

Do Allergens Induce Type-2 Immune Responses?

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Key Words

Allergen · Th1/Th2 · Immune response polarization ·
IL-4 · IFN- γ · Adjuvant

In the past 10 years we have witnessed exciting progress in understanding different facets of the complex biological phenomena that participate in an allergic reaction. However, most of the very basic questions that were formulated 20 or more years earlier are still valid, since no answer has been given to them. Characteristically, in 1978, in an article entitled, 'What makes an allergen an allergen', Aas [1] wrote 'why do some substances and some molecules act as allergens while others apparently do not?' This is a question of outmost importance since its elucidation will have an important impact on patient treatment and eventually on prophylaxis.

A domain in which great progress has been accomplished is that of T-cell biology. Indeed, we know a lot more about the mechanisms of T-cell activation and about T-helper cell subsets producing cytokines with opposing effects in the regulation of IgE. Today it is understood that the production of IgE is stimulated by cytokines IL-4 and IL-13 [2, 3] and inhibited by IFN- γ [4]. These cytokines are produced by distinct subsets of T-helper cells, the so-called Th2 and Th1, respectively [5]. The association between the IgE response against allergens and the deviation of the immune response (i.e.) to type 2 has been supported by studies showing that allergen-specific T-cell clones from allergic patients

produce type-2 cytokines when stimulated in vitro [6, 7]. Therefore, we can reformulate the above question as follows: why does the immune response against some substances (allergens) deviate to a type-2 cytokine profile?

One hypothesis is that allergens themselves possess some particular molecular characteristics that are responsible for the deviation of the immune response. Alternatively, since in nature allergens enter the organism in association with a multitude of substances, the possibility exists that some other factors, associated with the allergen, possessing immunomodulatory properties drive the immune response to type 2. In other words, we have to discriminate between two possibilities, and to redefine the term allergen accordingly: (1) allergens actively induce the cytokine profile that promotes IgE production and therefore are responsible for the initiation of a series of biological events that lead to the allergic reaction, and (2) the immune response is driven to a type-2 profile independent of the nature of allergen; the role of the allergen is limited to crosslink Fc ϵ R1-bound IgE and therefore is situated at the end of the chain of events that make an allergic reaction.

Determination of whether allergens possess Th2-polarizing capacity in vivo is complicated by the fact that immunization in adjuvants is often needed in order to elicit an immune response against antigens, and that adjuvants themselves may influence the type of the immune response generated [8]. This question was examined in initial experiments in which mice were immunized with a single dose of *Schistosoma mansoni* 28 kD glutathione S-transferase (Sm28-GST) or with the tetanus toxoid fragment c of

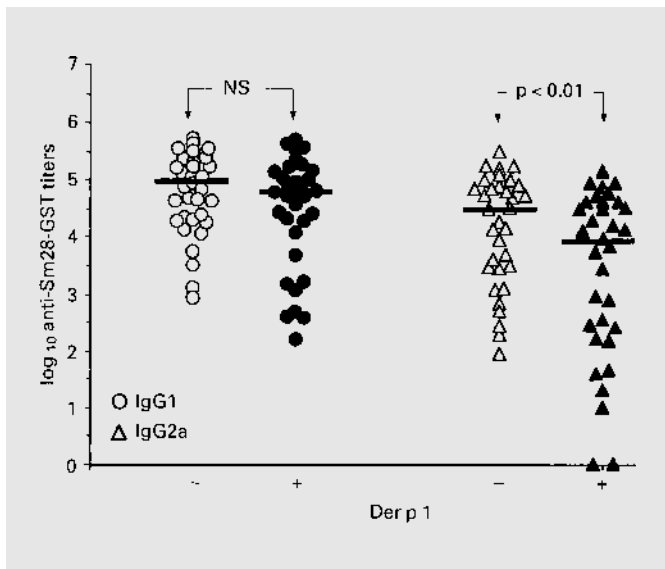


Fig. 1. Anti-Sm28-GST IgG1 (○, ●) and IgG2a (△, ▲) responses induced by immunization with Sm28-GST in CFA in the presence (●, ▲) or absence (○, △) of Der p 1. Mice were immunized s.c. with a single dose of Sm28-GST (20 µg/mouse; in CFA) alone or in the presence of 20 µg of Der p 1. Sera were collected 28 days after immunizations, and anti-Sm28-GST IgG1 and IgG2a levels were measured by ELISA. Results from 6 independent experiments each comprising 5–10 mice are shown. Each symbol represents the Ig titer of an individual mouse. The bars show the median.

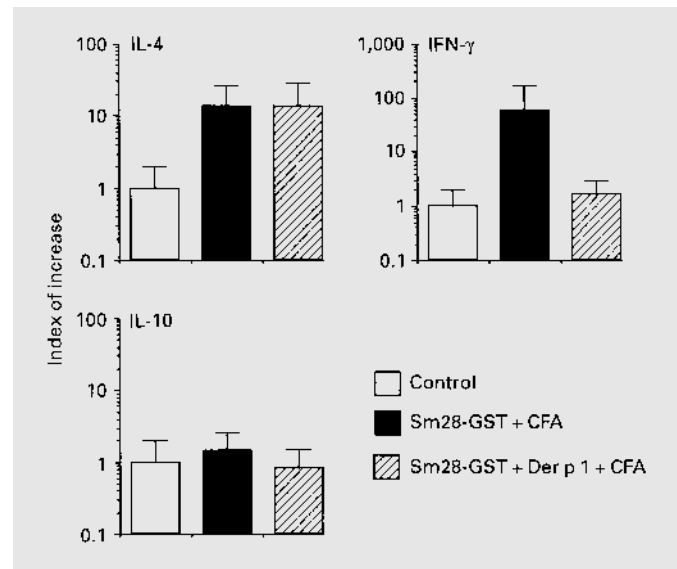


Fig. 2. Levels of IL-4 (A), IFN-γ (B), and IL-10 (C) mRNA after immunizations with Sm28-GST in the presence or absence of Der p 1. Mice (5/group) were either immunized s.c. with Sm28-GST (20 µg/mouse; in CFA) alone (■), or in the presence of 20 µg of Der p 1 (▨). Seven days after immunizations, mice were sacrificed and RNA from inguinal lymph nodes was assessed by semiquantitative RT-PCR for cytokine mRNA expression. Results are expressed as stimulation indices, relative to control mice injected with 0.9% NaCl (□; arbitrarily considered as 1). Mean ± SE of three experiments are shown.

Clostridium tetani (TTC) in Complete Freund's Adjuvant (CFA) or in alum or with recombinant *Salmonella typhimurium* expressing these proteins in a fusion protein. These protein antigens were chosen because they are major antigens of phylogenetically distant infectious organisms, inducing Th2 (parasites) and Th1 (bacteria) responses.

Antigen-specific IgG isotypes and cytokine mRNA expression in vivo, as well as cytokine secretion after in vitro antigen stimulation was studied (data not shown). Immunizations with either protein in alum induced a strong Th2-associated antibody (IgG1) and cytokine (IL-4) response. In contrast, the recombinant *S. typhimurium*, expressing the TTC/Sm28-GST fusion protein, induced a Th1-like response associated with the production of IFN-γ and IgG2a antibodies against both antigens. When CFA was used, a nonpolarized profile was observed, characterized by expression of both IL-4 and IFN-γ, as well as strong specific IgG1 and IgG2a antibody responses. Low IgE titers were observed only with the alum protocol. These results were interpreted to mean that some protein antigens play a weak role in polarizing the immune response and that opposing cytokine profiles could be induced against the same antigen, depending on the adjuvant employed [9].

Inasmuch as the immune profile is strongly influenced by adjuvants, testing the polarizing capacity of an allergen in a classical immunization protocol that makes use of adjuvants will be inappropriate, because any proper effect of the allergen on the immune profile would be obscured by the adjuvant. On the other hand, in previous studies it has been shown that a bystander immune response could influence the type of the response against an irrelevant antigen [10]. Based on these considerations we reasoned that it would be possible to test the polarizing capacity of an allergen in a heterologous immunization system. To do that, we examined whether coadministration of the major allergen of the house dust mite, *Dermatophagoides pteronyssinus*, Der p 1, influenced the immune response against an irrelevant antigen, for instance, the Sm28-GST.

Our results showed that the coadministration of Der p 1 with Sm28-GST in the CFA immunization protocol was associated with diminished anti-Sm28-GST IgG2a antibody titers (fig. 1), reduced IFN-γ mRNA expression (fig. 2), and reduced frequency of IFN-γ-producing cells (data not shown). In these same experiments similar Sm28-GST IgG1 antibody titers (fig. 1) and IL-4 and IL-10 mRNA (fig. 2) were observed in Sm28-GST/Der p 1 and in

Sm-28GST alone immunized mice. When the alum was used as adjuvant, both isotype and cytokine profiles were similar in mice immunized with Sm28-GST/Der p 1 and Sm-28GST alone (data not shown). In addition, coimmunization with TTc did not affect the profile of the response against Sm28-GST, demonstrating that the effect of Der p 1 was specific. These results suggest that Der p 1 has the capacity to induce type-2 responses by modifying the IL-4/IFN- γ balance in favor of the former, and thereby to influence isotype profiles [11].

Der p 1 was chosen in the above experiments, not only because it is a major allergen, but also because, like a number of aeroallergens, it possesses proteolytic activity. This common feature of some allergens, as well as the observation that several parasite antigens are proteases that also induce type 2 i.r., has raised the possibility that the proteolytic activity of some antigens may be essential for inducing type-2 immune responses [12]. Therefore, we decided to test whether abrogation of Der p 1's enzymatic activity either by heating or by treatment with the specific cysteine protease inhibitor E-64 would affect its ability to modulate the immune response in coimmunization experiments. Indeed, these treatments significantly diminished the ability of Der p 1 to inhibit anti-Sm28-GST IgG2a antibody titers (data not shown). In addition coimmunization with heat-inactivated Der p 1 had a significantly lesser effect in inhibiting IFN- γ production, as measured by in vitro, antigen stimulation experiments (fig. 3). These results support the hypothesis that proteolytic activity of allergens is important in their capacity to modulate the immune response to type-2 profile [11].

Even though it is widely accepted that allergens induce type-2 i.r., very little direct evidence is available in support of this allegation. Recently it has been shown that active phospholipase A2 (PLA2) is able to induce IgE-independent mast cell degranulation and IL-4 release. Also immunization with the active but not the inactive form induced PLA2-specific IgE [13]. In both this study and the present one, no evidence is provided explaining why the enzymatic activity of allergen is important to induce type-2 immune responses. Furthermore, it is difficult to imagine any common mechanism by which molecules possessing so different activities shift the immune response in the same direction. It is most probable that allergens play an immunomodulatory role by virtue of their very different activities that interfere at various levels and modulate the outcome of the immune response. In this respect, recent findings are of importance showing that Der p 1 is able to cleave two immunologically relevant molecules the CD23 and CD25 [14]. It still has to be demonstrated in vivo that

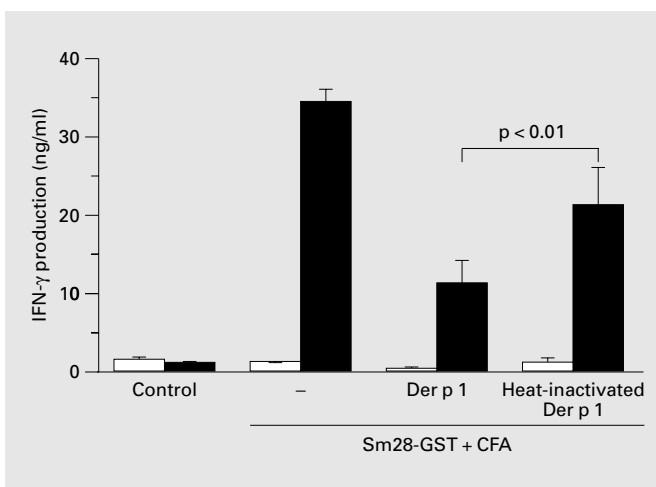


Fig. 3. Effect of Der p 1 coimmunization on IFN- γ production after antigen-specific stimulation in vitro. Mice (3/group) were either immunized s.c. with Sm28-GST (20 μ g/mouse; in CFA) alone or in the presence of 20 μ g of native or heat-inactivated Der p 1. Seven days after immunization, mice were sacrificed and spleen cells were stimulated in triplicate in vitro (4×10^6 cells/ml) with either Sm28-GST (20 μ g/ml, ■) or conalbumin (20 μ g/ml, □) as an irrelevant protein. Supernatants were assessed for IL-4 and for IFN- γ by ELISA. Results are expressed as the mean of triplicates ($\pm$ SE).

cleavage of these molecules is responsible for the deviation of the i.r.

Today, with an important number of major allergens cloned and available in pure form [15], these questions become very pertinent. Undoubtedly, the challenge of understanding why allergens are allergens lays ahead.

Acknowledgement

We thank Dr. H.M. Moutsopoulos for critical reading of the manuscript.

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