricted lymphokines (36) selectively suppressing the synthesis of specific IgE.

IMMUNOLOGICAL CHANGES DURING IMMUNOTHERAPY

IgE

During IT allergen-specific IgE initially increases followed by a modest decline (131). The reductions in symptoms are not likely to be caused by a lower level of IgE (15, 100), even though the seasonal rise in IgE, which is seen with seasonally occurring allergens, may become eliminated during IT (76). It has become a general assumption that IT does not induce formation of IgE towards components in allergen extracts to which the patient has not hitherto reacted. There are, however, examples of induction of IgE following injection of allergen extracts in non-allergic subjects (32, 62, 79, 117). In an IT study Østerballe demonstrated by means of the CRIE-technique that no new IgE-specificities were induced by a timothy grass pollen extract (131). A re-analysis of these samples has, however, questioned these findings, as several IgE-responses to new antigens in the extract have now been identified. A semiquantitative summation of the IgE-responses to the individual antigens in the extract suggests that the total amount of specific IgE is slightly decreased during IT (111). An investigation of the sera by means of an alternative method (immunoblotting) is now being performed.

Allergen-specific IgG – blocking antibodies

Development of an IgG immune response during IT has long been known, cp. above. Recently, it has also become clear that the natural exposition of allergens might lead to an IgG response not only to insect venom (17) but also to grass pollen (74), animal dander (119)

and moulds (55). The IgG immune response during IT is restricted mainly to the subclasses IgG1 and IgG4 (16). In spite of the close relation between successful IT and development of IgG (93), it is still a matter of controversy whether it is possible to predict the clinical outcome on the basis of IgG (-subclasses), as varying implications of different allergen systems have emerged (16, 18, 55). Preliminary results (111) suggest that the IgG response developed during IT is “broader” than IgE, i.e. more antigens are covered by IgG than by IgE response. Possible clinical implications of these findings are currently being analysed.

Cellular events

Histamine release from basophil leucocytes has been investigated during IT, and even though the basophil sensitivity to allergen seems to decrease, no clearcut picture has emerged (93, 100). Several investigators have demonstrated that allergen-specific T-suppressor lymphocytes are induced by IT (70, 101, 112). The significance of these findings remains obscure, as the level of specific IgE does not decrease during IT. A reduction of IgE by means of modified forms of IT is further discussed below.

MODIFICATION OF ALLERGENS

Many efforts have been made to modify allergen extracts for IT, the aim being a reduction of allergenicity (i.e. side effects) while retaining the immunogenicity (evidenced by induction of an IgG response). As early as 1940, Stull et al. were able to compare different methods of allergen modifications including formaline treatment, acetylation, heat inactivation, UV-irradiation and alum-precipitation (108). Since 1970 a vast amount of publications have emerged on possible improvements of IT by means of allergen modifications. From an immunochemical viewpoint it seems appropriate to divide modified allergen extracts into three groups:

1. *Mixture, emulgation or adsorption of allergens*, i.e. extract of *Ascaris suum*, Freund's Complete Adjuvant or alum.

- 2. *Covalent binding of allergen to non-immunogenic material* (Table 1). This material may be of biological (alginate) or of synthethic (polyethylene glycol (PEG), polyvinyl alcohol (PVA) or polymers of amino acids) origin.
- 3. *Modifications of allergens* by means of chemical (denaturation with urea or formaldehyde, crosslinking by means of glutaraldehyde) or physical methods (UV-irradiation, heat) in order to make them low or non-allergenic.

Mixture, emulgaion or adsorption

In rodents it is possible to raise a humoral immune response restricted to distinct immunoglobulin classes by means of different adjuvants. Extracts of *Ascaris*, *Nippostrongylus brasiliensis* or *Bordetella pertussis* and lipopolysaccharide, alum or aluminiumhydroxide will potentiate an IgE-response, while Freund's complete adjuvant will principally induce IgG and suppress the IgE-response (5, 105). In humans, IgE is similarly potentiated by extracts of bacteria and parasites mentioned above (5), whereas alum- and Al(OH)3-adsorbed allergen in two controlled immunotherapy studies have demonstrated clinical effects comparable to aqueous extracts (66, 79). Compared with aque-

ous allergen extract no higher levels of specific IgE were observed (66), whereas the levels of specific IgG were slightly increased (66) or unchanged (79). As opposed to this, immunization of healthy subjects with alum-adsorbed native allergens or "allergoids" leads to formation of specific IgE, allergen specific histamine release from leucocytes and skin reactivity to the allergens, although no clinical evidence of allergy was observed (58). Due to the depot effect, alum- or Al(OH)3-adsorbed allergens can be given in higher doses, thus reducing the number of injections in an immunotherapy regimen. Accordingly, adsorbed extracts have gained widespread clinical use (54).

A depot effect has further been achieved by adsorption of allergens to tyrosin-base (63), and these extracts have demonstrated a clinical effect comparable to alum-adsorbed extracts (38). Adsorption to tyrosine has also been combined with glutaraldehyde-polymerized extracts as described below (38, 61).

Covalent linking of allergen to non-immunogenic material

Hapten experiments. Animal experiments have demonstrated that when haptens are covalently

Table 1
Non-immunogenic carriers used for coupling to allergens. References given in text.

Name	Composition
<i>Biological origin*</i>	
Alginate	copolymer of mannuronic and guluronic acid
Dextran	carbohydrate (glucose)
IgG	homologous protein
Serum albumin	homologous protein
<i>Synthetic origin</i>	
Polyethylene glycol (PEG)	H-(O-CH ₂ -CH ₂) _n -OH
Methoxy-polyethylene glycol (m-PEG)	CH ₃ -(O-CH ₂ -CH ₂) _n -OH
Lauryloxy-polyethylene glycol	C ₁₂ H ₂₅ -(O-CH ₂ -CH ₂) _n -OH
Polyvinylalcohol (PVA)	H-(CH-CH ₂) _n -OH OH
D-GL	copolymer of D-glutamic acid and D-lysin
Polysarcosine (Poly-(N-methylglycine))	CH ₃ -NH-(CO-N-CH ₂) _n -COOH CH ₃

*This classification merely indicates that the substance can be found in biological systems, not that it is actually derived from biological material.

linked to non-immunogenic carriers, they can act as tolerogens. Thus, IgE synthesis is inhibited or abrogated when the tolerogen is given to the animal either prior or subsequent to the hapten in a form otherwise known to lead to formation of anti-hapten IgE antibodies. This has been shown with 2,4-dinitrophenyl-group (DNP) or benzylpenicilloyl-group (BPO) coupled onto homologous IgG (6, 43); with BPO linked to copolymers of D-glutamic acid and D-lysine (D-GL) (11) and with trimellityl coupled onto polyvinylalcohol (PVA) (123, 124).

PEG-linked allergens. These results led to investigations in which experimental antigens and allergens were coupled to polyethylene glycol (PEG). Experiments in mice showed that the primary and also the ongoing IgE immune response could be inhibited not only for experimental antigens as ovalbumin but also for well-known allergens as ragweed, timothy grass pollen, dog serum albumin, *Ascaris suum* and *Microspora faeni* (44, 45, 128). Later studies indicated that the inhibition of the IgE synthesis was caused by antigen-specific T-suppressor cells, which were stimulated by PEG-coupled antigens (46, reviewed in 104).

In spite of these promising results it has not been possible to observe an abrogation of the IgE synthesis when PEG-coupled allergens have been utilized for immunotherapy in man. Bee venom coupled to PEG was shown to produce fewer side effects compared with native allergen, but as a larger number of treatment failures were observed in the PEG-group the authors suggested that the maintenance dose might have been too low. A decline in bee-specific IgE was observed which, however, did not differ from that of the group treated with native venom (68). Another study showed only a modest decrease of specific IgE after immunotherapy (73).

Ragweed antigen E (AgE) conjugated to different polymers: PEG, methoxy-PEG, lauryloxy-PEG or D-GL, did not induce any significant decrease of the IgE level. Instead, an initially small rise was followed by a slow decline during the prolonged treatment (83). In

all three studies the conjugates have effectively stimulated an IgG-response to the allergen, indicating that the immunogenic potential of the allergens has been preserved in spite of the PEG-conjugation.

Amino acid polymers. The same discrepancy between animal experiments and human studies was seen with D-GL-conjugates, which abrogated both primary and ongoing IgE-responses to ovalbumin and AgE in mice (51). However, D-GL-AgE produced only a minor decrease in specific IgE (83) when used for immunotherapy in humans. When D-GL was conjugated with fraction A of short ragweed and compared to immunotherapy with native extract, a substantial increase in specific IgE antibody was indeed observed (9). In spite of the rise in IgE the clinical effect was comparable to the native extract and significantly better than placebo (9).

Another poly-amino acid, polysarcosine (poly-(N-methylglycine)), had the ability to inhibit the primary but not the secondary IgE-response to grass pollen allergen in mice (126, 127). In their discussion of the results the authors stress the important point of the dose given to experimental animals, and it is suggested that the discrepancy between this and other animal experiments is caused by differences in dosage (126).

Carbohydrates. Turning finally to the carbohydrates as possible carriers of tolerogenicity, it again appears that alginate (Conjuvac®, a copolymer of mannuronic and guluronic acid) suppressed the grass pollen-specific IgE response in rats (114), whereas a multicenter immunotherapy study showed clinical effect without concomitant decrease in IgE (8).

Relevance of animal models. Although clinical results comparable to treatment with native allergen extracts have been achieved with different conjugates between non-immunogenic carriers and allergens, it is unlikely that the observed effects are correlated to a major decrease in specific IgE. Thus, the optimism imposed by animal experiments has not been rewarded. Two main points should be men-

tioned as possible explanations of the discrepancies. One is the nature of the IgE response raised in experimental animals. It is usual to sensitize small rodents by means of one or two parenteral injections of admixtures of Al(OH)₃ and antigen – often at a dose of 1–10 µg/animal. It is clearly different from the IgE response in man, in whom sensitization is believed to occur through the airways. Marsh has estimated the amount of AgE inhaled per year to be in the order of 1 µg, and the amount actually extracted and absorbed could easily be 10–100 times smaller (56). Another point is the dose utilized in the animal experiments which is in the order of 100–500 µg of antigen per injection. This should be compared with human skin sensitivity where 100 pg of whole ragweed extract or 1 pg of AgE have been reported to elicit reactions in a large panel of ragweed-sensitive allergic patients (39). In immunotherapy, a maintenance dose (which can be reached only after several months of careful dose increments) of 100–200 µg of bee venom, 1–3 mg of whole pollen or animal dander extract, or 20 µg of AgE has been reported (54, 60, 66, 67 and own calculations).

Low-allergenic extracts

UV-light. Allergen extracts have been irradiated with light in the ultraviolet spectrum in order to diminish the allergenicity but not the antigenicity (4). Reduction of the allergenicity of house dust extract was demonstrated by intradermal skin tests and bronchial provocation tests but only a brief report of clinical effect in immunotherapy was provided (34).

Urea. The allergenicity of ragweed antigen E (AgE) was reduced about 10,000 times in direct skin tests when the protein was denatured with urea (40). In spite of the loss of allergenic determinants, subsequent animal studies showed that the denatured AgE had the capability of priming T cells with suppressor activity (35). However, when urea-denatured AgE was utilized for treatment of humans, no clinical improvement occurred, and the seasonal rise in IgE was only marginally suppressed (75, 80).

Formaldehyde. Marsh et al. prepared “allergoids” by treatment of Group I allergen from rye grass pollen with formaldehyde (57). The allergenic potency of these preparations was determined by means of the leucocyte histamine release assay, and less than 1% of the potency of the native extract was found. Still, the preparations were immunogenic in animals and crossreacted with guinea pig antibodies to native extract (33, 57). When native extract or two of these preparations were adsorbed onto alumina gel and injected in non-allergic humans, both IgG and IgE immune responses developed. The subjects developed skin sensitivity and leucocyte histamine release reactions to the allergen, but they developed no symptoms (58). The development of an IgE-response is likely to be caused by the use of alumina as adjuvant as discussed above. The treatment with formaldehyde has been modified to other allergens, and with ragweed it was shown that a polymerization occurs during treatment with both formaldehyde and glutaraldehyde. Both reagents produced material with a molecular weight of 100–500 kD, the main difference being that glutaraldehyde-treated extracts still contained some non-dialysable, low molecular weight material (59). A blinded clinical comparison between ragweed-allergoid and native extract showed a significantly lower symptom score among patients treated with allergoid. The rise in IgG-antibodies seemed to occur more rapidly in allergoid-treated patients (81). In a study including treatment of three groups with ragweed allergoid, native extract and placebo, the clinical outcome was similar in the first two groups and significantly better than the placebo group. Again, a higher IgG-response was observed in the allergoid-treated group, whereas the antigen-specific IgE showed no major changes (82).

Glutaraldehyde. In a series of studies glutaraldehyde was used for polymerization of AgE. It was shown that polymerized AgE reacted with antibodies raised in rabbits and that several molecular weight fractions were at least as immunogenic as the native allergen (84). The polymerized materials were slowly absorbed from skin injection sites and showed an aller-

genicity in leucocyte histamine release assay and skin tests respectively, that was 10–100 and 100–10000 times lower than that of native AgE (85). Among different molecular weight fractions the lowest induction of rabbit homocytotropic antibodies was found in a fraction of 200–4000 kD (86), which also expressed a long storage life (87). Different dose regimens of this fraction were compared with native AgE in a clinical study (60). Here it was demonstrated that higher doses (in terms of AgE content) of polymerized AgE could be given with fewer side effects. The symptoms in the two groups were comparable, whereas the IgG response was increased in a polymerized AgE-group treated with a tripling dose-increment regimen (60). In a later study Bacal et al. showed comparable clinical efficacy of native and polymerized whole ragweed antigen extract, whereas the latter produced fewer side effects (2).

Glutaraldehyde treatment of timothy grass pollen extract was compared with other procedures for modification: acetoacetylation by means of diketene, maleination or succinylation by means of maleic or succinic anhydrides, and guanidization by means of O-methyl isourea (64, 125). Although all reagents produced a depressed allergenicity and retained antigenicity, the most favourable allergenicity:antigenicity ratio was obtained with glutaraldehyde. Opposite to findings by Patterson's group (84), new antigenic determinants were introduced to a small extent by the different treatments (125). Different molecular weight fractions of glutaraldehyde-polymerized timothy extract have been compared. With 4 fractions of increased molecular weight a uniformly decreasing allergenicity and antigenicity was found (65), and in skin tests it was demonstrated that a fraction below 200 kD was 10^5 – 10^8 times more potent than a fraction of 200–2000 kD (29, 89).

Other allergen systems have been modified by means of glutaraldehyde: grass pollen mixture (88), bee venom (90), tree pollen (91) and house dust mite (71). The superiority compared with placebo has been established in a number of investigations for ragweed (25, 27, 31, 61) and grass pollen (26, 30). In a comparison between native aqueous grass pollen extract and

glutaraldehyde-polymerized extract patients noted a lower degree of improvement which coincided with a lower rise of IgG-antibodies in the polymer-group. Fewer side effects were recorded in this group (118).

Degree of modification. It should not be overlooked that covalent linking or even adsorption or emulga-tion of an allergen are likely to change the protein conformation and the availability of certain groups or sidechains. This is reflected in skin prick tests, where PEG-coupled extracts may show a biological activity considerably lower than that of the corresponding native extracts (42). However, most (but not all (51)) procedures for covalent coupling of allergens to polymers involve a preactivation of the non-immunogenic carrier before addition of antigen (41), and procedures have been developed avoiding cross-linking between antigen molecules (127, 128). Accordingly, the proteins in the covalently linked allergen extracts have preserved at least part of their ability to cross-link membrane-bound IgE leading to an allergic reaction.

This is contrary to low or non-allergenic preparations, for which the aims have been a profound reduction in the allergenic potency of the extract while still retaining the immunogenic properties, i.e. the ability to produce an IgG immune response upon injection (28). Thus, allergen preparations have been subject to conditions that may cause severe modifications of the proteins. Different reagents and procedures have been utilized for treatment: formaldehyde, glutaraldehyde, diketene, maleic anhydride, succinic anhydride and o-methyl isourea will all bind to primary amino groups, e.g. the ϵ -amino-group in lysine, while chaotropes like urea probably act via unfolding of the tertiary structure. Physical treatment like heat or UV-irradiation produces a variety of changes including disruption of intramolecular bindings with unfolding of the secondary and tertiary structure and possible destruction of histidine, tryptophan, tyrosine and cysteine (4).

Effects of modification: polymerization or epitope destruction? Many proteins tend to aggregate if

they become denatured, and this partly accounts for the increase in molecular weight that is often observed for modified allergens. Further, some of the reagents have a potential for cross-linking proteins. Moran & Wheeler have drawn attention to the importance of separating the effect of glutaraldehyde modification from the cross-linking which is also seen after glutaraldehyde treatment (64). Attempts to separate effects of glutaraldehyde-polymerization and mere aggregation have not been successful (65). From the data presented it seems that the highest reduction in allergenicity combined with retained immunogenicity has been achieved with material with a higher molecular weight than native allergen molecules, most of which are believed to be below 60 kD (56). Several explanations can be offered for the reduced allergenicity of these preparations. First, from stoichiometric considerations it follows that a smaller number of allergenic molecules is injected, and as each molecule probably only reacts with a few IgE-antibodies on a single cell, this will lead to a smaller reaction. Secondly, as shown by Patterson et al., the absorption from the injection site in the skin is reduced for polymers (85), providing the polymer with a depot effect and low diffusibility. At the same time this may reduce the number of mast cells and basophils having the possibility to encounter an allergen molecule and increase opsonization by macrophages, thus stimulating antigen presentation.

Even though modified allergens have greatly reduced allergenic potencies, they have often retained their antigenicity. It seems less likely that the relatively crude chemical modifications should selectively destroy epitopes towards which IgE is directed, leaving IgG-binding epitopes untouched. It should rather be emphasized that the antigen processing by macrophages might produce minor fragments of polypeptide chains that would resemble the IgE-binding epitopes of the native allergen. It should also be considered that even though a protein is modified in a way that reduces binding of the F(ab)-parts of the immunoglobulins, other receptors, e.g. the T-cell receptor, may still recognize it (35).

ALLERGEN-SPECIFIC IMMUNOPHARMACOLOGY

Passive immunization – administration of hyperimmune IgG

Due to the IgG immune response induced by IT, it has been hypothesized that administration of hyperimmune IgG *per se* might lead to symptom alleviation. Indeed, Cooke's discovery of blocking antibodies was based on observations that the transfusion of blood from hypersensitized persons to untreated hay fever patients reduced symptoms in 16 out of 20 cases (13). However, later investigations have not rewarded aspirations of passive IT. In a controlled study on hay fever patients reacting to ragweed, hyperimmune (anti-ragweed) IgG or ordinary IgG were injected (102). No differences were observed regarding skin reactivity or specific or total serum IgE, whereas a marginal reduction in nasal reactivity and basophil histamine release was noted in the hyperimmune IgG-treated group. These changes were normalized within 1–2 weeks after injection (102).

As mentioned above, injection of ragweed allergen extract into non-atopic subjects may lead to induction of an IgE immune response (62). Injection of hyperimmune IgG 1 week prior to until 2 weeks after injection of allergen eliminated the IgE response. However, no effect on IgE-response was seen if hyperimmune IgG was given 4 weeks after allergen (62). This indicates that specific IgG does not *per se* inhibit IgE synthesis, but must be combined with allergen either by acting as a blocking antibody neutralizing the injected allergen, or by the formation of a tolerogenic allergen-IgG complex *in vivo*.

Due to the high levels of bee venom-specific IgG that is often encountered in non-allergic beekeepers, preparation of hyperimmune IgG towards bee venom has been relatively simple. Jarisch administered hyperimmune IgG to a patient who had experienced anaphylactic shock after unsuccessful bee venom IT. In 2 weeks the treatment made the patient tolerant to allergen challenge, but no extended protec-

tion was reported (37). A number of investigators have tried to increase dosage or accelerate up dosing of bee venom by giving hyperimmune IgG prior to or concomitantly with bee venom injections. In some, but not all (130) cases, it has been possible to significantly increase the dose, leading to a reduced skin reactivity (47) or reduced side effects (7, 68). All investigations show that the diminishing of the reactivity is temporary, being equivalent to the catabolism of IgG in serum. A later IgG response caused by active immunization is not inhibited by administration of hyperimmune IgG. The same principle has been applied to ragweed allergy, where hyperimmune IgG was given prior to injections. It was possible to give higher doses of allergen to passively immunized subjects, thus leading to a better clinical result than in subjects receiving IT alone (3). The observed difference may not be solely attributable to the administration of IgG as a higher frequency of side effects in this group indicates a more aggressive dosing of the IgG-treated patients.

*Complexes between IgG and allergen:
a way forward?*

Several problems have emerged from the different forms of conventional IT, and from IT using altered allergen extracts. We shall briefly discuss a new approach taking into consideration two new concepts: i) utilization of *patient-related allergens* and ii) the use of *IgG-allergen immune complexes produced in vitro* for IT.

Different patients react to different allergenic components, and the content of an "average allergic patient extract" may not fulfil the needs of the individual patient. This problem has been approached by means of the idea of individually tailored patient-related allergens (109, 110). This concept involves purification of a commercially available allergen extract by means of the patient's own antibodies, which of course recognize only those allergen components to which the patient reacts. It has been shown that with IgE from an allergic patient it is possible to purify complexes between IgE and allergen (109). Further, by means of IgG from

a hyposensitized patient it has been possible to produce not only IgG-antigen complexes (110) but also patient-related antigens devoid of IgG (96). That should be applicable to the IgE system too, involving patients that have not previously been hyposensitized.

Thus, after firmly establishing the exact diagnosis of the patient, a blood sample is drawn from which patient-related (IgE-reacting) allergens are prepared. These are used for initiation of IT with careful increments of doses. Gradually, the patient develops an IgG immune response towards *only those allergens to which he or she had IgE*, and by means of a new blood sample complexes between IgG and the patient-related allergen are prepared. These complexes are used for continuation of the treatment; if necessary, the production of IgG-allergen complexes is repeated. It could be argued that the patient might not develop IgG towards all allergens injected, but preliminary investigations made in our laboratory (111) suggest that the IgG-response is always "broader" than IgE response.

If a person expresses multiple allergies, several allergen extracts could be used for the preparation, thus giving an extract exactly matching the complex antibody pattern of the multiallergic patient.

Amount of allergen. A crucial point of the approach is the amount of purified allergen to be produced from human antibodies. It has been estimated that it is possible to produce in the order of 120 ng of allergen/ml serum in the form of IgE complexes (109). Further, it has been estimated that from 100 ml blood (50 ml serum) about 5 µg of allergen devoid of IgG could be produced (Poulsen, unpublished results). It should be pointed out, however, that these estimates are based on the weight of materials with unknown biological activity. Evaluation of the method should be based on biological activities of the preparations, and such studies are in progress.

Rationales for utilization of IgG-allergen complexes. The idea of using IgG-allergen complexes stems from the fact that these complexes have several

features in common with the more successful attempts to alter allergen extracts, i.e. the glutaraldehyde- and formaldehyde-modified extracts. The molecular weights of these complexes have been determined to be in the range of 100 to 1000 kD (96), and thus the diffusibility and availability of the complexes to mast cells in the skin will be reduced as discussed above. Further, these complexes would be suspected to have low allergenicity *per se*, as several antigenic determinants are covered by IgG. The exact mechanism by which IT acts is not known. It is conceivable, however, that the allergens should be presented to the immune system in order to make the protection systemic. In this context, it could be suspected that immune complexes would easily be opsonized by macrophages, thus favouring antigen presentation. We have made a comparison between active, passive and combined (IgG-allergen complexes) IT in a guinea pig model, in which the animals could be inflicted with allergic asthma and monitored clinically (53). The results show that whereas passive IT did not clinically improve the animals, both active IT (comparable to conventional IT) and IgG-allergen complexes had a striking effect on the asthma elicited by allergen provocation (Poulsen et al., in press).

In investigations by Filion et al. it was found that in mice, OA covalently coupled to autologous IgG suppressed both the primary and secondary IgE response (19). Earlier we have emphasized that animal models in which IgE synthesis is suppressed, may not be applicable to human IT, partly because of the larger doses used in animal experiments. It is possible, however, that a combination of specific allergens with non-specific immunopharmacological agents could overcome this problem, and manipulate the cell clones responsible for the synthesis of allergen-specific IgE. Cyclophosphamide and cyclosporin A are drugs now utilized in rheumatology and transplantation immunology, and it is conceivable that immuno-modulatory agents more restricted to the IgE system will be available in future (36). Allergen-specific immunopharmacology will necessitate allergen preparations closely matching the needs of the individual patient, and in

this context patient-related allergens might offer a possibility.

Possible side effects of IgG-allergen complexes. It might be argued that when injected in the skin, IgG-immune complexes would lead to a Type III reaction. Whether this will happen probably depends on the size of the complexes. In conventional IT some circulating immune complexes (CIC) could be suspected to form *in vivo*. However, no higher levels of CIC have been found in patients undergoing IT, even though the majority of investigators find that, in general, atopic subjects have higher levels of CIC than non-atopic individuals (10, 12, 48, 106, 107, 129). The lack of Type III reaction caused by injected IgG-allergen complexes was confirmed in guinea pigs, in which immune complexes produced from hyperimmune IgG and an experimental allergen did not produce Arthus-like reactions, even though they were administered for an extended period of an animal lifetime. However, on the injection sites reversible formations of small granulomas were observed, comparable to those seen when Al(OH)₃-gel is injected (Poulsen et al., unpublished results).

If some of the IgG subclasses are homocytotropic in nature, complexes between allergen and IgG could cause degranulation by combination with and crosslinking of the putative Fc γ -receptors on the surface of mast cells or basophils. IgG₄ has been suggested to have homocytotropic properties (120, 121) but these results have not been confirmed by us (95) or others (115, 116).

CONCLUSION

Apart from seeking better forms of therapy, many investigations have also revealed interesting features of the allergic reaction, thus leading to a better understanding of some of the basic allergic mechanisms. However, in spite of almost 80 years' experience with IT, we still do not know the mechanisms by which symptom alleviation occurs. From the literature reviewed in this article it seems reasonable to conclude that no form of IT has yet been developed that will cause clinical improvement due to a suppression of the

IgE biosynthesis, even though this goal has been readily achieved in a number of animal models.

Modifications of allergen extracts by means of glutaraldehyde or formaldehyde have shown promising results in reducing side effects, but more controlled studies, in which conventional IT is used for comparison, should be presented. There are indications that the aldehyde-modified extracts largely owe their reduced allergenicity to an increased molecular weight, giving them a form of depot effect. Taking this into consideration, we have suggested an approach in which the patient's own antibodies are used for preparation of individually tailored allergen extracts for IT. From a theoretical point of view, this concept presents several advantages, and this has been confirmed in animal experiments. However, it has become increasingly clear that confirmation from studies in humans is necessary before a new form of IT should be considered as an improvement on the existing armamentarium.

IT has been performed since 1911, and its efficacy has been documented. Standardization of allergen extracts has improved tremendously during the last 10–15 years, even though most extracts are standardized only with respect to the major allergens. Evaluation of the efficacy of new preparations or regimens for IT today relies heavily on the clinical monitoring, as immunological measurements of F(ab)-specificities of IgE and IgG are unreliable predictors of the outcome of the treatment. To speed up the development of new and improved forms of IT it is important to disclose immunological parameters more closely related to the hitherto unknown mechanism behind IT. Obviously needed in this context are investigations of cellular receptors and functions.

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