

Microencapsulation

The Future of Oral Immunotherapy?

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Summary

Subcutaneous immunotherapy has been used successfully to treat atopic diseases for many years. However, its use is associated with several problems, the most bothersome being systemic allergic reactions. This has led to evaluation of alternative methods of treatment, including oral immunotherapy. For the most part, oral administration of allergens has not proven an effective substitute because of the lack of potency. Extraordinarily large oral doses were required to achieve clinical improvement, but even these doses were ineffective with some allergens.

To resolve this difficulty, we have developed a novel system of encapsulating allergens which protects them from the destructive effects of gastric acid and pepsin. Most importantly, encapsulated allergens administered orally to mice elicited vigorous systemic immune responses, with stimulation of predominantly CD4⁺ T helper type 2 lymphocytes.

Three studies of encapsulated short ragweed pollen extracts (eSRW) in short ragweed (SRW)-sensitive humans have been done. Treated patients developed high titres of SRW IgG antibodies, had blunting of the expected anamnestic increase in SRW IgE antibodies, downregulated the expected seasonal increase in nasal SRW IgA antibodies and had a decrease in nasal sensitivity to SRW on provocative testing. Symptoms were significantly decreased in treated patients if they could tolerate a high dose of eSRW. This was accomplished without inducing any systemic reactions, with an oral dose only slightly higher than that used in high dose subcutaneous immunotherapy.

These encouraging results suggest that encapsulated allergens have the potential to become important therapeutic agents in immunotherapy.

The objectives of this review are to examine the potential of oral allergen immunotherapy to treat allergic diseases, the rationale for considering it as an alternative to subcutaneous immunotherapy, and how the use of encapsulated allergens may eventually make oral immunotherapy the preferred mode of immunotherapy.

Subcutaneous allergen immunotherapy has been in use since 1911. Its efficacy has been confirmed in patients with allergic rhinitis, allergic

asthma and in individuals with anaphylactic sensitivity to hymenoptera stings.^[1-3] Despite the recent introduction of many potent pharmacological agents which can significantly alleviate allergic symptoms, interest remains high in this treatment as it is the only biological disease modifier that can produce long term improvement of allergic diseases, thereby reducing the need for pharmacological intervention. Furthermore, the salutary effects of immunotherapy usually persist after its discon-

tinuation.^[4] The classic technique for subcutaneous allergen immunotherapy consists of subcutaneous injections of allergen extracts at frequent intervals (usually weekly or biweekly) with small increments in dose with each injection until the highest tolerated dose or an arbitrary maximum dosage is reached. Although this treatment usually results in a significant decrease in allergic symptoms, they do not necessarily entirely disappear.

1. Immunological Effects of Immunotherapy

The mechanism(s) responsible for the observed decrease in symptomatology with subcutaneous immunotherapy has been and remains a contentious issue. The earliest reported immunological change associated with immunotherapy was an increase in allergen-specific IgG antibodies.^[5] These antibodies were called 'blocking' antibodies and were thought to bind the inhaled allergen before it could reach the mast cell surface and trigger the release of mast cell mediators by cross-linking of allergen-specific IgE antibodies bound to the high affinity FcεR1 receptors on the mast cell surface.^[6,7] The dilemma with 'blocking' antibodies was that some patients with a successful clinical outcome with immunotherapy had low titres of allergen-specific IgG antibodies, whereas other patients with high IgG titres had a poor result, implying that other immune mechanisms could be playing a part in generating the observed clinical benefit.^[8] Also, it was reported that some improvement was seen with immunotherapy before any rise in IgG antibodies could be detected.^[9] More recently, emphasis has been given to increases in antibodies in the IgG4 subclass of IgG as the major source of blocking antibodies. Although this interpretation of IgG4 antibodies is disputed, this is the principal subclass of IgG antibodies which increases during long term immunotherapy.^[10-12]

Two further potential roles for immunotherapy-induced IgG antibodies in producing clinical benefit are the inactivation of mast cells by cross-linking of FcγRII and FcεRI receptors on the cell surface, and the attenuation of the late phase of the

allergic reaction.^[13,14] This is the phase to which many investigators attribute the inflammatory component of atopic diseases, particularly asthma.^[15]

However, regardless of the actual role IgG antibodies play in reduction of symptoms by immunotherapy, a successful immunotherapy regimen, when conventional allergens are used, is always accompanied by a vigorous IgG antibody response.^[16]

There are many other immunological phenomena associated with the administration of subcutaneous immunotherapy which could contribute to the observed benefit. These include:^[12,17-24]

- abrogation of the expected seasonal anamnestic increase in specific IgE antibodies
- decrease in *in vitro* allergen-specific peripheral blood T cell proliferation
- decrease in histamine-releasing factors
- decrease in histamine release from peripheral blood basophils by specific allergen
- reduction of the late-phase skin test reaction
- eventual decrease with time in levels of allergen-specific IgE antibodies
- inhibition of allergen-induced mediator release
- decrease of tissue eosinophils and mast cells.

Most recently, emphasis has been given to the potentially beneficial changes in the T cell cytokine profile induced by immunotherapy. Atopic diseases are classic examples of the T helper cell types 1 and 2 (T_H1/T_H2) paradigm.^[25] Prior to initiation of immunotherapy, the cytokine profile detected in studies with stimulated peripheral blood T lymphocytes from atopic patients is that of a typical T_H2 pattern with production of interleukins (IL)-4, -5, -9, -10 and -13 and low levels of IL-2 and interferon-γ.^[25,26] With long term immunotherapy, a change to a partial T_H1 pattern may occur, with production of both IL-4 and interferon-γ.^[27,28] It is most likely that immunotherapy induces the development of allergen-specific T_H1 cells from the undifferentiated population of CD4⁺ T cells, as the original highly polarised T_H2 cells are probably resistant to change in phenotype.^[29] This shift in cytokine production is modulated by the generation of IL-10, predominantly by T_H2 cells.^[30] The

presence of IL-10 prevents excessive activation of T_H1 cells, and, thus, would preclude long term immunotherapy from inducing an autoimmune disease. The outcome is a mixed cytokine pattern. This altered cytokine profile may be involved in producing many of the immunological alterations associated with immunotherapy that are listed above.

2. Limitations of Standard Subcutaneous Immunotherapy

The major adverse effect of standard subcutaneous immunotherapy is the induction of systemic allergic reactions. These can be severe enough to result in an occasional fatality.^[31] Other lesser concerns are its relative high cost because of intensive usage of medical personnel for administering injections, the inconvenience of weekly injections and the length of time required to reach a therapeutic dose.

3. Alternatives to Standard Subcutaneous Immunotherapy

To overcome the limitations of subcutaneous immunotherapy as listed in section 2, investigators have pursued 2 alternative approaches.

The first is to modify chemically the structure of the allergens used in subcutaneous immunotherapy to decrease their reactivity with specific IgE antibodies bound to mast cells while maintaining their ability to stimulate production of specific IgG antibodies. Examples of such modifications include aggregation of allergens by glutaraldehyde or chemical modification by formaldehyde.^[32,33] This approach has not as yet yielded an approved immunotherapeutic product in the US.

The other alternative is to change the route of allergen administration, in particular to an oral form. With oral immunotherapy, the administration of allergens is simplified, and the involvement of medical personnel with treatment is curtailed. Up to 1986, many uncontrolled studies of oral immunotherapy were done with variable results and claims (reviewed in Taudorf^[34]). However, when

double-blind placebo-controlled trials of oral immunotherapy were performed, the potency of orally administered allergens was called into question. In 2 placebo-controlled studies in Sweden with oral birch pollen, the oral allergen dose needed to produce statistically significant improvement proved to be extremely high, up to 200 times the cumulative subcutaneous immunotherapy dose for birch pollen extract.^[35,36] Increases in birch pollen-specific IgG antibodies did occur, but were less than the levels usually attained during subcutaneous immunotherapy.^[37] Serum birch pollen-specific IgE antibodies rose prior to the birch pollen season with oral immunotherapy, and seasonal blunting of further rises of birch-specific IgE antibodies did occur in the treated patients. Some of these studies were performed with the oral birch pollen extract encased in enterosoluble capsules, but, surprisingly, this did not affect the requirement for very high doses. The reason for the lack of protection of the allergen contained within enterocoated capsules is not apparent.

In a study with oral ragweed pollen extract conducted in the US, the dose requirement for definite clinical improvement with increases in allergen-specific IgG antibodies was also excessive, 100 times the cumulative subcutaneous dose.^[38] During one oral immunotherapy study with dust mite extracts, 5 times the cumulative subcutaneous dose was required to induce a significant beneficial effect on patients with mite-related rhinitis and asthma.^[39] In the majority of studies with grass pollen extract and in one study with cat allergen, even high doses have been ineffective.^[40-43]

One encouraging finding in the successful double-blind studies of oral immunotherapy was the rare occurrence of systemic reactions despite using very high oral doses. Nonetheless, oral immunotherapy seemed economically impractical because of the high dose requirement, and of equivocal effectiveness as only a few allergens could be administered by this route with the expectation of clinical success.

No studies of the changes in cytokine profile induced by oral immunotherapy have been performed as yet.

4. Premise for the Use of Encapsulated Allergens in Oral Immunotherapy

We theorised that the requirement for excessive oral doses of allergen extract with oral immunotherapy is because of degradation of the allergen extract proteins in the hostile environment of the stomach by low pH and pepsin, which is followed by further digestion by enzymes in the small intestine. Based on the previous discussion of the immune events associated with immunotherapy, subcutaneous immunotherapy with conventional allergens results in active immunity to the injected allergen. To initiate activation of the immune system in the gut, we postulated that a protein had to be presented unaltered in its native conformation, and in adequate local concentration, to Peyer's patches. To establish the validity of these hypotheses we performed a number of studies to explore the role of gastric digestion on the ultimate immunological outcome to a fed antigen.

First, we showed that pepsin digestion of ovalbumin, bovine serum albumin, honey bee venom phospholipase A and short ragweed pollen extract under conditions comparable to gastric digestion resulted in the generation of small antigenic fragments. Whether administered to mice intraperitoneally, intravenously or orally, these fragments induced a tolerogenic response rather than an immunogenic one.^[44-46] The mechanism for tolerance induction by antigenic fragments has not been elucidated as yet.

A second line of evidence for the important role of gastric activity in induction of oral tolerance came from experiments in which mice were fed ovalbumin, but in whom the digestive effects of the stomach were bypassed by H₂ blockade of gastric acid with cimetidine.^[47] This resulted in priming the animals to respond partially with a low level of antibody production to an injection of ovalbumin. Control mice, which were fed water and injected with ovalbumin, made a more substantial immune

response. Other control mice, which did not receive cimetidine but were fed ovalbumin and then challenged, made no response to an injection of the antigen, indicating the induction of oral tolerance.

The final and the most convincing confirmation of the importance of gastric digestion on the immunological outcome of a fed antigen was provided by experiments in which either native bovine serum albumin or peptic fragments of bovine serum albumin were injected directly into the ileum of mice. Ileal injection of native bovine serum albumin induced an immune response in the mice, whereas direct injection of pepsin-derived fragments of bovine serum albumin into the ileum produced immunological tolerance.^[48]

The tolerisation of CD4⁺ cells, particularly T_H1 cells, by pepsin-induced allergenic fragments could be an adverse occurrence for a successful outcome with immunotherapy. Thus, when an allergen, which lacks protection from gastric digestion, is fed, a mixed signal is given to the immune system. Gastrically created allergen protein fragments may generate immune tolerance to their contained allergenic epitopes, whereas the surviving intact allergenic proteins would engender active immunity to them. Also, the intact proteins may have some difficulty achieving sufficient local concentration for activating the gastrointestinal mucosal immune system because of the dilutional effect of gastric secretions. Therefore, feeding allergens which are unprotected from gastric digestion would produce a suboptimal outcome for oral immunotherapy. In addition, this impaired immune response explains the necessity for using large doses of oral allergen for an effect with oral immunotherapy.

5. Development of Encapsulated Allergens and Antigens

To obtain an immunogenic signal by oral administration of allergens/antigens, several approaches have been taken, including the use of mucosal adjuvants such as cholera toxin, incorporation of the allergens into genetically engineered live bacterial vectors, integration into liposomes and presenta-

tion in biodegradable microspheres.^[49-53] We have elected to take the last approach to avoid the toxicity of the known mucosal adjuvants such as cholera toxin, the potential difficulty of including allergens in bacterial genomes and the instability of liposomes.

The technique developed by us for encapsulating allergens and antigens involves a water-soluble system with a pH-dependent coating which protects the contents during passage through the harsh acidic environment of the stomach. Upon passing into the duodenum, the encapsulated allergens/antigens are released explosively in a 'burst' in sufficient amounts locally to initiate an immune response in Peyer's patches. Briefly, allergens/antigens are solubilised in a solution containing polyvinylpyrrolidone as a binding agent. This solution is then spray coated onto a sugar/starch core using a Wurster bottom spray process. Then, the allergen/antigen-covered cores are enterocoated with an aqueous dispersion of a pH-sensitive methacrylic acid copolymer (Eudragit L30D). The resulting encapsulated allergens are protected from dissolution at pH 1.2 (gastric pH) for 24 hours but dissociate rapidly at pH >5.5, releasing the allergens in their native state. Most importantly, this procedure avoids the use of organic solvents which frequently denatures allergens and antigens. This is a major hurdle for many biodegradable encapsulation processes such as those which employ DL-lactide-co-glycolide.^[54] Thus, the final product is able to evade the destructive events in the stomach and permits the allergenic/antigenic proteins to be presented in their native state and in high concentration to Peyer's patches located in the duodenum and elsewhere in the intestine.

Murine studies with a variety of orally administered antigens/allergens encapsulated by this technique, including ovalbumin, bovine serum albumin and a number of inhalant allergen extracts, have confirmed that such encapsulation produces significantly enhanced immunological potency when these antigens/allergens were given orally. Vigorous primary and secondary responses were elicited.^[54,55] Even previously established immune

tolerance to ovalbumin was abrogated by an oral challenge with encapsulated ovalbumin.^[56] The murine serum antibodies induced by orally fed encapsulated antigens/allergens in mice were composed of IgG1, IgE and IgA immunoglobulin classes and subclasses, implying that encapsulated antigens/allergens given orally predominantly activate CD4+ T_H2 lymphocytes.^[57] Also, antigen/allergen-specific secretory IgA antibodies were stimulated in the gastrointestinal tract of the mice.^[55] Furthermore, antibody-secreting cells measured by the ELISPOT assay, and induced by orally administered encapsulated ovalbumin, were found more frequently in the Peyer's patches located in the proximal third of the small intestine, although they were present in lesser numbers in the lower small intestine.^[57] This is in accord with the dissolution characteristics of the encapsulated allergens. Murine ovalbumin-specific B and T lymphocytes originating in Peyer's patches migrated to mesenteric lymph nodes, the spleen and the gastrointestinal lamina propria, in accordance with the concept of the common mucosal immune system.^[57,58]

It is probable that the exceptional potency of this novel system of antigen/allergen encapsulation involves additional factors beyond evasion of gastric destruction and maintenance of native antigen/allergen structure during manufacture. The study with cimetidine in mice with an oral unencapsulated antigen (section 4) detected low antibody titres which were considerably less than those usually elicited by encapsulated antigens.^[47] Also, enterocoated capsules containing antigens or allergens have not proven very successful.^[36] As yet untested hypotheses to explain the increased potency of encapsulated antigens/allergens include:

- encapsulated antigens/allergens may retain a partial particulate state after dissolution
- antigen/allergen uptake is facilitated by core components such as polyvinylpyrrolidone
- the 'burst' of antigen/allergen release is so compact that a very high local antigen/allergen concentration is available at a Peyer's patch.

6. Clinical Studies with Orally Administered Encapsulated Short Ragweed Pollen Extract

The achievements with oral immunisation with encapsulated antigens/allergens in mice described in section 5 led to consideration of encapsulated allergens as immunotherapeutic agents for treatment of human atopic diseases. Initial experiments in mice demonstrated that encapsulated short ragweed pollen extract (eSRW), dispensed orally to mice, was capable of producing potent primary immune responses in naive mice and significant secondary immune responses in mice that had been primed with short ragweed pollen extract (SRW) administered intraperitoneally in alum.^[55] The preparation of eSRW used in these and subsequent studies showed the typical dissolution profile of an encapsulated antigen/allergen, releasing the SRW proteins in solutions with pH >5.5. The released proteins were in their native form as confirmed by RAST inhibition, ELISA inhibition and isoelectric focusing patterns. Repeated testing of eSRW stored at 4 and 21°C showed that the Amb a 1 component of eSRW, which is the major protein allergen present in short ragweed pollen, was stable for more than 4 years. We next attempted to employ this oral encapsulated allergen as an immunotherapeutic agent in atopic humans with ragweed allergic rhinoconjunctivitis (hay fever).

In a phase I open clinical trial, 9 ragweed-sensitive patients were treated with oral eSRW containing doses up to 36µg of Amb a 1 (doses of eSRW in this review are expressed in terms of the major allergenic protein contained in short ragweed pollen, Amb a 1).^[59] The effectiveness of this treatment both in serological results and symptom/medication scoring was gauged by comparison with the results obtained in a group of 9 matched SRW-sensitive patients who received no treatment. Treated patients developed high titres of SRW-specific IgG and IgE antibodies prior to the ragweed season. The average increase in IgG antibodies was 440% from baseline to peak titre. The large increase in SRW IgE antibodies observed

prior to the ragweed pollination season is an anticipated occurrence in immunotherapy studies employing potent allergens.^[60] Also, during the ragweed season, the expected seasonal anamnestic increase in serum SRW IgE antibodies was blunted. There was no change seen in IgG titres of control individuals. The mean highest daily dose of eSRW (27.8 µg of Amb a 1) required to achieve these effects was only slightly higher than the dose of SRW used in high dose subcutaneous immunotherapy (20 to 24 µg of Amb a 1). Doses greater than 30µg of Amb a 1 caused a disproportionate incidence of gastrointestinal adverse effects. The maximum dose was achieved within 7 weeks and the treated patients experienced no reactions other than mild gastrointestinal ones. In this open study, the 9 treated patients fared clinically better during the ragweed pollination season than the untreated patients.

One explanation suggested for the lack of systemic reactions in this study was that the onset of the rise in SRW IgG and IgE antibodies was simultaneous in 7 out of 9 patients. This is contrast with patients treated with subcutaneous SRW immunotherapy, in whom the onset of rise in SRW IgE antibodies preceded the rise in SRW IgG antibodies by several weeks.^[60] This delay in the development of IgG blocking antibodies associated with subcutaneous immunotherapy exposes patients to a heightened risk of systemic reactions during this period of time.

These positive results from the first investigation encouraged us to perform a double-blind placebo-controlled trial the following year.^[61] 21 SRW-sensitive patients were treated with oral eSRW while 20 matched SRW-sensitive patients received oral placebos. Doses were given daily. The average highest dose was 23.8µg of Amb a 1 (24µg was the contemplated highest dose). The treated patients developed high titres of serum SRW IgG antibodies (geometric mean 1.15 mg/L at baseline increasing to 21.2 mg/L at the peak), as well as increased SRW IgG4 subclass antibodies in 14 of 21. The magnitude of the increases in SRW IgG antibodies found in this study and the previous

one is comparable with the increases in IgG antibodies found in studies with subcutaneous immunotherapy when a comparable IgG antibody assay is used (ELISA).^[12] Placebo-treated individuals had no change in their IgG titres. Furthermore, the treated patients developed increased titres of allergen-specific IgE antibodies (prior to the pollination season), and experienced regulation of the expected seasonal increase in SRW IgE antibodies (9% increase in treated patients, compared with 59% increase in placebo-treated patients; $p = 0.009$). Although only the treated patients made a small amount of nasal secretory SRW IgA antibodies prior to the ragweed pollination season, during the season the natural exposure to ragweed pollen produced very large increases of SRW nasal secretory IgA antibodies in both groups of patients (117% increase in treated patients, compared with 717% increase in placebo-treated patients; $p = 0.01$). This downregulation of the seasonally induced increase in allergen-specific secretory IgA antibodies with eSRW treatment may be relevant in treatment of the mucosal atopic diseases, as secretory IgA antibodies are the most potent of the immunoglobulins for inducing eosinophil degranulation.^[62] Despite insubstantial pollen counts during the ragweed pollination season (the lowest pollen counts recorded in a period of 10 years in Cincinnati), the symptom/medication scores in the treated patients were less during the peak weeks of the ragweed pollination season in the treated patients than in the placebo group (4.28 versus 6.18; $p = 0.059$). These differences became statistically significant ($p = 0.03$) in the subgroup that could tolerate the highest dose of eSRW, $>20\mu\text{g}$ of Amb a 1, which included 19 of the 21 treated patients. Gastrointestinal adverse effects occurred in only 4 patients. Two of these eventually tolerated the highest dose of eSRW. No systemic reactions occurred. Again, the onset of the rise in serum SRW IgG and IgE was concurrent in 16 of the 21 treated patients. While SRW IgE antibodies did rise initially in 4 patients, their SRW IgG antibodies began to increase during the next 2 weeks.

Another double-blind study of eSRW administration to ragweed-sensitive patients was performed at the University of Pittsburgh, where nasal provocation studies with SRW were also performed.^[63] In this study, 10 patients received eSRW with a slightly more aggressive administration schedule (highest dose $24\mu\text{g}$ of Amb a 1) and 9 patients received placebo. At the end of the study, treated patients showed statistically significantly less symptoms and signs following intranasal SRW challenge ($p < 0.05$). In this small study, the treated patients had fewer symptoms during the ragweed season than placebo-treated patients, but this difference did not reach statistically significant levels. Again, SRW IgG and IgG4 levels rose with treatment without any change occurring in placebo-treated individuals. No reactions occurred during treatment except gastrointestinal ones (25% of the treated patients).

7. Conclusions

Can encapsulated allergens at the present be considered the future mode of oral immunotherapy or, eventually, even the replacement for subcutaneous immunotherapy? It is obviously premature at this time to attempt to make any such inference. In the second clinical trial discussed in section 6, the 19 of 21 patients who received high dose oral immunotherapy ($>20\mu\text{g}$ of Amb a 1/dose) had significant improvement in their symptom/medication scores. This confirms the need for high dose immunotherapy for success with potent oral allergens, which has been the case for subcutaneous immunotherapy.^[64,65] The third study had too few patients included and thus too low a statistical power for a positive clinical result to be detected. Obviously, more double-blind studies will be required to establish the therapeutic adequacy of oral encapsulated SRW extracts, as well as studies which encompass other major allergens such as grass pollen and dust mites.

Some of the immunological changes that occur during subcutaneous immunotherapy, as enumerated above, were found during oral immunotherapy with an encapsulated allergen. High titres of

IgG-blocking antibodies were detected, which were quantitatively comparable with those found in studies where subcutaneous immunotherapy was employed. Production of IgE antibodies was stimulated by treatment, and the seasonally incurred increase in allergen-specific IgE antibodies was blunted.^[12] In addition, it was shown that:

- this new encapsulation technique preserves the allergenic epitopes, at least those in SRW
- allergen-induced nasal reactivity diminishes with treatment
- there is a unique partial suppression of the seasonally induced increase in nasal allergen-specific secretory IgA
- the dose of oral allergen needed to produce these immunological changes and clinical benefit was only slightly higher than that used in high dose subcutaneous immunotherapy.

This last finding is very different from the experience with unencapsulated allergens. Also, the time to reach the highest oral dose was short (7 weeks). Finally, and very significantly for the widespread application of oral immunotherapy with encapsulated allergens, administration of this improved oral immunotherapeutic agent was not accompanied by any systemic reactions. More than 40 patients have received this new oral allergen without any reactions other than mild gastrointestinal ones. In a review of 20 published studies of conventional subcutaneous immunotherapy, 12.9% of the patients developed at least one systemic reaction during their course of treatment.^[66] With rush immunotherapy and with very sensitive patients, the incidence was even higher.

Thus, based on the data generated at present, encapsulated allergens do have the potential to become important therapeutic agents in immunotherapy. But, as stated throughout, many more clinical and immunological studies, including observations of the changes induced in the CD4⁺ profile of cytokines by oral immunotherapy with encapsulated allergens, must be performed before such optimism translates into reality.

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