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Key Words

IgE
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To E or not to E?

Can an IL-4-Induced B Cell Choose between IgE and IgG4?

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Abstract

Parasite immunologists had known for some time that IgE-mediated hypersensitivity reactions are rare in patients with chronic helminth infections, even though basophils and mast cells in these patients are sensitized with antiparasite IgE and exposed, often continuously, to parasite antigens. The inhibition of allergic reactivity in chronic helminth infections is mainly due to IgG4 'blocking antibodies' in the serum of the infected individual. IgG4 do not fix complement and bind weakly to Fcγ receptors. Thus, antigen binding by IgG4, unlike IgE, is likely to have no or minimally harmful consequences. The discovery that, similar to IgE, expression of IgG4 is IL-4-dependent and is an intermediate step in sequential switching from IgM to IgE makes it imperative to understand how the two isotypes are coregulated and whether the two responses can be uncoupled, selectively boosting IgG4 over IgE. The ultimate goal is to apply to allergy the lesson we learnt from helminth infections.

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'Learning how to duplicate in atopic individuals IgG4 responses that parallel the specificities of their pathogenic IgE responses (as occurs naturally in clinically asymptomatic patients with chronic helminth infection) may well be essential for improving the success of allergy control strategies' [1].

As a basic immunologist strongly interested in IgE regulation, I have to confess that for a long time I paid very little attention to other immunoglobulin isotypes and to the conditions that lead to their production in vivo or in vitro. The discovery that isotype switching is directed by cytokines [reviewed in ref. 2, 3], and that interleukin-4 (IL-4) is specially responsible for the induction of switching to IgE [4–7] spurred a vast effort in many laboratories to try and understand the cellular and molecular mechanisms underlying the effects of this cytokine. Not surprisingly, this task kept everybody busy for a long time, and we aren't through yet.

In looking systematically at the effects of IL-4 on human B cells, it had been pointed out very early on that this cyto-

kine seemed to induce not only IgE, but also IgG4 [8]. Later on, a rather sophisticated analysis showed that simultaneous production of IgM, IgG4 and IgE in various combinations could be easily detected in B cell clones stimulated by IL-4 and activated CD4⁺ T cell clones. This observation was taken to suggest (quite correctly, as it was soon to be proved) that 'during clonal expansion switching might occur in successive steps from IgM to IgG4 and IgE' [9]. However, for a while, despite these sparkles of interest, IgG4 remained the underdog among human immunoglobulin subclasses. After all, why bother about an isotype that normally represents only 4% of total IgG [10]?

Actually, there were at least two cases in which IgG4 were thought to be major players in immune reactions. Para-

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site immunologists had known for some time that IgE-mediated hypersensitivity reactions rarely occur in patients with chronic helminth infections, even though basophils and mast cells in these patients are sensitized with antiparasite IgE and are exposed, often continuously, to parasite antigens. The inhibition of allergic reactivity in chronic helminth infections was known to be due to 'blocking antibodies' in the serum of the infected individual. After a long and difficult search, the blocking activity was found predominantly in the IgG4 subclass [1]. IgG4 are unusually predominant among specific antifilarial antibodies, representing 50–95% of the total IgG response [11]. Indeed, depletion of IgG4 by adsorption on anti-IgG4 affinity columns specifically removed the blocking activity from the sera of microfilaremic patients. Furthermore, IgG4-blocking antibodies inhibited the binding of specific IgE to *Schistosoma mansoni* by 95% [12]. It was soon clear that the blocking activity resulted from a direct competition for allergen binding between IgG4 in the serum and IgG bound to high-affinity Fcε receptors on cells [13]. IgG4 do not fix complement and bind weakly to Fcγ receptors [10, 14]. Thus, antigen binding by IgG4, unlike IgE, is likely to have no or minimal harmful consequences.

Allergists, on the other hand, had evidence that patients receiving immunotherapy for insect venom and house dust mite hypersensitivity developed IgG4-blocking antibodies [reviewed in ref. 15]. However, a thorough understanding of the role of IgG4 in immunity was still prevented by serious methodological problems (IgG4-specific monoclonal antibodies became available only the mid-eighties) [16] and perhaps even more, by the somewhat magic nature of the IgE/IgG4 relationship.

This (in hindsight) deplorable state of affairs came abruptly to an end with the cloning and sequencing of the switch fragments amplified from sIgE-negative B cells stimulated to switch to IgE by a combination of hydrocortisone and IL-4 [17]. Most switch fragments contained sequences derived from the switch (S) μ region directly joined to S ϵ sequences and were therefore the product of direct switching from μ to ϵ . Several clones, however, contained insertions between the S μ and S ϵ sequences. A close examination of these insertions revealed that they originated from the S γ 4 region, the sequence of which had just been reported [18]. The presence of those S γ 4-derived fragments inserted between S μ - and S ϵ -derived sequences couldn't but mean that those composite switch regions were the results of sequential switching from IgM to IgG4 to IgE. This interpretation was soon confirmed by data obtained through a specular approach, i.e., by sequencing switch circles excised from B cells that had undergone isotype switching to IgE [19].

With the demonstration that human B lymphocytes switch sequentially from IgM to IgG4 to IgE, most (but by no means all) loose ends were finally tied up. It was almost a truth of reason that IgG4 and IgE could compete for the allergen *if and only if* the two antibody isotypes had a parallel specificity of antigen recognition [13]. The model of sequential switching within one and the same B cell clone now provided a molecular mechanism for this old puzzle. Some believer in IgG4 has gone as far as proposing that a specific function of serum IgG4 may be to control antigen recognition by IgE, and consequently, to regulate anaphylactic reactions and IgE-mediated immunity [1]. Even if this is not a case of adaptation but rather an 'exaptive' function à la Steven Jay Gould [20], the fact is that the clinical findings in human filariasis – and in particular, the absence of symptoms in microfilaremic patients with high levels of antifilarial IgE and IgG4 [1, 21] – lend support to the potential importance of IgG4 as an antiallergic device.

All this begs the question, how are IgG4 and IgE coregulated, and are there limits to this coregulation? In other words, is it possible to uncouple the two responses in such a way IgG4 is selectively boosted at the expense of IgE? While this question remains as yet unanswered, we are getting somewhat closer to a more articulate view of this issue.

Much (albeit not as much as we would like) is known about the molecular mechanisms of IgE regulation [reviewed in ref. 3]. By contrast, IgG4 was a black hole: partly – as discussed above – because of a lack of motivation, partly because of technical problems deriving from the high degree of homology between IgG subclasses and their genes. The major stumbling block was the lack of information about the crucial level at which the isotype specificity of switching is determined, i.e., the series of events that culminate in immunoglobulin germline transcription. The expression of germline transcripts reflects the cytokine-dependent activation of transcription from an I_H region located upstream of the switch region subsequently targeted by recombination [22, 23]. The germline transcripts proceed from the I_H region through the switch region itself and then all the way through the immunoglobulin constant heavy chain (C_H) gene expressed as a result of switching. The germline transcripts produced after splicing contain the I_H region directly joined to the C_H transcript. It is by now generally accepted that germline transcripts per se and/or the mechanism of germline transcription are required to increase the accessibility of a specific switch region (the one through which transcription is activated by the cytokine), that thus becomes the preferred target for the recombination machinery. Even though chromatin accessibility remains a model that still awaits direct confirmation [24], it is strong-

ly supported by an overwhelming amount of data from different experimental systems, first and foremost mice in which genes that are essential for germline transcription have been deleted by homologous recombination [25–31].

The regulation of ϵ -germline transcription in human B lymphocytes has been worked out in some detail. In a nutshell, we know where $I\epsilon$ and its promoter are [32, 33], we understand (although not completely) some of the crucial signals (IL-4, CD40 crosslinking) required to activate ϵ -transcription [34], and we have identified a number of transcription factors (Stat6, NK- κ B and BSAP) that bind the promoter and get it to fire. Stat6 seems to be essential for ϵ -IL-4 response [35], whereas NF- κ B [36] and BSAP [37] mediate both the IL-4- and the CD40-delivered signals. We also know that the effect of IL-4 and CD40 crosslinking on the ϵ -promoter is peculiar in that IL-4 by itself induces a relatively modest activation and CD40 crosslinking almost none, but these two signals synergize quite impressively [37]. The molecular basis of this synergism is not understood, but it may have a lot to do with the stepwise build-up of a transcription-activating complex that requires a strict cooperation between independently regulated transcription factors.

By contrast, γ 4 germline transcription was the dark side of the street. Approaches to detect these transcripts had been described by a number of groups [38, 39], but were invariably and understandably troubled by the homology between the loci for the four IgG subclasses. The most recent effort [40], predicted on the structure of γ 1- and γ 3-germline transcripts [41] and on the putative position of a shared splice donor site upstream of the switch γ 4-region [42], seemed to come quite close to success. Indeed, the product amplified by the reverse transcriptase polymerase chain reaction (RT-PCR) had the expected size and was digested by the expected restriction enzymes [40]. Regrettably, however, not even this approach was validated beyond reasonable doubt.

Like our predecessors, we used RT-PCR to amplify the putative γ 4-germline transcripts, but unlike them, we cloned and sequenced the products we got. Finally, satisfied that what we had amplified was indeed a γ 4 germline transcript (because it contained part of $I\gamma$ 4 correctly spliced to C γ 4 and all the diagnostic nucleotides were where they should be), we determined the 5' boundaries of $I\gamma$ 4 using primer extension analysis. With $I\gamma$ 4 finally identified and localized 0.6-kb upstream of the γ 4-switch region, we then tentatively identified the γ 4-germline promoter in a 1.5-kb region upstream of $I\gamma$ 4. We are now in the process of identifying the transcription factors that bind to it. We are also

developing a subclass-specific assay to quantitate γ 4-germline transcripts by competitive RT-PCR [A. Agresti, C. Di Bartolo and D. Vercelli, in preparation].

Although our work is still in its infancy, one important qualitative difference between the γ 4 and ϵ -germline promoters has already emerged. Indeed, whereas synergism is the name of the game in ϵ -germline transcription upon stimulation by IL-4 and CD40 crosslinking [37], the same signals seem to elicit only an additive response from the γ 4 promoter, and never as brisk. It remains to be seen whether this is truly the best the γ 4-promoter can do. Perhaps, we are still missing some elements and/or signals that could lead to a more efficient activation.

In conclusion, as we understand more and more about IgG4 regulation, our hope – our holy Graal – is to find out that the lesson we learnt from helminth infections can be applied to allergy [1]. Perhaps we will discover that the B cell has at some point a choice between the two IL-4-dependent isotypes, IgE and IgG4. Perhaps we will identify factors or signaling pathways critical for the one but not the other. Should this be the case, in the end we may be able to manipulate IL-4-induced responses in such a way as to make them less harmful or even harmless. We know that the scenario we propose may sound somewhat heretic. After all, how many times have we heard (and said) that IL-4 is the foe to search and destroy? Nevertheless we think that this possibility is too intriguing to leave it unexplored. And if that be madness, we devoutly hope that there is a method in it.

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