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Allergen specific immunotherapy: is it vaccination against toxins after all?

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Abstract

IgE-mediated allergies, in particular allergic rhino-conjunctivitis and asthma, have reached epidemic proportions, affecting about one third of the population in developed countries. The

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most effective treatment for allergies is specific immunotherapy (SIT), which involves the injection of increasing doses of an allergen extract to allergic individuals.

The current form of SIT was first introduced 1911 and recently celebrated its 100th birthday for the treatment of hay fever. The concept of this therapy at the time was straight-forward, since it was believed that pollen contained toxins against which the patient could be vaccinated. However, the understanding became blurred with the discovery that IgE antibodies were the effector molecules of the allergic response. Subsequent research focused on the idea that SIT should induce tolerance keeping the IgE antibodies at bay. In this review, we will discuss the various hypotheses for the mechanism of SIT and we will put forward the concept that allergens may be viewed as “protoxins” which need to be activated by IgE antibodies. Within this framework, protoxin-neutralizing antibodies are the key effector molecules while a shift to Th1 or Treg cells mainly contributes to the efficacy of SIT by helping B cells to produce neutralizing IgG antibodies.

Box 1:
A short history of Specific Immunotherapy

The birth place of allergen-specific immunotherapy was in England. There, since the age of 8 years physician John Bostock suffered every June and July of a whole basket of symptoms. These included nasal discharge, paroxysms of sneezing, itching of the eyes, difficulty of breathing and malaise. Recognizing that this was a syndrome distinct of common cold he described the first case of hay fever in 1819¹. At the time Bostock did not relate his condition to pollen but believed that its seasonal incidence was due to physical factors, such as temperature. He therefore named the disease catarrhus aestivus, summer catarrh. The discovery that summer catarrh was caused by

pollen was later made by Charles Blackley, another English physician who himself suffered from hay fever. Blackley collected grass pollen in summer and stored them in a bottle. In winter, he then opened the bottle and inhaled the pollen, which immediately caused the symptoms². He concluded that toxins in the pollen were the cause of hay fever.

Based on these important discoveries and the idea that hay fever was a toxin-mediated disease, in 1900 Henry Holbrock Curtis, an American researcher, tried to induce immunity by subcutaneous injections of pollen from flowers and ragweed³. The project had to be discontinued, however, due to significant side-effects. At the Pasteur Institute in Paris in 1907, the Ukrainian-French biologist and physician Alexandre M. Besredka was the first to establish an experimental animal model, where he could protect animals against anaphylaxis by repeatedly injecting them with a diluted dose of the substance causing the anaphylaxis, a procedure he called “anti-anaphylaxis”^{4,5}. In Hamburg, the American physician William P. Dunbar in 1903 adapted the concept of protective immunity to the treatment of hay fever^{6,7}. First, he showed that a local reaction induced by pollen grains could be imitated by an extract of pollen in alcohol or brine. He then developed a hyperimmune serum in animals vaccinated with pollen extract and subsequently treated hay fever patients with this animal serum, sold as nasal drops called “Pollantin”. By this antibody-based immunotherapy, Dunbar was able to “cure” some patients, but “admittedly in exceptional cases”⁸.

It was in this environment where the English physician Leonard Noon in 1908 began investigating the causes and treatment of hay fever. Three years later, Noon then reported in the *Lancet* the first successful prophylactic inoculation against hay fever, a publication that today is considered the birth of allergen-specific immunotherapy⁸. Unfortunately, Noon died soon

thereafter at young age from tuberculosis, but his work was continued by his colleague John Freeman, who published a follow-up article also in the Lancet in 1911⁹. The concept behind these studies is clearly explained in the first part of Noons paper. By quoting the work of Bostock, Blackley and Dunbar, Noon describes that hay fever is a catarrh of the mucosa and caused by soluble toxins contained in the pollen (Fig. 1). With respect to the work of Dunbar, Noon appreciates the use of passive immunotherapy with “Pollantin” as a “reasonable method of treatment, but one which is difficult and laborious, and one which is not calculated to bring a permanent cure⁸.” Noon therefore postulates that “on general grounds a much more satisfactory result would be expected from the induction of an active immunity and it seemed worth while to put this expectation to the test of the experiment⁸.” Noon and Freeman were two young doctors working at the Inoculation Department at St. Mary’s Hospital in London, which had been established by Sir Almroth Wright who was pioneering the development of active prophylactic, rather than passive, vaccination against infectious diseases, focusing particularly on typhoid¹⁰. Thus, allergen immunotherapy was introduced the same way as any other prophylactic vaccine.

Introduction

Allergic rhinitis has a relatively short history (Box 1). Indeed the first documented case of hay fever was 1819. John Bostock, a physician who himself suffered from the hay fever called the disease catarrhus aestivus, summer catarrh. At the time, this catarrhus aestivus was a rare disease¹¹ and after reporting his own case, it took John Bostock nine more years to find another 28 cases for publishing a second article¹². This has dramatically changed. Today, IgE-mediated

allergies such as allergic rhinoconjunctivitis and asthma have reached a worldwide prevalence of 25%¹³, and are therefore called ‘the new epidemics of the 21st century’¹⁴. This increase in prevalence is associated with improved hygiene, which led David P. Strachan in 1989 to introduce the hygiene hypothesis¹⁵. In essence, it is postulated that stimulation of the immune system by infection with bacteria or parasites protects from developing IgE mediated allergies¹⁶. In addition, epigenetic changes have been shown to reduce the risk of developing allergies¹⁷.

Specific Immunotherapy: How it’s currently performed and how it might be improved

There are a number of possibilities to treat IgE mediated allergies, mostly consisting of symptomatic medications such as anti-histamines or corticosteroids. However, the only disease modifying therapy is allergen-specific immunotherapy (SIT). Allergen immunotherapy is defined as the repeated administration of specific allergens to patients with IgE-mediated allergies for the purpose of providing protection against the allergic and inflammatory reactions associated with natural exposure to the same allergens^{18,19}. SIT was not widely accepted for many years until its efficacy in the treatment of both allergic rhinoconjunctivitis and allergic asthma was demonstrated in appropriate double-blind, placebo-controlled trials. Today, allergen-specific SIT has become an established treatment option against respiratory allergies and hymenoptera venom allergy^{18,19}.

In addition to the method of subcutaneous allergen immunotherapy (SCIT) introduced by Noon and Freeman, other routes of allergen administration have been investigated. Local nasal immunotherapy (LNIT) and local bronchial immunotherapy (LBIT) were reported not safe or ineffective²⁰. In the 1920ies and then again in the 1960ies, scarification of the skin with allergens

was successfully performed to suppress the distressing symptoms of hay fever and asthma²¹⁻²⁴. Such epicutaneous allergen immunotherapy (EPIT) recently celebrates a revival after efficacy was demonstrated in double-blind placebo-controlled clinical trials²⁵⁻²⁹. Another novel approach which appears to produce rapid symptom amelioration is the direct intralymphatic administration of allergens (ILIT)³⁰⁻³⁷.

Despite insufficient symptom control with pharmacotherapy, only 5% of patients suffering from allergic rhinoconjunctivitis decide to undergo allergen-specific immunotherapy (SIT)³⁸⁻⁴⁰, which is the only disease-modifying and long-lasting treatment option^{18,19}. Yet, the requirement for numerous visits to a medical office and the risk of treatment-associated systemic allergic side effects limit the broad use of the more than 100 years old subcutaneous allergen specific immunotherapy (SCIT). Aiming to increase acceptance of SIT, tremendous effort has been put into the development of more patient-convenient and self-administrable treatment routes. With the consented approval of sublingual allergen-specific immunotherapy (SLIT) as a valid needle-free treatment alternative to SCIT^{41,42}, a new area of SIT has been initiated. In recognition of the excellent safety profile with rare systemic allergic side effects^{41,42}, SLIT can be self-administered and therefore represents an increasing percentage of SIT treatments in Europe⁴³. However, up to 75% of patients report local oral and gastrointestinal side effects^{41,42}. Also, treatment duration is not reduced but requires daily intake of high allergen doses for three years, leading to considerable treatment costs and low treatment compliance⁴⁰. When covered by health insurance, merely 25% of patients finish three years of treatment, and only 15% of the patients finish the treatment if they have to cover the cost themselves⁴⁴. Also, recent meta analyses demonstrate that the treatment effect is modest⁴⁵.

The above numbers make a clear case for the development of more patient-friendly and faster SIT regimens in order to increase patient convenience and compliance. The main obstacle to improvement of SIT over the last decades has been lack of a clear understanding of the mechanism of action. While Noon and Freeman in 1911 had the seemingly straight-forward task to develop a vaccine against a toxin which protected via antibody formation, today's immunological views on SIT are far more complex. And because allergies are caused by a misguided immune response, therapeutic concepts typically center around tolerance induction. In the current review, we would therefore take a step back, compare SIT side by side with classical vaccination (Table 1) and then discuss whether or not SIT leads to tolerance induction or simply the induction of neutralizing antibodies. We propose that comparing allergens to pro-toxins facilitates the analysis and conclude that the primary goal of any SIT for the treatment of allergic rhinitis should be the induction of allergen-specific IgG antibodies.

Comparison of SIT with classical flu vaccination

The disease

Although allergies and infectious diseases seem to have little in common at first sight, a comparison of hay-fever symptoms with those seen after infection with influenza virus or common cold viruses may suggest otherwise. Hay fever was characterized by Blackley to be associated with nasal discharge, paroxysms of sneezing, itching of the eyes, difficulty of breathing and malaise. Rather obviously, most of these symptoms are also seen if someone comes down with flu or a common cold. Indeed, it was only due to the yearly recurrence of these

symptoms in summer that Bostock concluded that the disease was not an infectious catarrh¹.

Thus, in terms of symptoms, influenza and hay fever are rather similar diseases (Table 1).

Different immune responses or simply different nomenclature

In both hay fever and influenza, immunization with pollen extract and influenza virus, respectively, leads to long-lived protection⁴⁶. In case of hay fever, such immunization today is called “immunotherapy to induce tolerance”, but in case of influenza, one speaks of “protective vaccination”. Why is this? As defined in 1906 by Clemens von Pirquet, allergies are caused by a immunological hypersensitivity reaction against otherwise innocuous substances⁴⁷, in case of hay fever, by IgE against allergens contained in pollen. Therefore, the notion is that allergen immunotherapy must aim at reducing this immune response, hence to induce tolerance against the allergen. In contrast, influenza is caused by a pathogen, which is certainly not innocuous, and the notion is that vaccination must protect against the pathogen. However, already 100 years BC, Mithridates VI, king of Pontus, rendered himself more tolerant to poisons and toxins by exposing himself to small doses of them. And the term “immune”, was first described by Marcus Annaeus Lucanus 60 BC and referred to North African tribes who made themselves resistant to snake venom by injections of venom glands⁴⁸. Hence, immunity and tolerance have been known to go hand in hand for a long time.

Pathogenesis

It is well accepted that allergy symptoms are caused by the immune system by mast cells after allergens have cross-linked surface bound IgE. In addition, allergen-specific Th2 cells also contribute to allergy symptoms. However, also the symptoms of influenza virus are not only

caused by the virus itself, but mostly by immune mechanisms. These include lysis of infected cells, cellular infiltration and inflammation, as well as production of cytokines, chemokines, and other pro-inflammatory molecules that cause a flurry of symptoms including fever and malaise. Interestingly, also in the case of influenza and rhinoviruses, the most frequent cause of common cold, the viruses lead to mast cell degranulation, in part by IgE-independent mechanisms^{49,50}, but likely also due to virus-specific IgE⁵¹⁻⁵³. This is the reason why most composite influenza and common cold medications contain anti-histamines. Yet, despite the fact that influenza symptoms are also partly caused by the immune system, and some even by the same immune mechanisms, nobody would postulate that vaccination with influenza should aim at inducing immune tolerance.

Immune mechanism of vaccination versus immunotherapy

When neither the disease nor the immune mechanisms causing the symptoms of hay fever and flu are much different, why then is influenza vaccination thought to induce a protective immune response, and allergen immunotherapy thought to induce immune tolerance? The answer to this question apparently lies in the discovery of novel immune mechanisms. As soon as these mechanisms emerged in the literature, they were searched for and always found in patients undergoing SIT (Box 1).

In 1911, immunity just had been explained by the “humoral theory” proposed in 1891 by Emil von Behring and Kitasato Shibasaburō, and in 1900 by Paul Ehrlich’s “antibody formation” theory⁵⁴. Therefore, allergen immunotherapy was interpreted as a vaccine working by inducing protective antibody responses, as soon after demonstrated 1903 by Dunbar in animals. In 1935, Robert A. Cooke demonstrated that antibodies induced by SIT could be passively transferred to

block the allergic reaction produced by intradermal injection of allergens⁵⁵. After the discovery of IgE by Ishizaka and Ishizaka⁵⁶ and Johansson⁵⁷ in 1968, allergy and SIT research focused on potential immune mechanisms that could counteract or tolerize this misguided immune response. Soon after the discovery of suppressor cells⁵⁸, such cells were demonstrated to be induced under allergen immunotherapy⁵⁹. With Th1/Th2 cell subsets being characterized⁶⁰, it was also found that allergen immunotherapy induced a Th1 type response⁶¹⁻⁶⁵ which presumably counteracted the Th2 type response underlying the IgE mediated allergy. Consequently, after the discovery of regulatory T cells⁶⁶ allergen immunotherapy was and is currently explained by induction of allergen specific Treg^{64,67-71}.

All these effector mechanisms certainly play a role in allergen immunotherapy, but these characteristics do not at all differentiate SIT from vaccination against pathogens. The same immune mechanisms in the same order of appearance were also demonstrated in the immune response against pathogens. Also in infectious settings, Treg are induced where they help to resolve the proinflammatory response, thereby minimizing tissue damage by immunopathology⁷²⁷³. Also childhood diseases, for example measles, induce antibodies, Th1 and Treg⁷⁴⁻⁷⁶. Yet, nobody would argue that immunity after childhood disease is “tolerance induction” to the pathogen.

Box 2: Allergen Specific Immunotherapy and IgE

While the term “sensitization” refers to production of IgE, it must be first clarified that the effect of “desensitization” does not diminish IgE. In contrast, in patients receiving immunotherapy, initially there is an increase in specific IgE antibody levels⁷⁷, followed over the years by a slow gradual decrease in IgE levels toward or below baseline levels. Clinical improvement occurs

before IgE decreases, so that efficacy is not dependent on reductions in specific IgE levels^{78,79}. Thus, SIT appears to induce a protective immune response, rather than to work via diminishing IgE. Only in the long term this protective immune mechanism may then lead to a reduction in IgE¹⁸.

Revisiting the mechanism of SIT

Taking the outlined similarities between classical vaccination and SIT into account, the following sections will revisit the various postulated mechanisms of action of desensitization and discuss possible implications for the design of optimized therapies.

Mechanism of desensitization: Tregs and Th1 cells

As discussed above, SIT has been shown to induce allergen-specific Treg cells^{64,67-71}. It has been postulated that Treg cells exert this therapeutic effect via direct suppression of Th2 cells⁸⁰, by inhibition of allergen-specific IgE production, or by upregulation of IgG4 and IgA⁸¹. Mast cells could also be the direct target of the suppressive activity of Tregs⁸². However, from a mechanistic and in particular kinetic point of view, it is unlikely that Treg cells are the key effector cells induced by SIT. Allergic reactions, such as rhinoconjunctivitis, occur within minutes upon allergen exposure. When allergens cross-link IgE molecules bound to the surface of tissue mast cells, this rapidly triggers the release of preformed mediators, including histamine. These mediators quickly cause the local symptoms of itching, reddening and swelling. It is difficult to envisage how regulatory T cells could inhibit this process. To become activated, Treg cells need to interact with their cognate antigen, i.e., allergen-derived peptides presented on MHC class II molecules on dendritic cells or other antigen presenting cells (APCs), such as

macrophages or B cells. This implies that before Treg may become activated and enabled to suppress activation of mast cells, allergens need to be taken up and processed by APCs. In addition, once activated, Treg cells need to produce suppressive cytokines or may even physically interact with mast cells in order to inhibit the release of the mentioned mast-cell derived mediators. However, how these two processes should occur within minutes in order to block mast-cell activation remains enigmatic. On the contrary, our current knowledge on antigen uptake and processing and subsequent T-cell activation suggests that this is simply not possible within this time frame.

Alternatively, Treg cells may also block mast cell degranulation by producing a largely non-inflammatory or “suppressive” milieu that reduces mast-cell activity^{82,83}. However, such a process would not be allergen-specific and therefore contradict clinical experience. As the name says, “allergen-specific desensitization” is “allergen-specific”, as first demonstrated in grass and ragweed allergic patients, where SIT with ragweed had no effect on the grass allergy⁸⁴. In addition, we recently established a murine model of SIT for cat-allergy. Using this model, an effector role for Tregs or Th1 cells for blocking allergic symptoms could be excluded, as CD4- or regulatory T cell depletion had no impact on efficacy of SIT⁸⁵.

It is important to realize that different effector mechanisms cause allergic rhinitis and asthma^{18,86}. The most important mediators of allergic rhinitis are IgE antibodies causing mast-cell activation. Asthmatic reactions occur in two phases, an early phase occurring within minutes upon allergen-exposure followed by a late phase, peaking 12-24 hours later. Similar to rhinitis, the first phase is mainly mediated by IgE antibodies. In contrast, a major effector cell during the second phase is the infiltration of Th2 cells into the lung tissue⁸⁷. This second or late phase of asthma may be an important target for Treg cells, and it is possible that Tregs are able to suppress Th2 cells by

secreting anti-inflammatory cytokines, such as IL-10 or TGF- β . Induction of Tregs may therefore be an important component for immune-mediated therapy of asthma^{18,88}. Similar considerations may apply to Th1 cells, which are known to inhibit Th2 responses. Hence, both Tregs as well as Th1 cells may inhibit the late phase of the asthmatic reaction while a direct effect on the early, IgE-dependent allergic reaction seems less likely a target of these T cells. However, it should be kept in mind, and as discussed below, both Tregs and Th1 cells may help controlling IgE-dependent allergic reactions by regulating the production of allergen-specific IgG antibodies.

Mechanism of Allergen Specific Immunotherapy: Antibodies

When Robert A. Cooke investigated the protective mechanisms underlying allergen-specific immunotherapy⁵⁵, he found that the allergic reactions observed upon injection of ragweed pollen extract into the skin of ragweed-allergic patients could be blocked by pre-incubation of the extract with serum from patients that had received ragweed-specific immunotherapy. Shortly after, Mary H. Loveless, who attended Cooke's laboratory at the Department of Allergy at New York's Roosevelt Hospital, found that these blocking antibodies bound to the allergen and therefore inhibited the reaction of the allergen with "sensitizing antibodies"⁸⁹, today known as IgE. Loveless further found that the presence of blocking antibodies correlated to symptom amelioration in ragweed-allergic patients undergoing SIT⁹⁰. In 1968, Lawrence M. Lichtenstein and co-workers at the Johns Hopkins Asthma and Allergy Center in Baltimore found that blocking antibodies were IgG⁹¹, and they also showed that pre-incubation of ragweed allergen with blocking antibodies inhibited histamine release from the basophils of ragweed-allergic patients.

Already Lichtenstein saw a correlation between the rise in blocking IgG and the decrease in specific IgE over two years of SIT⁷⁷, which was later confirmed by others⁷⁹. Interestingly, Tada and Okumara have shown in a rat model that blocking IgG antibodies and allergen-specific IgE do not just inversely correlate, but that the passive transfer of blocking IgG antibodies eventually caused a decrease in IgE production⁹².

Today it is undisputed that SIT induces allergen-specific IgG production, but there has been doubt about its importance, since it has been difficult to establish a correlation between IgG antibody levels in serum and clinical outcome⁹³⁻⁹⁶. Only 2 out of 6 clinical trials found a positive correlation between induction of allergen-specific IgG4 and amelioration of allergy⁹⁶. However this absence of a correlation may have several reasons.

i) IgG4 levels are measured using whole allergen extracts, e.g. a grass pollen extract, which include allergens but also many other proteins against which the treated individual is not allergic. An increase in anti-allergen-IgG4 will therefore often be masked by an increase of IgG4 against the many irrelevant proteins in the extract, as well as by pre-existent IgG4. By way of analogy, immunization with a whole-cell pertussis vaccine will result in a multitude of antibodies specific for a vast number of different antigens. Protection will, however, be mediated by few antibody-specificities, such as the pertussis toxin-specific antibodies. Measurement of all antibodies against the whole bacterium may therefore not necessarily correlate with protection against disease. The same is true for influenza virus, where protection is mediated by a fraction of antibodies specific for hemagglutinin and perhaps neuraminidase, while antibodies against other virus proteins will be irrelevant.

ii) The above might lead to the conclusion that measurement of IgG against a purified or recombinant major allergen should then unmask the correlation of IgG with the treatment effect.

But this is not the case, because also a single allergenic protein contains relevant and irrelevant epitopes. While blocking IgG antibodies inhibit IgE binding, other IgGs may bind the allergen without interfering with binding of IgE⁹⁷.

iii) The affinity of blocking antibodies is certainly of clinical importance, as they have to bind soluble allergens present at low concentration and in addition compete against IgE binding. SIT has been demonstrated to induce high affinity blocking antibodies with somatic mutations in their complementary determining region as an indication for affinity maturation^{97,98}. However, the affinity of the IgG response has only rarely been assessed^{99,100}. It is therefore likely that low affinity antibodies without biological activity will also be picked up by the typically used ELISA IgG measurements. In fact, if IgG is measured by functional assays, which require high affinity, such as inhibition of IgE-facilitated antigen presentation to T cells, the correlation appears better¹⁰¹.

iv) The IgG/IgE ratio in the patient may be more important than total IgG levels¹⁰².

On the relevance of IgG Isotype

While most early studies concluded that IgG4 is the predominant isotype induced by SIT^{103,104}, more recent studies using purified allergen preparations found that SIT also induced allergen-specific IgG1 antibodies¹⁰⁵⁻¹⁰⁷. It seems likely that the preference for IgG4 is mostly due to the vaccination protocol used for SIT. Indeed, when covalently linking an allergen to a virus-like particle, we found that the predominant allergen specific IgG subclasses were no longer IgG4, but IgG1 and IgG3¹⁰⁸. Furthermore, while inactivated influenza virus induces IgG2a in mice, subunit vaccines or viral particles in the absence of viral RNA induce a similar

isotype pattern as allergens⁸². Induction of IgG4 therefore is not a peculiarity of allergens but rather normal for B cell responses triggered in the absence of viral RNA or other TLR-ligands¹⁰⁹⁻¹¹¹. IgG1 and IgG4 antibodies also show little mechanistic differences. Using recombinantly produced murine and human antibodies we have recently shown in mice and human basophile cultures, that IgG4 and IgG1 were equally potent at inhibiting the allergic reaction¹¹².

Mechanism of IgG-mediated inhibition of the allergic reaction

Numerous preclinical studies have addressed the potential of allergen-specific antibodies to inhibit mast cell activation (reviewed in¹¹³). We have recently established a mouse model for cat allergy in which allergy could efficiently be treated by immunization with Feld1, the major cat allergen, coupled to virus-like particles. In this model, allergen-specific IgG antibodies induced by vaccination were able to fully block local as well as systemic allergic symptoms⁸⁵. Importantly, treatment of allergic mice with allergen-specific IgG fractions was able to alleviate all allergic symptoms, supporting the old findings of Dunbar^{6,7}. In contrast, depletion of all Th cells or specific Treg-depletion prior to challenge of de-sensitized mice, did not influence protection against anaphylaxis⁸⁵. Interestingly, allergen-specific IgG antibodies were able to inhibit mast cell activation in vitro and vivo by two independent mechanisms (Fig 2). Polyclonal allergen-specific IgG antibodies were able to “neutralize” allergens and simply block binding of allergens to IgE on mast cells. Hence, this mechanism follows the classical paradigm of toxin-neutralization induced by vaccination as postulated by Dunbar and Noon. The second mechanism of IgG-mediated inhibition of mast cell activation followed a different principle. Rather than simply neutralizing the allergen, IgG antibodies were able to engage the inhibitory receptor FcγRIIb on mast cells, abrogating cellular activation⁸⁵. This additional mechanism has

the important implication that IgG antibodies specific for a single epitope on the allergen are able to block mast cell activation induced by polyclonal, multi-epitope-specific IgE antibodies.

A role for Tregs in driving isotype switching

While a direct role for Tregs in halting mast cell activation remains unlikely, it is possible that Tregs may promote the production of allergen-specific antibodies. This possibility is supported by the findings that IL-10 enhances generation of IgG4 while TGF- β promotes isotype switching to IgA⁸¹. In addition, control of Th2 cells by Tregs may block the generation of novel IgE producing plasma cells, resulting in the eventually observed downregulation of IgE levels¹⁸. Interestingly, toll-like receptor ligands also promote the generation of IgG as well as IgA antibodies without necessarily inducing Tregs¹¹⁰. Thus, induction of Tregs by SIT may not be a prerequisite for induction of specific antibodies or clinical efficacy, but simply reflect the way the allergens are applied; at low doses and in a frequent manner. It should be kept in mind that the immunization regimen used in SIT has not necessarily been established to enhance efficacy of the therapy, but is important for the safety of the treatment in order to avoid strong local and anaphylactic reactions. In support of this, combining allergens with the TLR-containing adjuvants MPL, a TLR4 ligand, has recently been shown to shorten SIT¹¹⁴, and encouraging initial results were also reported for ragweed allergen covalently linked to the TLR9 ligand CpG¹¹⁵. Furthermore, formulation of allergens in microcrystalline tyrosine as an adjuvant has been shown to strongly enhance efficacy of SIT¹¹⁶. In addition, and as discussed above, preclinical models have shown that display of allergens on TLR-ligand-containing VLPs render SIT highly efficient with symptom amelioration after a single injection⁸⁵. In summary, these considerations suggest that the goal of improved SIT is the generation of allergen-specific

IgG/IgA antibody levels in the absence of a IgE antibody boost, and not the induction of increased Treg responses. The latter may go along with stronger Treg responses but may also be driven by other T cell subsets, such as Th1 cells or follicular Th cells.

Allergens as Protoxins

Within the paradigm of IgG-mediated inhibition of allergen-induced mast cell activation, it may be useful to look at allergens as pro-toxins. It is well established that most allergens are not toxic per se. Nevertheless, allergens are able to intoxicate an allergic individual, and this is due to binding of allergen-specific IgE. Hence, in a way similar to pro-enzymes, which only become active upon proteolytic cleavage, or many classical toxins (e.g. aflatoxin), which are innocuous unless chemically activated by liver enzymes, allergens only become pharmacologically active after binding to IgE. SIT therefore essentially reflects active immunization against the allergen protoxin.

Viewing allergens as protoxins may therefore allow outlining a path for the development of more efficient immunotherapies. 1) SIT should aim at maximizing allergen-specific IgG responses. With this respect, SIT is comparable to any other classical prophylactic vaccine. 2) SIT should avoid conversion of the protoxin (allergen) into the active toxin (IgE-allergen complex able to trigger mast cells). With this respect, SIT fundamentally differs from other vaccination principles. Several possibilities exist to inhibit generation of active IgE complexes. Most straight-forward would be the elimination of IgE epitopes on allergens. In general, this may be difficult, since IgE and IgG antibodies use the same variable regions, and there is evidence that isotype-switching to IgE may be secondary event following isotype-switching to IgG1¹¹⁷. A

related concept would be to generate vaccines containing a single B cell epitope. IgG antibodies induced by such a vaccine may be able to block some of the specific IgG and block mast cell activation additionally by engaging FcγRIIb. A slightly different strategy has been followed by us. By coupling allergens to virus-like particles, it was possible to abrogate mast cell activation while enhancing induction of allergen-specific IgG responses⁸⁵. Rather than changing the drug, it may also be possible to optimize drug delivery. Injection of allergens into lymph nodes may allow using sufficiently low doses of the allergen to avoid allergic responses while IgG responses are induced nevertheless³⁰. Formulating allergens in appropriate particles^{118,119}, which can prevent systemic spread or adjuvants which allow for the use of low doses or fewer injections are another possibility. Yet another option is to pulse APC with allergen at sites which are not vascularized, so that high allergen doses can be utilized without the risk of allergen inadvertently reaching the circulation are another strategy, which is pursued in epicutaneous immunotherapy (EPIT), where allergens are administered by a patch to pulse Langerhans cells in the epidermis, lacking vascularization^{26,29}. In summary, the challenge of novel strategies for the treatment of allergy is the elimination of the allergic potential of vaccine while preserving their ability to trigger strong IgG reactions, a strategy that has long been pursued¹²⁰, by the use of so called allergoids, i.e., allergens chemically modified to reduce IgE binding, but with the difficulty not to destroy the IgG epitopes. Consideration of T cells other than their involvement in isotype-switching may not be necessary.

Thus, when aiming to improve allergen-specific immunotherapy, interpretation of SIT as a vaccine against a toxin or protoxin may be a helpful working hypothesis, i.e. going back to same working hypothesis that also led to the invention of SIT a century ago.

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Figure Legends:

Fig. in Box: Important discoveries in immunology changed the interpretation of SIT. As long as allergies were thought to be mediated by toxins, SIT was interpreted as a vaccine. With the discovery of IgE the interpretation of SIT changed towards “tolerance induction”.

Fig. 1: Invention of allergen specific immunotherapy. When introduced 100 years ago, SIT was thought to be a vaccine against toxins in pollen⁸.

Fig. 2: Neutralizing and non-neutralizing antibodies inhibit mast cell activation. Top: allergens crosslinking IgE bound to high affinity IgE receptors FcεRI causes degranulation and release of mediators. Middle: crosslinking of IgE can be inhibited by neutralizing/blocking IgG. Bottom: non-neutralizing IgG can inhibit mast cell activation by engaging the inhibitory receptor FcγRIIb on mast cells.

Table 1

Comparison of hay fever and influenza virus infection

	Symptom	Pathogenesis	Immune response induced by Vaccination/SIT	Duration of protection induced by vaccination/SIT
Influenza	Rhinitis, Cough	Viral replication Immune- mediated malaise	IgG/IgA/T cells	long
Hay Fever	Rhinitis, Asthma	Immune-mediated malaise	IgG/IgA/T cells	long

PROPHYLACTIC INOCULATION AGAINST
HAY FEVER.

BY L. NOON, B.C. CANTAB., F.R.C.S. ENG.

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Inoculation, St. Mary's Hospital.*)

HAY fever is a form of recurrent catarrh affecting certain individuals during the months of May, June, and July. It is caused by a soluble toxin found in the pollen of grasses.

Fig 1

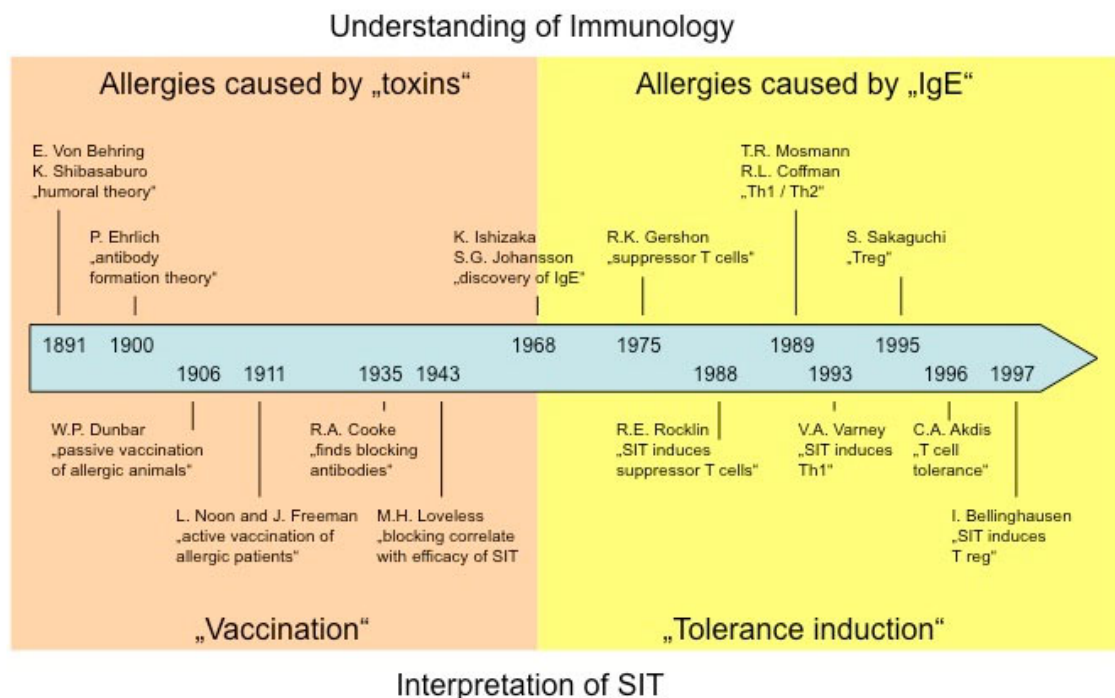


Fig 2

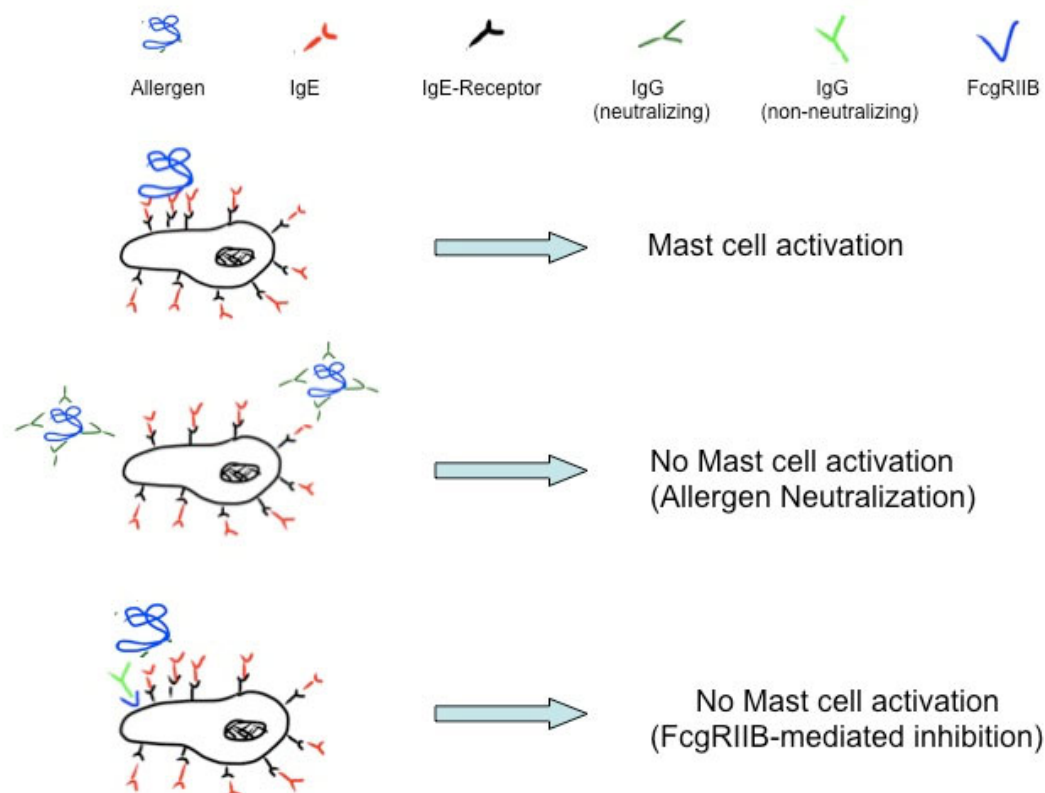


Fig 3