Yin outwits Yang at the IgE locus

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Serum IgE concentrations are kept low to avoid potential allergic complications. New data show how Id2 suppresses class switching to the ε isotype and reduces IgE expression.

Normally, serum immunoglobulin E (IgE) concentrations are far lower than those of other Ig isotypes. This makes biological sense because IgE is the isotype that may do more harm than good. Although IgE antibodies provide protection against a limited spectrum of parasites, they also stimulate mast cells and basophils to release an array of mediators, including histamine, leukotrienes and prostaglandins, with physiological consequences that range from hay fever and food allergy to asthma and anaphylaxis[1,2]. How are IgE concentrations kept low? In this issue, Shimizu and colleagues[3] identify Id2 as a negative regulator of IgE expression, acting at the level of class switching, and further show that the cytokine transforming growth factor-β1 (TGF-β1) induces Id2 expression, thereby suppressing IgE.

The starting point for these exciting results was the demonstration that Id2$^{-/-}$ mice are impaired in specific aspects of immune function[4,5]. In Id2$^{-/-}$ animals, splenic architecture appears normal, with typical B and T cell compartments and germinal centers, but numbers of natural killer cells are reduced, lymph nodes and Peyer's patches are absent and the distribution of serum antibody isotypes is perturbed, with IgE concentrations dramatically elevated. Asking what accounts for the elevation in IgE serum antibodies, Shimizu and colleagues[3] document a dramatic increase in the number of IgE^{+} splenic B cells produced in response to immunization and show that this reflects increased switch recombination to the ε isotype.

Switch recombination is activated and targeted by transcription of switch (S) regions, short (2–10-kb) stretches of guanine-rich DNA found just upstream of those constant regions that participate in switch recombination[6]. S region transcription produces a germline transcript that does not code for protein, and the critical role of S region transcription in the recombination mechanism is thought to reflect increased accessibility of transcribed regions and/or formation of DNA structures that are targets for nuclease attack. Germline transcription of each S region is driven by a dedicated promoter containing a specific combination of *cis*-regulatory elements, so the identification of factors that regulate S region transcription is key to understanding regulation of isotype expression at the molecular level. Shimizu and colleagues[3] show that in Id2$^{-/-}$ mice increased recombination to ε correlates with increased levels of $S_ε$ germline transcripts, thus establishing the role of Id2 in IgE regulation.

Id family proteins such as Id2 are negative regulators that counterbalance activator proteins of the E family by sequestering them in Id-E heterodimers[7,8]. E family proteins bind to E box sequence motifs in duplex DNA to activate transcription. DNA binding and dimerization require a specialized basic helix-loop-helix (bHLH) domain, which mediates protein-protein interactions and also contains a positively charged region that contacts DNA. Id proteins contain HLH domains for dimerization, but lack the basic region necessary for DNA binding. Thus Id proteins can dimerize with E proteins, but the resulting Id-E heterodimer cannot bind DNA to activate transcription.

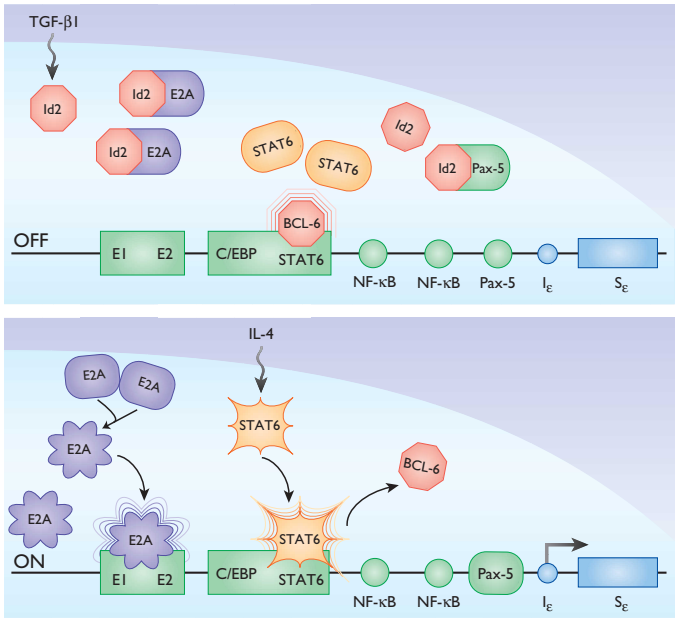

Figure 1. The ε switch region ($S_ε$) must be transcribed in order for class switch recombination to ε and IgE expression to occur. $S_ε$ transcription is driven by a dedicated promoter ($I_ε$). Upstream binding sites for several different factors have been identified, including E2A, C/EBP, STAT6, NF-κB and Pax5. Id2 acts as a negative regulator of $S_ε$ transcription by sequestering E2A to prevent interaction at the E1 and E2 boxes. Id2 expression is activated by the cytokine TGF-β1. IL-4 induces the positive regulator STAT6, which competes for DNA binding with the negative regulator BCL-6. Positive regulators, green; negative regulators, red; cytokines, blue.

Once Id2 was shown to regulate $S_ε$ transcription, the Id-E paradigm predicted that E family members would act at the $S_ε$ germline promoter. Consistent with this, two adjacent E box motifs could be identified in the promoter region for $S_ε$ germline transcription (**Fig. 1**) and deletion of either E box was shown to abolish activation by E2A, the predominant E family member in B cells. The $S_ε$ promoter contains sites for factors other than E2A, including C/EBP, signal transducers and activators of transcription 6 (STAT6), NF-κB and Pax5 (**Fig. 1**). Id2 can interact with Pax5 to inhibit its DNA binding[9], a property that may further contribute to suppression of $S_ε$ germline transcription. STAT6 is activated by the cytokine interleukin 4 (IL-4), and it has long been known that culture with IL-4 causes primary murine B cells to switch to ε and to γ1. Somewhat paradoxically, IL-4 has a greater stimulatory effect on γ1 switching than ε. Shimizu and colleagues offer an explanation, showing that although the inhibitory effect of Id2 on switching to ε is pronounced, there is not a comparably strong effect on switching to γ1[3]. In particular, deletion of either E box causes transcription to become refractory to the otherwise synergistic effect of IL-4 and E2A.

Shimizu and colleagues also provide a mechanistic insight into regulation of the immune response by the pleiotropic cytokine, TGF-β1, which manifests both anti-inflammatory and immunosuppressive effects[3]. TGF-β1 was thought somehow to regulate

switching to ε because TGF-β1–deficient mice are characterized by dramatically elevated serum IgE concentrations[10]. Shimizu and colleagues now show that treatment of B cells with TGF-β1 induces Id2 expression, thereby down-regulating S_ε germline transcription and switching to ε[3].

These results have exciting implications. The specific functions described for Id2 and TGF-β1 in IgE regulation suggest that defects in these factors may contribute to human diseases characterized by IgE overproduction, a possibility that can be directly tested by genetic analysis. Identification of Id2 as a critical suppressor of ε switch transcription raises the possibility that other genes in activated B cells may be targets of negative regulation by Id2. The demonstration that TGF-β1 induces Id2 expression suggests that this may prove to be a general mechanism for relaying signals by this pleiotropic cytokine. And finally, there may be practical ramifications. Because the negative consequences of IgE production can be so profound, suppression of IgE is an important therapeutic goal, and the specificity with which Id2 regulates ε switching makes Id2 an intriguing target for therapy.

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Novel interferons

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Type I IFNs are important in antiviral immunity. Two studies report the identification of another family of molecules that have similar properties to the type I IFNs but are otherwise structurally and genetically distinct.

Interferons (IFNs) form an important group of cytokines that are best known for their ability to induce cellular resistance to virus infection[1–3]. However, IFNs also affect many other cellular functions, such as cell growth, and they possess immunomodulatory activities. IFNs include the type I IFN family (also termed the IFN-αβ family) and a single member type II or IFN-γ family. In humans, type I IFNs comprise at least 13 functional nonallelic genes encoding IFN-α, one gene encoding IFN-β and the less extensively studied genes encoding IFN-ω, IFN-κ and limitin[4,5]. All type I IFNs bind to the same heterodimeric receptor (IFN-αβR), whereas the IFN-γ protein binds to IFN-γR. The extracellular domains of the IFN-αβR and IFN-γR subunits contain conserved amino acid residues, including several cysteine residues, that are also found in the subunits of the interleukin 10 receptor (IL-10R) and in receptors for the emerging family of IL-10–related proteins, all of which belong to the class II cytokine receptor family[6,7]. In this issue of *Nature Immunology* Sheppard *et al.*[8] and Kotenko *et al.*[9] describe a novel family of cytokines that are structurally related to the type I IFNs and to the IL-10 family. Like other IFNs, the newly described cytokines protect cells from virus infection and induce major histocompatibility complex (MHC) class I antigen expression, suggesting that these previously unknown mediators contribute to the antiviral defenses and perhaps carry out other functions similar to those of the type I and type II IFNs. Although functionally similar to type I IFNs, these cytokines can be viewed as members of a distinct family: the first novel IFN family defined in over 20 years.

The three members of this newly identified cytokine family, termed IFN-λ1, IFN-λ2 and IFN-λ3 by one group[9] and IL-28A, IL-28B and IL-29 by the other[8] (HUGO has tentatively used the interleukin nomenclature), bind to a heterodimeric receptor, in which one subunit is a novel member of the class II cytokine receptor family and the other is identical to the second chain of the IL-10R (**Fig. 1**). These newly described cytokines are functionally similar to the type I IFNs because their synthesis is induced by virus infection or double-stranded RNA, they render cells resistant to virus infection and they activate the same intracellular signaling pathways as type I IFNs. Despite the similarities to type I IFNs, a number of observations indicate that the IFN-λs represent a distinct family. (For simplicity, the terminology proposed by Kotenko *et al.*[9] will be used here.) The sequence similarity of IFN-λ to IFN-α (15–19 % amino acid identity) is significant but lower than among even the most distant members of the type I IFN family. In addition, the genes for all three members of the IFN-λ family are clustered on chromosome 19 (q13.13 region), whereas the genes for all type I IFNs are clustered on human chromosome 9 (the gene encoding IFN-γ is located on chromosome 12). Finally, the IFN-λ genes contain multiple exons, whereas type I IFN genes are encoded within a single exon. As pointed out by Sheppard *et al.*[8], the IFN-λ family represents an interesting evolutionary link between the type I IFNs and the IL-10 family: although IFN-λ proteins are structurally more closely related to the type I IFNs than to IL-10, their genomic structure resembles that of the IL-10 family.

One of the most interesting findings reported by the two groups[8,9] is that all three IFN-λ proteins utilize a heterodimeric class II cytokine receptor composed of the newly identified class II cytokine receptor subunit IFN-λR1 (also termed IL-28Ra[8]) and a second chain, IL-10R2, that also serves as a subunit of the IL-10R and of the receptor for the IL-10–related cytokine IL-22[7]. Promiscuity in the usage of cytokine receptor subunits is a common vice among cytokines: for example, several members of the IL-2 family share the receptor γ-chain and the IL-6 family cytokines share the gp130 subunit[10]. Similar to other class II cytokine receptors, IFN-λR1 appears to determine the specificity of binding and it probably also drives much of the recruitment of intracellular signaling molecules.

Type I and II IFNs and all other known cytokines that utilize class II cytokine receptors signal by activating the Jak tyrosine kinases–signal transducers and activators of transcription