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Molecular Mechanisms of Immunoglobulin E Regulation

Key Words

IgE · Isotype switching · Interleukin-4 · Interleukin-13

Introduction

Immunoglobulin (Ig) E plays a key role in the pathogenesis of allergic disease. The studies on the cellular basis of IgE regulation have provided important insights into a disease process which affects a considerable proportion of the population worldwide. More recently, the molecular events underlying IgE synthesis have been actively investigated. In this review, we will discuss the cells, the cytokines, the signal transduction events and the molecular mechanisms involved in human IgE synthesis as well as therapeutic approaches directed at the abrogation of these processes.

B-Cell Gene Rearrangements Leading to IgE Expression

The Ig molecule is composed of heavy and light chains encoded by distinct gene clusters. The gene cluster for the lambda (λ) light chain is on human chromosome 22, the kappa (κ) light chain is on human chromosome 2, and the Ig heavy chain locus is on chromosome 14. These loci are configured such that a series of gene rearrangements is required in order to generate the extensive repertoire of Ig specificities [reviewed in 1]. The variable regions of the κ and λ light chains are generated through somatic recombination of the variable ($V\kappa$ and $V\lambda$) and joining ($J\kappa$ and $J\lambda$) segments while the heavy chain variable region results from recombination of variable, joining and diversity (D) segments [2]. In the case of the heavy chains, joining of D to J_H segments precedes the joining of V_H to the previously rearranged (D) J_H segments. The processes of (D) J_H and V_H (D) J_H recombination involve the juxtaposition of conserved heptamer and nonamer repeats located upstream to the

V genes, downstream of the J genes and on both sides of the D genes. When these recombination signal sequences are brought together, the intervening DNA is looped out and recombination occurs with joining of the (D) J_H or V_H (D) J_H sequences and excision of the intervening DNA. These recombination events are accomplished by the products of the recombination activator genes *RAG-1* and *RAG-2*, which have topoisomerase-like activity and result in DNA breakage and rejoining. The critical role for these gene products is exemplified by mice genetically deficient in *RAG-1* or *RAG-2*, which are unable to rearrange their immunoglobulin genes due to an inability to initiate V(D)J recombination [3, 4]. In addition, a form of human autosomal recessive severe combined immunodeficiency has been linked to defective *RAG-1* and *RAG-2* function with a marked reduction or absence of V(D)J recombination activity [5].

During the development of an immune response, a B lymphocyte can express different immunoglobulin heavy chain isotypes which share the same antigen-binding region. The phenomenon of isotype switching allows a single B-cell clone to produce antibodies with the same fine specificity but different effector functions determined by the heavy chain isotype. The genes encoding the Ig heavy chain isotypes form a cluster located 3' to the J_H region genes and contains segments for $C\mu$, $C\delta$, $C\gamma3$, $C\gamma1$, a pseudogene for epsilon ($C\psi\epsilon$), $C\alpha1$, $C\psi\gamma$, $C\gamma2$, $C\gamma4$, $C\epsilon$, and $C\alpha2$ arranged in tandem. The 5' intronic region of each heavy chain isotype gene, except $C\delta$, includes a switch (S) region which contains repeated sequences of GAGCT and GGGGT [6]. Following the signal which activates the switch machinery (see below), these switch regions undergo recombination and result, in the case of switching to IgE, in the formation of an $S\mu/S\epsilon$ junction with deletion of the intervening DNA segments [7, 8]. This process occurs during switching to all

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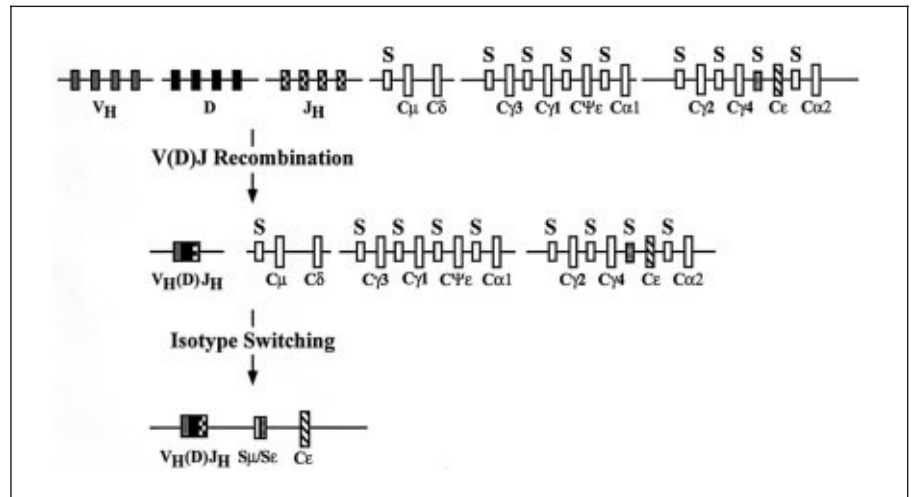


Fig. 1. DNA rearrangement events in B-cell ontogeny and maturation. The top panel shows a schematic arrangement of the Ig heavy chain genes in the germline. This consists of an array of V_H genes, D genes, J_H genes and genes encoding for the constant portion of the Ig heavy chains (C_H). Each of the C_H genes consists of several exons including exons encoding the transmembrane region and the secreted region of each chain. Each C_H gene, except C_{δ} , is flanked on its 5' side by a switch region (S) containing repetitive DNA sequences. Differentiation into a mature B cell involves V(D)J recombination with the formation of a V(D)J segment that encodes for the antigen-binding domain of the Ig molecule. This V(D)J segment lies in proximity of C_{μ} and is separated from it by only those J_H genes and intervening se-

quences that were not deleted during V(D)J recombination. The B cell then transcribes an RNA that reads through V(D)J, C_{μ} and C_{δ} . Alternative splicing results in IgM and IgD mRNA which are then translated into IgM and IgD proteins. The bottom panel depicts the Ig gene organization in a B cell that has switched to IgE. This B cell has rearranged its heavy chain genes by deletional switch recombination involving S_{μ} and S_{ϵ} . All intervening DNA has been deleted and now the V(D)J region is the same proximity to C_{ϵ} as it was to C_{μ} . An RNA is transcribed through V(D)J and C_{ϵ} and spliced to form an mRNA that contains V(D)J- C_{ϵ} and encodes for IgE. Since the V(D)J segment is conserved, the antigen specificity of the Ig remains unaltered.

isotypes except for IgD, which results from splicing variations of the C_{μ} - C_{δ} transcript. It should be noted that class switching can occur sequentially, such that a B cell which had previously switched to C_{γ} can later switch to an isotype located 3' to the C_{γ} gene, such as C_{ϵ} , by repeating this same process. RNA transcription proceeds from the $V_H(D)J_H$ segment to the C_{ϵ} segment following by splicing out of the intronic and switch regions and results in a mature mRNA species for an ϵ heavy chain. Therefore, in order to produce a functional immunoglobulin molecule, B cells must undergo two DNA rearrangement events. The first rearrangement involves $V_H(D)J_H$ recombination and gives rise to an IgM^+IgD^+ B cell. The second rearrangement involves isotypes switching and gives rise to a B cell expressing IgG, IgA, or, as illustrated in figure 1, IgE.

The Two-Signal Model for the Induction of IgE Synthesis

In order to undergo isotype switching, B cells require two distinct signals [9, 10]. The initial signal is cytokine-

dependent and results in the activation of transcription at a specific region of the Ig locus, thus determining isotype specificity. The second signal activates the recombination machinery, resulting in DNA switch recombination.

The two signals required for switching to IgE are delivered to B cells by T cells through a complex series of interactions. These are illustrated in figure 2 [reviewed in 11]. Antigen-specific IgM on the B-cell surface binds antigen, leading to internalization of the antigen-receptor complex. The antigen then undergoes endosomal processing and subsequent presentation to an antigen-specific T cell in the form of peptide fragments associated with HLA (human leukocyte antigen) class II molecules. Recognition of the antigen/HLA class II complex on the surface of the B cell by the T-cell receptor (TCR) complex leads to two crucial events: (1) the secretion of lymphokines, in particular interleukin (IL)-4 and/or IL-13, and (2) the expression of the CD40 ligand (CD40L, CD154) by the T cell. Binding of IL-4 to its receptor on the B cell delivers the first signal for isotype switching and induces transcription of the C_{ϵ} heavy chain gene locus [12]. Binding of the TCR complex to an appropriate peptide fragment in the context of HLA class II re-

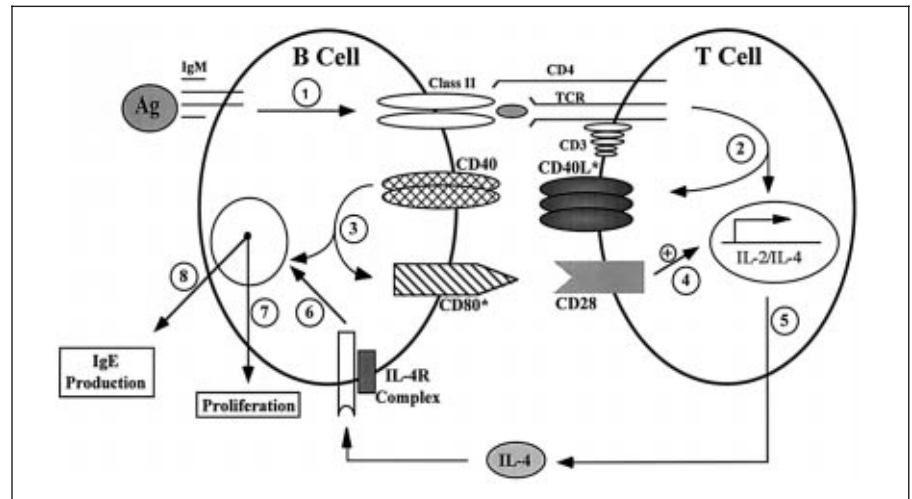


Fig. 2. T- and B-cell interactions involved in IgE synthesis. Binding of an antigen (Ag) by a B cell with surface IgM specific for the Ag leads to (1) internalization of the IgM/Ag complex and is followed by endosomal processing and presentation on the surface of the B cell in the context of HLA class II molecule. (2) Recognition of this complex by an Ag-specific TCR complex induces CD40L expression. (3) Ligation of CD40 by CD40L results in upregulation of CD80 (B7) expres-

sion. (4) Engagement of CD28 by CD80 delivers a co-stimulatory signal to the T cell which, together with the TCR signal (2), leads to (5) cytokine synthesis and T-cell proliferation. (6) The cytokine IL-4 binds to its receptor on B cells and synergizes with the CD40 signal (3 and 6) to induce (7) B-cell proliferation and (8) IgE isotype switching and synthesis. Asterisk denotes molecules that are not constitutively expressed but which are inducible by the above signals.

sults in activation of the T cell and expression of CD40L is a 39-kDa type II membrane glycoprotein which is absent on resting naive T cells [13]. The transient expression of CD40L following T-cell activation renders T cells fully competent to induce isotype switching to IgE [14]. CD40L engages CD40, a 50-kDa integral membrane glycoprotein member of the tumor necrosis factor (TNF) receptor superfamily that is constitutively expressed on B cells, macrophages, dendritic cells, and endothelial cells [13]. CD40L-induced oligomerization of CD40 on B cells delivers the second signal that triggers deletional switch recombination to IgE. Ligation of CD40 on the B-cell surface by CD40L results in the upregulation of several B-cell surface molecules. Expression of B7 (CD80) is increased following CD40 activation and serves as a counterreceptor for CD28, a co-stimulatory molecule constitutively expressed on T cells. The simultaneous engagement of the TCR and CD28 results in optimal T-cell activation and secretion of cytokines, including IL-4 [11, 15].

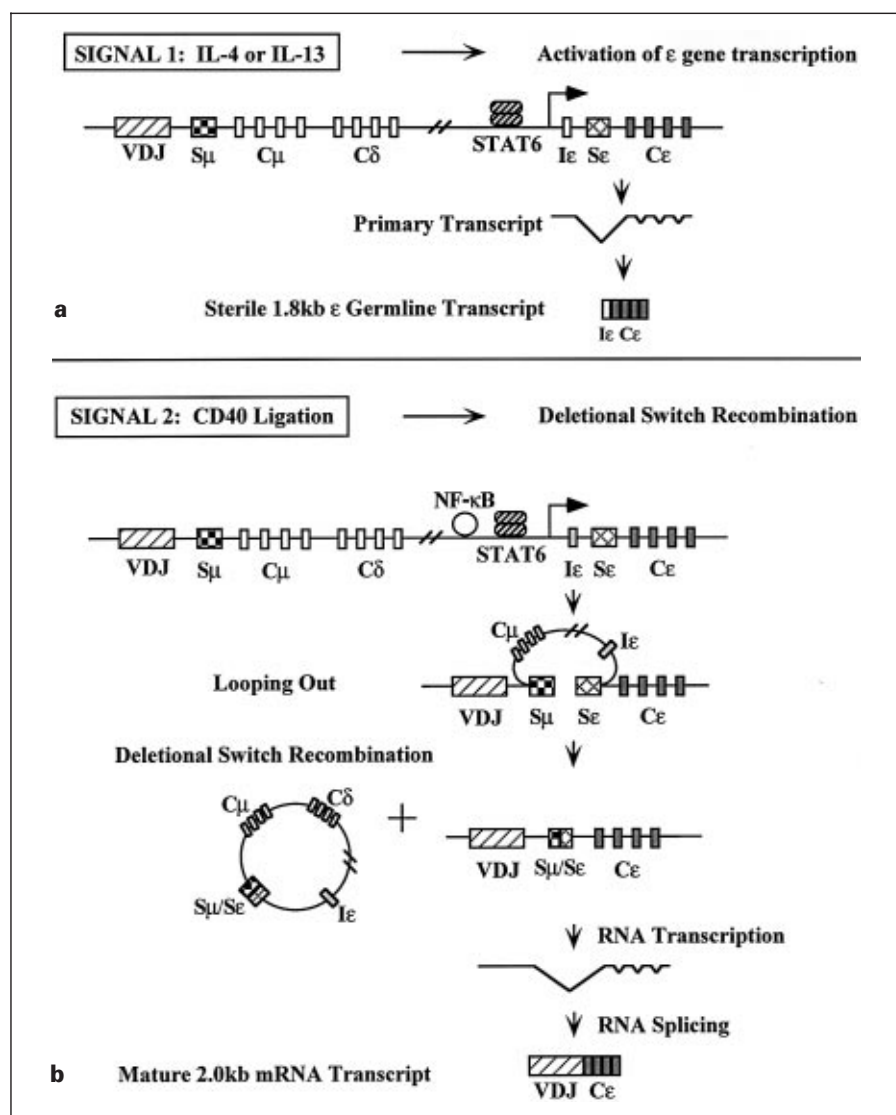
The importance of CD40-CD40L interactions in isotype switching is illustrated by several observations. Monoclonal antibodies directed against CD40 eliminate the need for T cells in IL-4-driven isotype switching to IgE in vitro [16]. Soluble human CD40 inhibits in vitro T-cell-driven IL-4-dependent IgE synthesis [17]. Patients with the X-linked hyper-IgM syndrome (X-HIM) are deficient in CD40L and

lack serum IgG, IgA and IgE due to defective isotype switching in vivo [18–22]. In addition, mice with targeted disruption of the CD40L [23] or CD40 [24, 25] genes lack serum IgE and fail to undergo isotype switching in vivo and in vitro following immunization with T-cell-dependent antigens but are capable of mounting IgM and IgG responses to T-cell-independent antigens.

Stimulation of the IL-4 receptor (IL-4R) by IL-4 delivers the first signal by inducing transcription of C ϵ RNA which initiates upstream of the I ϵ exon and proceeds through the S ϵ and the C ϵ exons (fig. 3a). The S ϵ is then spliced out and results in the ϵ germline transcript (GLT) consisting of the 140-bp I ϵ exon and exons C ϵ 1–C ϵ 4 [26]. The ϵ -GLT is sterile due to the presence of stop codons in each of the three reading frames of the I ϵ exon. IL-4 or IL-13 alone are capable of inducing ϵ germline transcription without the requirement for previous B-cell stimulation [26, 27]. The exact role of the germline transcript is presently unclear, but it may serve to promote specific recombination events.

Following the second signal via CD40, deletional switch recombination occurs [7, 8] (fig. 3b). DNA is cut in the switch region that is upstream of the C μ gene (S μ) and in the switch region that is upstream of the C ϵ gene (S ϵ). The two switch regions are then joined together to form an S μ /S ϵ junction. The intervening DNA is circularized and deleted. This rearrangement brings the V_H(D)_H segments encoding

Fig. 3. Molecular events in IgE isotype switching. **a** In an IgM B cell, the V(D)J segments have been rearranged and lie in proximity to the constant region genes for the IgM and IgD heavy chains $C\mu$ and $C\delta$. The $C\mu$ gene is flanked at its 5' side by a switch region $S\mu$. Transcript initiates 5' of V(D)J and extends downstream of $C\delta$. This transcript can be spliced into a V(D)J+ $C\mu$ or VDJ+ $C\delta$ mRNA which encode for IgM and IgD, respectively. Signal 1 is delivered by IL-4 or IL-13 and results in the activation of transcription factors that bind upstream to the $I\epsilon$ exon. This initiates transcription through $I\epsilon$, the switch region $S\epsilon$, and the $C\epsilon$ exons. The spliced $I\epsilon$ + $S\epsilon$ mRNA germline transcript is sterile and does not encode for a full-length protein because there are stop codons in all three reading frames within the $I\epsilon$ exon. **b** Signal 2 is delivered via CD40 and targets the transcriptionally active ϵ gene for switch recombination. This occurs by deletion of the DNA region between $S\mu$ and $S\epsilon$ with the formation of an $S\mu$ / $S\epsilon$ switch junction by looping out and deletional switch recombination. This brings the V(D)J segment close to $C\epsilon$. Transcription followed by splicing results in a V(D)J+ $C\epsilon$ mRNA that encodes for an IgE molecule that retains the exact antigen specificity of the original IgM because they both use the same V(D)J segment that encodes for the antigen-binding site.



the antigen-binding site into proximity of the $C\epsilon$ -encoding segments and results in the transcription of a message that encodes for a full ϵ heavy chain.

A detailed understanding of the mechanisms of isotype switching is still lacking. It is thought that transcription through an Ig heavy chain locus renders that region accessible to switch recombination, although the mechanism by which this occurs is unclear [28]. Recent data suggests that the recombination machinery is constitutively active in B cells, since an artificial substrate containing DNA sequences that encompass murine $S\mu$ and $S\gamma 2a$ undergoes deletional switch recombination when integrated into the genome of murine B-cell lines [29]. Transcription through the switch region is not sufficient by itself to activate isotype switching, because replacement of the promoter of $C\epsilon$ and

of the $I\epsilon$ exon by an LPS-responsive promoter induces strong transcription through the switch region and of $C\epsilon$ but results in minimal IgE isotype switching [30]. This supports a role for the $I\epsilon$ -containing mRNA transcript and/or a putative short polypeptide derived from the $I\epsilon$ exon in rendering the $S\epsilon$ region a suitable substrate for switch recombination, perhaps by allowing the recruitment of the 'recombinase' [31]. The switch recombinase is likely to be a complex of several proteins, including the DNA-dependent protein kinase (DNA-PK), which is defective in *scid* mice [32], and the associated DNA-binding proteins Ku70 and Ku80 [33, 34]. The importance of the DNA-PK complex in isotype switching is illustrated by experiments in which B-cell precursors from *scid* mice express ϵ GLTs after stimulation with anti-CD40 monoclonal antibody (mAb) plus

IL-4 but fail to undergo S μ /S ϵ recombination [35]. In addition, nuclear extracts from resting B cells contain relatively low levels of the Ku heterodimer but, following activation through CD40 ligation plus IL-4, Ku expression is increased [36]. Thus, both DNA-PK and Ku70/80 appear to be integral members of the isotype switch machinery.

Signal 1: The Cytokines IL-4 and IL-13

CD4⁺ T-helper (T_H) lymphocytes are heterogeneous and can be divided into two subtypes based on their patterns of cytokine production [reviewed in 37]. T_H1 cells produce predominantly IL-2, TNF- β and IFN- γ and promote delayed-type hypersensitivity responses while T_H2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13 which favor humoral immune responses including isotype switching and the development of allergic diseases. The T_H1-derived cytokines especially IFN- γ , inhibit T_H2 cytokine production and T_H2-derived cytokines inhibit T_H1 cytokine production. Consistent with this paradigm, while IL-4 and IL-13 induce ϵ germline transcription, IFN- γ suppresses the IL-4 induction of ϵ GLTs [38].

The interaction between the cytokines IL-4 and IL-13 and their receptors delivers the first signal for switching to IgE. Evidence from different lines of inquiry consistently shows that IL-4/IL-13 is essential for IgE production. IL-4 and IL-13 are the only known cytokines able to induce IgE synthesis in vitro when added in recombinant form [9, 27, 39]. Anti-IL-4 antibodies preferentially inhibit IgE synthesis without affecting IgM, IgG or IgA synthesis [12, 40, 41]. Injection of an anti IL-4 or anti IL-4 receptor antibody abolishes IgE production in parasite-infected mice [42, 43]. A recombinant extracellular domain of the IL-4R blocked switching to IgE by blocking IL-4/IL-4R interactions [44] and shifted the immune response of mice infected with *Leishmania major* from a T_H2-predominant response associated with disease susceptibility to a T_H1-predominant response resulting in disease resistance [45].

IL-4 is an 18–20-kDa glycoprotein produced by T_H2 cells, NK 1.1⁺ cells, mast cells and basophils [46]. Like other cytokines, it demonstrates pleotropic effects which include favoring development of T_H2-type T cells, increasing surface CD23 (Fc ϵ R2), MHC class II and IL-4R expression on B cells and monocytes, and increasing VCAM-1 expression on endothelial cells. The proto-oncogene *c-maf* has recently been described as a murine T_H2-specific transcription factor and appears to be an important element in the control of the IL-4 promoter [47]. *c-maf* synergizes with the nuclear factor of activated T cells (NF-AT) in transactivation of the IL-4 promoter, an effect which is markedly enhanced by the NF-AT-interacting protein NIP-45 [48].

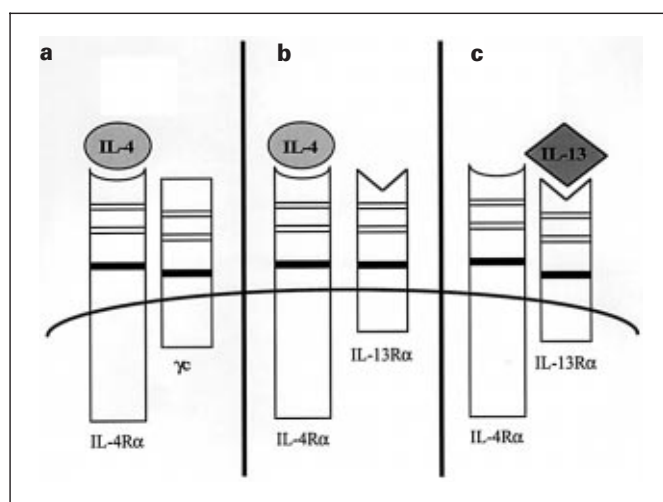


Fig. 4. Relationship of IL-4 and IL-13 receptors. **a** The IL-4R is comprised of two chains: IL-4R α chain that binds IL-4 and a γ c chain. **b, c** The IL-13R consists of two chains, an IL-13R α chain and IL-4R α . IL-4 can bind both IL-4R and IL-13R because it binds with high affinity to the IL-4R α chain used by both receptors. IL-4 has very poor affinity to γ c and none to IL-13R α . IL-13 binds only to the IL-13R because it has high affinity only to IL-13R α .

IL-13 is produced by activated T_H2 cells and mast cells [49]. The biologic effects of IL-13 overlap those of IL-4 with the notable exception that IL-13 does not activate human T cells [39, 50]. Like IL-4, IL-13 increases CD23 and MHC class II expression on B cells and monocytes and induces ϵ germline transcription and isotypes switching to IgE and IgG₄ [27, 39, 50]. However, IL-13 is less potent than IL-4 in most of these actions.

Figure 4 depicts the structure of the receptors for IL-4 and IL-13. The IL-4R complex is composed of two chains, the 145-kDa IL-4R α chain and the γ c chain which is shared with the receptors for IL-2, IL-7, IL-9 and IL-15 [51]. IL-4R α binds IL-4 with high affinity and is similar in structure to type I hematopoietin receptors with four conserved cysteine residues and a WSXWS motif in the extracellular domain. The IL-4R complex is expressed on multiple cell types including T cells, B cells, mast cells, endothelial cells and macrophages [46]. The IL-4 receptor exists in both a cell membrane bound form as well as in a soluble, circulating form in vivo [52]. In addition, recent data indicates that several allotypes of the murine IL-4R exist and that BALB/c mice possess an allotype characterized by a significantly enhanced rate of IL-4 dissociation from the receptor [53]. This may account for the diverse responses in different inbred mouse strains in IL-4-dependent disease models. The IL-13R is a heterodimer which consists of an IL-13 binding

(IL-13R α) chain and the IL-4R α chain [54–57]. IL-4 can bind to the IL-13R by virtue of its affinity to the IL-4R α chain, whereas IL-13 does not bind to the IL-4R.

Figure 5 depicts the mechanism of signal transduction via the IL-4R. The IL-4R α and γ c chains lack known kinase activity and are associated with the Janus family tyrosine kinases JAK1 and JAK3, respectively [58, 59]. Upon activation, presumably secondary to IL-4-induced heterodimerization of the IL-4R α and γ c chains, these kinases phosphorylate tyrosine residues in the intracellular domains of the receptor chains. The intracellular domain of IL-4R α contains two tyrosine residues which, upon phosphorylation, serve as docking sites for two units of the signal transducer and activator of transcription (STAT) molecules STAT6 [60, 61]. STAT6 molecules in turn undergo phosphorylation and form homodimers which subsequently translocate to the nucleus where they bind to specific DNA sequences (TTCNNN(N)GAA) in the promoter regions of IL-4-responsive genes, including C ϵ [62]. The importance of STAT6 in IL-4 signaling is reinforced by the lack of IgE class switching in mice deficient for STAT6 [63].

Although IL-13 activates STAT6 and induces C ϵ GLTs it does not activate JAK3 [64–67]. Consistent with this observation, IL-13R signaling is intact in B cells from JAK3-deficient patients [68]. There is evidence that the IL-13R α chain associates with JAK2 [65] and with the Janus kinase family member TYK2 [67]. Upon ligand binding, heterodimerization of the IL-13R chains initiates a cascade of events identical to that which follows heterodimerization of the IL-4R chains by IL-4, resulting in IL-4R α chain phosphorylation, docking and phosphorylation of STAT6 followed by STAT6 dimerization and nuclear translocation.

The adaptor protein insulin receptor substrate-1 (IRS-1) and the antigenically related 4PS also undergo phosphorylation following IL-4 treatment [69]. IL-4-dependent phosphorylation of IRS-1 may be mediated by JAK1, TYK2 or JAK2 [70]. The intracellular domain of the IL-4R α contains an I4R motif which is also present in the insulin and insulin-like growth factor-1 receptors. Phosphorylated IRS-1 binds to the I4R motif and then functions as a docking protein for many src-homology 2 (SH2) domain-containing molecules, thereby serving as a potential link between the IL-4R and other pathways implicated in cell growth, including the phosphatidylinositol-3-kinase (PI-3-kinase) and mitogen-activated protein kinase (MAPK) pathways [46, 49]. It has recently been demonstrated that IL-4 treatment of human B cells results in the dephosphorylation of tyrosine residues in the p85 component of PI-3-kinase, likely by the SH2-containing tyrosine phosphatase-1 (SHP-1), which co-immunoprecipitates with IL-4R and PI-3-kinase following IL-4 stim-

ulation [71]. The importance of SHP-1 in IL-4R signaling is demonstrated in a recent study which identified a gain-of-function mutation in the intracellular domain of IL-4R α which resulted in increased signaling via IL-4R, as measured by CD23 expression, along with a decrease in SHP-1 binding to IL-4R α [72]. Therefore, in addition to tyrosine phosphorylation, tyrosine phosphatase activity may play a role in the signaling pathways following IL-4R activation.

Activation of C ϵ gene transcription requires DNA-binding transcription factors other than STAT6. The promoter region upstream of the I ϵ exon also contains binding sites for NF- κ B/p50 and for one or more members of the C/EBP family of transcription factors [73]. Mice deficient for NF- κ /p50 are severely deficient in switching to IgE, supporting a role for NF- κ B in isotype switching [74]. Additionally, B cells from mice genetically deficient for the C-terminal transactivation domain of the NF- κ B family member c-Rel express normal levels of ϵ GLT but fail to switch to IgE in response to lipopolysaccharide (LPS) plus IL-4 [75]. The B-cell-specific activator protein BSAP (Pax 5) also binds to the I ϵ promoter and has been implicated in activating C ϵ germline transcription [76].

It was recently demonstrated that IL-4 induces C ϵ germline transcription and IgE isotype switching in B cells from patients with X-linked severe combined immunodeficiency (X-SCID) due to mutation of the γ c chain [77]. Induction of C ϵ gene transcription by IL-4 in B cells with γ c chain deficiency may involve signaling via the IL-4R α -containing IL-13 receptor. Alternatively, the IL-4R α chain may transduce intracellular signals that lead to C ϵ gene transcription independent of the γ c chain. We recently showed that homodimerization of the intracellular domain of the human IL-4R α chain induces C ϵ GLTs [78]. This was accomplished by expressing chimeric receptors consisting of the extracellular domain of the erythropoietin receptor and the transmembrane and intracellular domains of the IL-4R α chain in B-cell lines. Homodimerization of the chimeric receptor by erythropoietin resulted in JAK1 activation and induction of nuclear STAT6 DNA-binding activity which was abolished by disruption of the JAK1-binding region of IL-4R α .

Other cytokines, including IL-5, IL-6, IL-12 and IFN- γ , have modulatory effects on IgE synthesis. The T_H2-derived cytokines IL-5 and IL-6 increase IL-4-dependent IgE synthesis [79, 80]. IL-6 appears to play an essential role, since a polyclonal antibody against IL-6 inhibited IL-4-driven IgE production. It has also been demonstrated that IL-12, which favors T_H0 differentiation into T_H1 cells, inhibited IgE synthesis by IL-4-stimulated peripheral blood mononuclear cells but failed to inhibit IgE synthesis by purified B cells stimulated by anti-CD40 mAb plus IL-4. IL-12 treatment

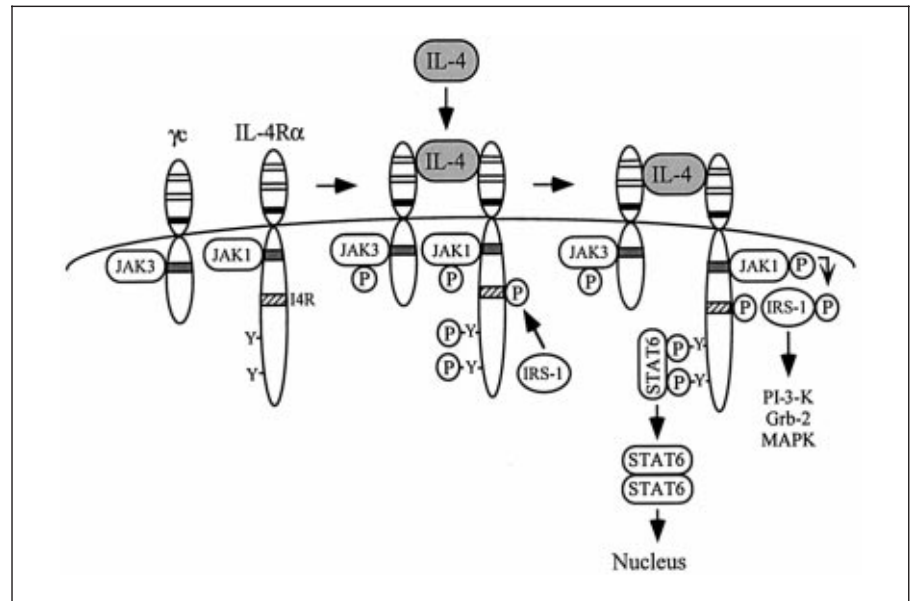


Fig. 5. Signal transduction through the IL-4 and the IL-13 receptors. Heterodimerization of the IL-4R chains follows binding of IL-4 to IL-4R α . IL-4R α and γ c undergo phosphorylation by the JAK kinases JAK1 and JAK3 that are associated with their intracellular domains. IRS-1 is also phosphorylated by Janus family kinases and then binds to the I4R domain of IL-4R α , potentially linking IL-4 stimulation to PI-3-K and MAPK pathways. Phosphotyrosines in the intracellular domain of IL-4R α serve as docking sites for the SH2 domains of STAT6 molecules. The docked STAT6 molecules in turn undergo phosphorylation, likely by the JAK kinases. The SH2 domains of STAT6 have higher affinity to phosphorylated tyrosines on STAT6 than

to phosphorylated tyrosines on IL-4R α . Thus, phosphorylated STAT6 molecules dissociate from IL-4R α and form homodimers. This results in the unmasking of a nuclear localization signal on the STAT6 molecule allowing nuclear translocation. In the nucleus, STAT6 binds to DNA sequences TTCNNN(N)GAA present in the promoter of IL-4-responsive genes, including *C ϵ* and, in concert with other transcription factors, activate transcription. A similar series of events occurs following homodimerization of the IL-13R chains upon binding of IL-13 to the high-affinity IL-13R α chain. Both JAK2 and TYK2 have been found to interact with IL-13R α .

resulted in decreased amount of mature ϵ transcripts but did not affect ϵ germline transcription [81]. Treatment of activated B cells with anti-CD40 mAb plus IL-12 and/or IL-18 leads to IFN- γ production, and this IFN- γ inhibits IL-4-dependent IgG1 and IgE responses in vitro [82].

Signal 2: CD40 Ligation

CD40 is a transmembrane glycoprotein member of the TNF receptor superfamily [83] which includes the two TNF receptors, Fas (CD95), the low-affinity nerve growth factor receptor, CD27, CD30, 4-1BB antigen and OX-40 [reviewed in]. CD40 activation by CD40L results in homotypic B-cell adhesion, increased B7, CD23, ICAM-1, and LT- α expression [84] and rescues germinal center B cells from apoptosis induced by Fas or surface IgM. CD40L is a 39-kDa glycoprotein member of the TNF superfamily which is expressed on activated T cells. The special capacity of CD40 to trigger isotype switching suggests that CD40 ligation activates a unique signaling pathway(s) or a unique combination of known signaling pathways. Four intracellu-

lar proteins which belong to the family of TNF receptor-associated factors (TRAF), TRAF-2, TRAF-3, TRAF-5 and TRAF-6, were found to associate with the intracytoplasmic domain of CD40 [85–89]. TRAF-2, TRAF-3 and TRAF-5 share a binding site which is distinct from the TRAF-6-binding site. TRAF-2, TRAF-5 and TRAF-6 have been shown to mediate activation of NF- κ B [86, 87, 89, 90], whereas the function of TRAF-3 is not known [85]. TRAF-3-deficient mice survive poorly and their T-cell-B-cell collaboration is abnormal resulting in failure of isotype switching [91]. It is, however, unlikely that TRAF proteins are sufficient to mediate isotype switching since all TRAF proteins associated with CD40 are also associated with other receptors which do not induce B-cell isotype switching.

Engagement of CD40 activates protein tyrosine kinases (PTKs) [92, 93] which play an important role in CD40-mediated aggregation, LT- α expression and Ig class switching. Indeed, CD40-mediated isotype switching is inhibited by herbimycin A and by cross-linking CD40 to the transmembrane tyrosine phosphatase CD45 [94, 95]. Previous studies

have shown that CD40 ligation results in the rapid tyrosine phosphorylation and activation of the src family PTKs lyn and syk, the phosphorylation of phospholipase C γ -2 and the activation of PI-3-kinase [92, 96]. However, there has been no demonstration of direct or indirect association of src family kinases with CD40.

Since CD40 lacks a kinase domain, CD40-associated proteins must be critical in CD40 signaling. Cytokines and growth factors which signal via JAK kinases contain, in their intracellular domain, two conserved sequences known as box 1 and box 2 [97]. Box 1 is a proline-rich motif which is critical for stable association of JAK kinases with cytokine receptors, whereas box 2 may play a crucial role in kinase activation. We observed the presence of both box 1 (-PTNKAPHP-aa 202–209) and box 2 (-PVTQETGKESR-aa 230–240) motifs in the membrane proximal region of the cytoplasmic domain of CD40. This suggested that JAK3 could bind to CD40 through the box 1 motif of this receptor. We recently demonstrated that JAK3 is constitutively associated with CD40 and that CD40 engagement induces tyrosine phosphorylation and activation of JAK3 and STAT3 [98]. Signaling through CD40 also results in the activation of the NF- κ B transcription factor system in B cells [99]. The activated NF- κ B resulting from CD40 stimulation may then synergize with IL-4-derived signals, such as STAT6, on the C ϵ promoter [100, 101].

The association of JAK3 with CD40 suggested that JAK3 may play an important role in CD40-mediated isotype switching. However, current studies using peripheral blood lymphocytes from JAK3-deficient patients stimulated with anti-CD40 mAb in the presence of IL-13, the action of which is independent of JAK3, reveal normal IgE isotype switching [H.H. Jabara and R.S. Geha, unpubl. data]. These results suggest that JAK3 is not critical in isotype switching. Thus, the nature of the PTK that is important for CD40-mediated isotype switching remains unknown.

T-Cell-Independent Second Signals

Two additional stimuli have been described which, when present along with IL-4, can induce IgE synthesis in a T-cell independent manner. The Epstein-Barr virus (EBV), in the presence of IL-4, is capable of inducing IgE synthesis by human B cells in vitro [102, 103]. This may be due to the EBV latent infection membrane protein-1 (LMP-1), a constitutively active TNFR superfamily member that is similar to CD40 in structure and activation patterns. Studies have demonstrated that TRAF-1, TRAF-2, and TRAF-3 associate with LMP-1 and activate NF- κ B [88, 104, 105]. The gluco-

corticoid hydrocortisone, in the presence of IL-4, is also capable of upregulating IgE synthesis by human peripheral blood mononuclear cells as well as inducing IgE synthesis by sIgE- B cells [106], although the mechanism of this effect is unclear.

Pharmacologic Modulation of IgE Synthesis

Based on our present understanding of IgE regulation, several modalities aimed at modulating the synthesis and function of IgE may be available in the future. These include:

(1) *Inhibition of IgE switching by interfering with the interaction between IL-4 and the IL-4 receptor:* A mutant form of human IL-4, IL-4.Y124D, which results from substitution of the tyrosine residue at position 124 by aspartic acid, binds to the IL-4R with high affinity but lacks detectable proliferative activity for T cells and inhibits IL-4-dependent T-cell proliferation [107]. This mutant form also inhibits IL-13 action on IL-4- and IL-13-responsive TF-1 cells [108] and inhibits both IL-4- and IL-13-induced IgE synthesis in vitro [109]. When administered to *scid* mice reconstituted with human B and T lymphocytes, IL-4.Y124D significantly reduced or eliminated in vivo IgE production without affecting IgG production [110]. There is also a naturally occurring isoform of IL-4, IL-4 δ , which arises from an alternatively spliced IL-4 RNA by omission of the second exon and inhibits IL-4-induced T-cell proliferation [111]. These functional antagonists of IL-4 and IL-13 may prove to be effective as therapeutic agents. A soluble form of the IL-4R (s-IL-4R) occurs naturally in mice and results from alternative splicing of the IL-4R RNA [52]. Administration of s-IL-4R to mice along with anti-IgD antibodies resulted in significantly decreased IgE production [112]. Recent studies have identified a protein present in normal mouse serum and urine that binds to IL-13 (IL-13-binding protein, IL-13BP) with greater affinity than does the IL-13R α chain [113]. Since s-IL-4R and IL-13BP exist in vivo and mice with nonfunctional IL-4 genes are essentially healthy [114], this form of therapy holds good promise.

Therapy with IL-4 antagonists may, however, need to be accompanied with therapy with IL-13 antagonists. By virtue of the fact that both the IL-4R and IL-13R use the IL-4R α chain for signal transduction, compounds that inhibit IL-4R α signaling would be extremely useful. This therapeutic approach is complicated by the finding that B cells that have already switched to IgE production are no longer dependent on IL-4 for IgE synthesis (see below). Thus, therapy directed towards IL-4 and IL-13 may need to be coupled with therapy directed towards eliminating IgE-secreting B cells.

(2) *Modulation by cytokines*: IFN- γ inhibits the induction of IgE synthesis by IL-4 [38]. IFN- γ has already undergone clinical trials in diseases associated with elevated serum IgE levels, such as atopic dermatitis and the hyper-IgE syndrome [115–117]. These studies demonstrated that, while recombinant IFN- γ therapy resulted in inhibition of spontaneous IgE production in vitro, it had minimal effect on serum IgE levels. This is perhaps explained by the observation that B cells that have already switched to IgE synthesis (IgE⁺ B cells) are no longer subject to inhibition by IFN- γ [118].

(3) *Neutralization of existing IgE and inhibition of IgE synthesis by IgE⁺ B cells*: Since IgE⁺ B cells are no longer dependent on IL-4 [119], the above modalities of therapy will not eliminate existing IgE, although they will prevent the further differentiation of B cells along the IgE pathway. Therefore, these therapies may need to be combined with therapies aimed towards the elimination of already existing IgE⁺ B cells and/or the neutralization of IgE antibody. Local administration of a recombinant fragment of the ϵ chain (ϵ 2–4) was capable of inhibiting the Prausnitz-Kustner reaction to ragweed antigen [120], likely by competing with IgE for binding to its receptors. Intravenous injection of a fusion protein consisting of the extracellular domain of the human high-affinity IgE receptor (Fc ϵ RI) α chain and a truncated human IgG heavy chain constant region was able to block passive cutaneous anaphylaxis reaction in mice [121]. Recently, high-affinity oligonucleotides have been described which competitively inhibit the association between IgE and the Fc ϵ RI in vitro [122]. These approaches, using peptides or oligonucleotides to interfere with IgE binding to its receptors, may be useful in eliminating the effects of existing IgE, but are unlikely to affect further IgE production.

Another approach in abrogating IgE-mediated disease would be the development of antibodies directed against IgE. These anti-IgE antibodies must be specific for IgE and not bind IgM, IgG or IgA. In addition, they must block binding of IgE to Fc ϵ RI yet avoid binding to, and subsequent triggering of, mast cell-bound IgE. These mAbs are typically of murine origin and thus may serve as immunogens when administered to humans. Humanization of mAbs with these properties has been achieved [123]. Administration of such a humanized anti-IgE antibody to atopic patients resulted in decreased serum IgE levels, downregulation of basophil Fc ϵ RI expression and decreased histamine release to *Dermatophagoides farinae* [124]. A phase I trial of CGP 51902, a humanized anti-IgE antibody that binds to free IgE and IgE on the surface of IgE⁺ B cells but not to IgE bound to Fc ϵ RI on mast cells or basophils nor to IgE bound to the

low-affinity IgE receptor (CD23), was conducted in a group of pollen-sensitive patients with elevated IgE levels [125]. This therapy was well tolerated with a significant dose-dependent decrease in free serum IgE. Only one patient developed low levels of anti-CGP 51902 antibodies. A mAb that recognizes an amino acid sequence present in IgE expressed on the surface of human B cells, but not present in secreted IgE, has also been described [126]. Properly conjugated, this type of antibody may be used to kill IgE⁺ B cells.

(4) *Inhibition of CD40-mediated isotype switching by pharmacologic agents*: We have found that the popular anti-asthma drugs disodium cromoglycate and nedocromil sodium inhibit, in a dose-dependent fashion, IgE production induced by anti-CD40 and IL-4 by peripheral blood mononuclear cells as well as by highly purified B cells [127, 128]. The inhibition is at the level of the B cell. Neither of these compounds had an effect on C ϵ germline transcription induced by IL-4. However, both drugs did inhibit switch recombination at the molecular levels as demonstrated by a PCR assay that amplifies S μ /S ϵ switch junctions [7].

Conclusions

Our understanding of the mechanisms of IgE synthesis continues to expand. However, several issues remain to be investigated. Foremost is the exact role of C ϵ germline transcription in rendering the S ϵ region accessible to switch recombination and the nature of the switch recombinase involved in isotype switching. A thorough understanding of the role and mechanisms of action of IL-13 in human IgE antibody responses is required because strategies that target solely IL-4 may be insufficient to inhibit these responses. The pace at which our understanding of the cytokines involved in IgE synthesis, their receptors, the signal transduction pathways triggered via these receptors, and the ensuing molecular mechanisms that result in IgE isotype switching, promises that successful strategies that selectively inhibit IgE synthesis are not too far in the future.

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