

Molecular regulation of the IgE immune response

D. VERCELLI

Molecular Immunoregulation Unit, San Raffaele Scientific Institute, Milan, Italy

Induction of IgE synthesis

T cells deliver to B cells the signals required for IgE induction through a complex series of interactions. Allergen-specific B cells capture antigen via their surface Ig molecules, internalize it, process it into peptides which are then presented on the B cell surface in association with MHC class II molecules. Recognition of the antigen/MHC class II complex by the T cell receptor leads to two crucial events: the secretion of lymphokines (in particular, IL-4, which provides the first signal for IgE induction), and the expression of CD40 ligand. CD40 ligand is not detectable on resting T cells, and it is the induction of this molecule that makes T cells finally competent to induce IgE. Engagement of CD40 by its ligand delivers the signal that triggers switch recombination, and results in IgE synthesis (reviewed in [1]).

The central role of IL-4 in IgE induction has been confirmed by gene targeting experiments. Mice in which the IL-4 gene had been knocked out by homologous recombination were unable to mount an anti-parasite IgE response [2]. Furthermore, genetic linkage analysis has shown that IL-4, or a nearby gene on chromosome 5q31.1, is responsible for the regulation of overall IgE production [3]. More recently, IL-13, another cytokine encoded on chromosome 5, has been shown to induce IgE synthesis by human B cells [4]. Receptors for IL-4 and IL-13 are thought to share a subunit that is involved in signal transduction (reviewed in [5]). The respective roles of IL-4 and IL-13 in IgE synthesis under physiological conditions remain to be established.

CD40 belongs to a newly defined family of cysteine-rich transmembrane receptors, which includes the receptor for nerve growth factor and TNF α , CD27, CD30, and the *fas* antigen. CD40L is a type 2 membrane glycoprotein, which is expressed on activated, but not on resting, TH-1 and TH-2 cells, and is part of a family that includes the ligands for CD27 and CD30, TNF α and lymphotoxin (reviewed in [6]). Cells transfected with CD40L induced IgE synthesis in both murine and human B cells in the presence of IL-4, whereas a soluble

CD40-Ig fusion protein inhibited IL-4-dependent IgE synthesis in human PBMC. These results clearly indicated that CD40/CD40L interactions are critical in delivering the second signal required for IgE production. The central role of the CD40/CD40L pathway in Ig switching has been confirmed by the finding that defective switching in patients with X-linked hyper-IgM syndrome results from an impairment in CD40L expression [7], and no IgG, IgA and IgE response to thymus-dependent antigens was detectable in CD40 knock out mice [8]. Recently, human basophils have been reported to express CD40L [9], and to secrete IL-4 [10]. Thus, basophils may provide both signals required for IgE synthesis. However, the role of basophils should be in IgE amplification, rather than induction: indeed, secretion of IL-4 is triggered by allergen-dependent cross-linking of receptor-bound, allergen-specific IgE, the production of which would involve allergen-specific T cells.

The TH-1/TH-2 dichotomy

Recent studies with T-cell clones obtained from patients with atopic disease and parasitic infections clearly indicate that the lymphokine profile of T-cell clones specific for an antigen is determined both by the nature of the antigen, and by the genetic background of the individual [11]. The majority of helper T-cell clones specific for parasitic antigens, isolated from patients with no history of atopic disease, had the TH-2 lymphokine profile described in mice, i.e. they consistently produced high levels of IL-4, and low IL-2 and IFN γ , and induced therefore vigorous IgE synthesis. On the other hand, allergen-specific CD4 T-cell clones established from atopic patients had a TH-2 lymphokine profile, and provided help for IgE synthesis, whereas helper T-cell clones from the same patients, specific for non-allergenic antigens, or CD4 clones from non-atopic individuals had a TH-1 profile. Thus, antigens such as those expressed on parasites seem to be able to evoke a TH-2/IgE response regardless of the genetic background. In contrast, the genetic background seems to be critical for the generation of a TH-2/IgE response against allergens.

The factors ultimately responsible for skewing the

Correspondence: Dr D. Vercelli, Molecular Immunoregulation Unit, DIBIT-H. San Raffaele, Via Olgettina 60, 20132 Milan, Italy.

T-helper repertoire towards TH-1 or TH-2 have not been fully identified. Complex cytokine amplification loops exist, which are likely to heavily influence T helper differentiation and IgE regulation. TH-2-derived IL-4 markedly inhibits IFN γ production by human T cells stimulated with mitogens or allogeneic cells, and furthermore directs the differentiation of precursor T cells into TH-2 cells that will secrete more IL-4. By contrast, IFN γ promotes the differentiation of CD4 T cells into TH-1 cells. It is therefore conceivable that, once an antigen has initially triggered a response — which will be TH-1 or TH-2 depending on the nature of the antigen and the genetic background of the individual — the skewing of the antigen-specific T-cell repertoire will be further amplified, and more T cells with the same lymphokine profile will be recruited. IL-12 has been shown to skew the profile of the TH response towards TH-1 [12]. IL-12 may provide a link between the 'natural' and the specific immune response, because some viruses and intracellular bacteria have the ability to stimulate macrophages to produce IL-12, that in turn induces IFN γ production by both T cells and NK cells. Thus, TH cells may be simultaneously presented with processed antigen plus cytokines that induce them to differentiate toward a TH-1 phenotype. Further understanding of the mechanisms underlying the TH-1/TH-2 dichotomy may be provided by ongoing studies, suggesting that human TH-2 cells may be defined by the presence of CD30 [13], and the absence of CD27 [14].

Molecular events in IgE switching

The effects of IL-4 on IgE switching result from the ability to activate transcription through the ϵ locus, with expression of ϵ germ line transcripts (GLT). Human ϵ GLT consist of a ≈ 134 bp I ϵ exon located ≈ 3.5 kb upstream from C ϵ , in a region which is deleted by switch recombination in the expressed allele of the U266 IgE myeloma. Transcription initiation sites are scattered within a stretch of 90 bp. The I ϵ exon is directly spliced to C ϵ 1 by removal of the intervening sequences from the primary transcript [15]. GLT contain all four C ϵ exons, as well as the 3' untranslated region. The human I ϵ exon does not contain an initiation codon in frame with the C ϵ region, and stop codons were observed in all three reading frames, suggesting that no protein is encoded by these transcripts. The region upstream from the initiation sites lacks well-defined TATAA motifs [15].

IL-4 dependent ϵ GLT expression is regulated by cytokines known to modulate IgE synthesis. TGF- β and PAF-acether block the expression of ϵ GLT in IL-4-treated human B cells. IFN γ profoundly suppressed

the expression of ϵ GLT in murine B cells stimulated with IL-4 and LPS, but no inhibition was observed in IL-4-stimulated human B cells. Thus, TGF- β and PAF-acether may inhibit IgE production by acting on GLT expression, whereas IFN γ seems to affect post-transcriptional events [1].

Although IL-4 is by itself sufficient for the initiation of transcription through the ϵ locus, switch recombination and expression of mature C ϵ transcripts (containing VDJ spliced to C ϵ 1-4) require engagement of CD40 on B cells by the CD40 ligand expressed on activated T cells. DNA recombination events underlying IgE isotype switching have been investigated using nested primer polymerase chain reaction (PCR) amplification of ϵ switch fragments, followed by cloning and DNA sequencing [16]. S μ /S ϵ switch fragments were amplified from B cells stimulated with IL-4 and EBV, or IL-4 and anti-CD40 MoAb, or IL-4 and hydrocortisone, but not from B cells stimulated with EBV, anti-CD40 MoAb, hydrocortisone or IL-4, alone. DNA sequencing revealed that deletional switch recombination with direct S μ /S ϵ joining was the predominant mode of switching. Occasionally, switch fragments contained insertions at the S μ /S ϵ junction which were derived from S γ 4 [17]. These findings provide evidence for sequential isotype switching from IgM to IgE via IgG4.

IgE regulation and lymphokine patterns in the aged

IgE production steadily declines with age. After reaching peak levels in the latter half of the first decade and at the beginning of the second decade of life, serum IgE levels both in normal and atopic subjects decline progressively to reach the lowest levels after the age of 60 years. In parallel, the incidence of onset of allergic symptoms, as well as their severity, decrease. Aging therefore represents an interesting *in vivo* model to study the waning of allergic responses.

We recently compared IgE production in the elderly (≥ 60 years old) and young (15–30 years old) non-atopic individuals [18]. Our studies showed that IL-4 responsiveness of B cells activated by cognate and/or contact-dependent signals is intact in elderly humans, because addition of exogenous IL-4 to mononuclear cells from aged and young subjects induced equivalent amounts of IgE. Lymphokine secretion is altered in ageing. Following surface receptor stimulation with Con A, IL-4 production by mononuclear cells from elderly subjects was $\approx 50\%$ as compared with the young, whereas IFN γ production was reduced threefold. The pattern of lymphokine secretion in ageing results from a defect in T-cell signal transduction. Indeed, when surface receptors were bypassed by stimulation with PMA + ionophore,

comparable secretion of IL-4 and IFN γ was induced in old and young individuals. Furthermore, Ca⁺² fluxes in response to CD3 crosslinking were significantly decreased in the elderly group.

Thus, a subtle defect in IL-4 due to an age-dependent impairment in the responsiveness to surface-mediated activation signals, may underlie the progressive decrease in IgE production observed in ageing. Allergen-specific human T cells are typically TH-2, i.e. they produce high amounts of IL-4. Because IL-4 is necessary for IgE isotype switching, a decreased availability of IL-4 would result in reduced IgE synthesis. The concomitant decrease in the IL-4 antagonist, IFN γ (due to the same age-related signalling impairment) could not compensate for the IL-4 defect.

The hypothesis that the decline of IgE responses in aging is due to a decrease in IL-4 production by TH-2 cells has found indirect support in the genetic linkage analysis of chromosome 5q31.1, and in the observation that the CD45RO⁺ T cell subset is reduced in elderly subjects. This subset includes memory and suppressor-inducer T cells, and is unique, in that it contains CD30⁺ T cells, which have been recently identified as TH-2 [13]. A reduction in CD45RO⁺/CD30⁺ TH-2 cells may therefore be responsible for the decrease in IL-4 production observed in ageing.

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