

EDITORIAL

Changes in interferon- γ production following specific allergen immunotherapy: biology vs methodology

Specific immunotherapy (SIT) with whole allergen extracts was first introduced by Noon in 1911 [1,2]. Although infrequently practised in the UK, SIT remains widely available as a treatment for allergic disorders in Europe and the Americas. SIT has been demonstrated to be effective in selected patients with immunoglobulin E-mediated disease including asthma [3,4]. Recently, Durham and colleagues demonstrated long-term benefit following withdrawal of 3 to 4 years of grass pollen immunotherapy [5]. Considerable interest has been expressed in the immunological mechanisms underlying successful SIT and this, in turn, has provided insight into the regulation of immune responses to exogenous environmental antigens [6].

In an appropriately sensitized individual, contact with allergen results in the cross-linking of mast cell-bound IgE leading to the activation of signal transduction cascades within the cell. Subsequently, degranulation and release of mast cell mediators such as histamine, cytokines such as interleukin (IL) -4 and IL-5, and arachidonic acid metabolites take place leading to a characteristic inflammatory response. Allergen molecules are simultaneously taken-up by antigen-presenting cells (APCs) such as macrophages, B cells and dendritic cells. Processing and presentation of antigenic fragments to T cells in the context of molecules of the major histocompatibility complex (MHC) leads to T-cell activation and elaboration of soluble mediators such as cytokines.

Atopic allergic responses are associated with a TH2 pattern of cytokine elaboration from activated allergen-specific T cells [7–9]. Interleukin-4 induces the transcription of the epsilon immunoglobulin heavy chain locus and, in combination with CD40/CD40L interaction, leads to B-cell isotype switching to produce IgE [10]. IL-4 is also important in T-cell development, inducing commitment to the TH2 pathway [11]. IL-5 is also produced by TH2-type T cells and is prominent in the development and survival of eosinophils and their release from the bone marrow [12].

Many of the biological actions of IL-4 are counter-regulated by the TH1 cytokine interferon-gamma (IFN γ). For example, IFN γ induces Ig isotype switching to complement fixing IgG isotypes rather than IgE and IgG4 [13]. For this reason it has been suggested that induction of IFN γ at sites of allergic inflammation may be beneficial in regulating IgE-mediated inflammation.

A number of recent studies have described molecular and cellular changes associated with successful SIT. Expression

of immunoglobulin isotypes has been shown to be modulated following the initiation of SIT. A transient and paradoxical increase is seen in serum IgE levels which gradually subsides in parallel with a substantial increase in levels of allergen-specific IgG4 [14]. Numbers of mast cells have been shown to decrease after SIT [15] as have numbers of eosinophils in the skin and nasal mucosa of rhinitics [16] and the bronchoalveolar lavage of patients with birch pollen-induced asthma [17].

Modulation of T-cell phenotype has also been documented in a number of studies. Investigating cytokine production by allergen-stimulated peripheral blood mononuclear cells following PLA2 SIT, Jutel and colleagues demonstrated a decrease in IL-4 and a concomitant increase in IFN γ secretion [18]. In contrast, Secrist, investigating cytokine profiles in allergen-specific CD4⁺ cell lines, found decreased IL-4 production but in the absence of changes in levels of IFN γ [19]. Furthermore, Varney and colleagues demonstrated increased IL-2 and IFN γ mRNA-positive cells in the skin [20] in response to allergen challenge after 12 months of SIT. Similar increases in IFN γ mRNA-positive cells were reported in nasal mucosal biopsies after grass pollen SIT [21]. Thus, there has been considerable variation in cytokine profiles observed after successful SIT but this is likely to reflect, at least in part, the differing systems studied. Taken together the majority of studies support modulation of TH2 responses towards TH1 responses.

Alternative mechanisms of modulation of cytokine responses after SIT have also been described. Akdis and colleagues described epitope-specific T-cell tolerance after immunotherapy with phospholipase A₂. In this study the authors clearly demonstrated that allergen-specific cells persisted but in an inactive state since addition of exogenous IL-2 restored proliferation and IL-4 production [22]. CD8⁺ T cells have also been implicated in immune modulation in animal models of allergic disease [23,24]. Evidence also exists for the presence of CD8⁺ suppressor T cells in peripheral blood after immunotherapy for ragweed allergy which were capable of suppressing proliferative responses of blood mononuclear cells and IgE [25,26].

Two studies in the current issue of the Journal make further contributions to our understanding of the mechanisms underlying successful SIT by analysing the cytokine responses of T cells before and after immunotherapy [27,28]. Upon superficial inspection, the findings of these

two studies appear to be in opposition. However, when one looks closely at experimental design, it becomes clear that the differences observed are more likely to be associated with methodology than biology.

O'Brien and colleagues [27] investigated IFN γ production by purified CD8 $^{+}$ T cells from nonatopic and atopic subjects when challenged *in vitro* with autologous peripheral blood mononuclear cells (PBMCs) as APCs. The cells from the atopic subjects made considerably more IFN γ than the nonatopic subjects. This observation is an important one which may appear counter-intuitive as nonatopic individuals are generally perceived as making stronger TH1 responses to allergens than atopics. There are, however, a number of possible explanations for these observations such as (a) a lower precursor frequency of allergen-specific CD8 $^{+}$ cells in the nonatopics, or (b) due to IgE-mediated allergen focusing in the atopic subjects, the relative concentration of allergen presented to the T cells on the surface of the atopic APCs is higher leading to stronger T-cell responses.

Interestingly, when IFN γ production was investigated in subjects who had undergone SIT with house dust mite extract, their IFN γ production was demonstrated to be lower than the atopic group (without SIT) but still higher than the nonatopics. In the absence of exogenous IL-2, however, virtually no IFN γ was made implying a dependence of the IFN γ response on IL-2 from CD4s. Since CD4 $^{+}$ T cells were not present in the cultures *in vitro*, a constant concentration of IL-2 was added exogenously. This culture system therefore takes no account of the qualitative differences in IL-2 production by CD4 cells in atopic and nonatopic individuals *in vivo*. Thus, the nonatopic subjects' CD4 $^{+}$ T cells may make much more IL-2 than the atopics', such that although in this system the amounts of IFN γ produced by CD8 $^{+}$ cells are greater in the atopics, this need not translate into higher levels *in vivo* since there may be more CD4 $^{+}$ T-cell-derived IL-2 in the nonatopics.

Antigen focusing by specific antibody bound to cell surface Fc receptors was first described by Lanzavecchia in 1985 [29]. Antigen-presenting cells capturing antigen in this way have been shown to be 100–1000 times more effective at presenting antigen than APCs without specific antibody. More recently it has been shown that allergen-specific IgE can focus allergen via uptake by APCs expressing high affinity IgE receptors [30]. Thus, APCs such as dendritic cells and monocytes from atopic individuals would be expected to be considerably more efficient presenters of allergen than those from nonatopic individuals. Since O'Brien and colleagues found similar IFN γ production in all groups following mitogen treatment, allergen focusing and also allergen-specific T-cell frequency would be expected to influence the quantity of IFN γ produced.

Thus, in allergic individuals, allergen-specific IgE would lead to improved presentation of allergen via focusing. This in turn would lead to a larger pool of allergen-specific T-cell precursors capable of producing larger quantities of IFN γ after allergen simulation. The observation that IFN γ was reduced following SIT would suggest that one of a number of potential downregulatory mechanisms was at work after SIT including clonal deletion of reactive T cells, induction of anergy or of a regulatory T-cell population which suppresses house dust mite responses.

The fact that SIT reduces IFN γ in this system suggests that it is unlikely to mediate its effects by increasing the amount of IFN γ that is produced, at least not CD8-derived IFN γ . Previous work from the same group and that of others, has shown reduced IL-4 and in some cases IFN γ , suggesting that it is either a wholesale reduction in reactivity that is induced (i.e. T-cell tolerance or deletion) or that there is a relative increase in IFN γ by virtue of the reduction in IL-4.

In the second of the two papers in this issue, Majori and colleagues looked solely at atopic allergic patients ($n = 12$) undergoing SIT. They used flow cytometry to assay IL-4 and IFN γ production in CD4 cells and in CD8 cells, following stimulation with the polyclonal cell activators PMA and ionomycin. The cytokine measurements were used to generate an IFN γ :IL-4 ratio. Cytokine production was evaluated at baseline, after 3 months of therapy and at the end of therapy at 1 year. Symptoms improved and drug use decreased indicating clinical efficacy. IFN γ :IL-4 ratios in CD4 cells were increased at 3 months and further increased by 1 year. No change was observed in the ratio in CD8 cells at either point.

The results of the two studies appear to differ, however, there are significant differences in experimental procedure. The Majori study investigated polyclonally activated T cells from atopic individuals undergoing SIT. In the O'Brien study both atopic and nonatopic individuals were investigated including a group of atopics receiving SIT. Cytokine production in the latter study was analysed following both polyclonal and allergen-specific activation. Production of IFN γ by CD8 $^{+}$ T cells pre- and post-SIT did not change in the Majori study when T cells were treated with the nonantigen-specific mitogen phytohaemagglutinin, in contrast with a gradual reduction of IL-4 and an increase in IFN γ expression in CD4 $^{+}$ cells. Interestingly, O'Brien and colleagues found that although allergen-specific CD8 IFN γ production was reduced following SIT, levels of this cytokine did not differ following polyclonal activation in broad agreement with Majori *et al.*'s findings. Thus, it appears that SIT can give rise to gross nonallergen-specific changes in cytokine phenotypes in CD4 cells (which are not mimicked in CD8 populations). By addressing allergen-specific responses, O'Brien and colleagues demonstrate subtle changes associated with

atopic status and immunotherapy which are not visible with mitogens.

It appears therefore that the combined message from these two studies is that SIT has multiple effects on different T-cell populations and some of these are gross and some more subtle. Thus SIT induces IFN γ production in CD4 cells and decreases IL-4 when assayed after mitogen stimulation. However, with mitogens, it is not possible to see changes in CD8 IFN γ production. However, if one looks more closely, at allergen-specific responses, one can see that there is actually a reduction in IFN γ produced by CD8 cells after 1 year of immunotherapy.

In conclusion then, two studies in which production of IFN γ by CD8⁺ T cells following SIT is addressed, demonstrate the constraints of methodology rather than significant differences in biology. Interesting points arising from these studies include:

- CD8⁺ allergen-specific T cells are present in atopic allergic individuals, probably in greater numbers (although we cannot say for sure due to lack of limiting dilution analysis) than in nonatopic subjects.
- CD8⁺ cells from atopic individuals make more IFN γ than cells from nonatopic individuals and this is reduced after SIT. However, since production of IFN γ appears to be dependent on IL-2 (from CD4⁺ cells), caution should be exercised when interpreting data from cultured CD8⁺ cells in isolation.
- CD8⁺ T cells from atopic subjects may produce more IFN γ *in vitro* due to a combination of both IgE-dependent allergen focusing and (resultant) higher allergen-specific precursor frequencies.
- Although at the level of CD4⁺ T cells as a population (i.e. regardless of allergen-specificity) IFN γ increases and IL-4 decreases, changes in CD8⁺ cells are more subtle. Thus, reduced IFN γ production by CD8⁺ T cells can be observed at the level of allergen-specific cells, but not at the level of CD8⁺ cells *per se*.

These studies provide further insight into the complex mechanisms of regulation which result in clinical efficacy after immunotherapy with allergen extracts. Clearly further research is required relating to issues of precursor T-cell frequency and the interaction between T cells of the CD4 and CD8 subsets.

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