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Review Article

Regulatory mechanisms of human IgE synthesis

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Abstract

The induction of allergen-specific IgE synthesis requires the cognate interactions between B and T helper (Th) 2 cells. The B cell-activating signal for IgE synthesis is delivered through interleukin (IL)-4 or IL-13 and CD40 ligand, which are provided by activated Th2 cells. Signaling through the IL-4 receptor α chain (IL-4Rα) triggers IL-4- or IL-13-dependent germline Cε transcription by activating signal transducer and activator of transcription (STAT)-6 through members of the Janus kinase (JAK) family. In addition to the known JAK–STAT pathway, two adaptor molecules associated with the IL-4Rα, which include Src homologous and collagen (Shc) and a product of the *fes* proto-oncogene family, are involved in the induction of germline Cε transcription. These adaptor molecules transmit the downstream signaling, leading to activation of PU.1, a product of the *ets* proto-oncogene family, which cooperates functionally with STAT-6 for germline Cε transcription. Ligation of CD40 in the presence of IL-4 or IL-13 leads to expression of activation-induced cytidine deaminase (AID). This novel RNA-editing enzyme plays a role upstream of the putative switch recombinase, activation of which results in IgE isotype switching, mature Cε transcription and IgE synthesis. Although CD40 signaling activates multiple pathways that are critical for the activation of the switch recombination machinery, none of the known second messengers and transcription factors generated by CD40 ligation is involved in AID expression and isotype switching. Elucidation of the merging point of IL-4Rα and CD40 signaling pathways required for IgE switching will provide potential new strategies for the isotype-specific regulation of IgE synthesis.

Key words: activation-induced cytidine deaminase, adaptor molecule, germline Cε transcription, IgE isotype switching, switch recombination machinery.

Introduction

The human immunoglobulin family consists of nine isotypes, each of which is involved in humoral immunity. Of these isotypes, IgE plays a key role in the pathogenesis of allergic disease. The production of IgE by B cells requires interactions with T cells and is induced through cytokines and cell surface molecules provided by activated T cells. Increasing understanding of the cellular and molecular events underlying IgE synthesis has allowed the identification of new targets for IgE regulation. The present article provides an up-to-date overview of the regulatory mechanisms of human IgE synthesis.

Immunoglobulin expression during B cell differentiation

The primary function of B cells is to produce antibodies, including the IgE isotype, against a vast array of environmental antigens. The production of such a large spectrum of antibodies is due to the generation of a large repertoire of B cells, each of which expresses antibodies of a different specificity.

Antibody molecules are composed of paired heavy and light chains and their variable regions have a unique antigen-binding specificity. Of clonally diverse B cells, a cell bearing a particular Ig receptor responds to the complementary antigen with proliferation and differentiation into antibody secreting plasma cells.

The B-lineage cells, which derive from hemopoietic stem cells that give rise to cells of other blood lineages, can be divided into five general stages of differentiation represented by pro-B cells, pre-B cells, immature B cells,

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mature B cells and plasma cells (Fig. 1). The generation of B cells and their maturation in the bone marrow are antigen-independent processes, whereas the terminal differentiation of mature B cells into plasma cells in lymphoid organs is antigen dependent.

The production of B cells by the bone marrow is a multifocal process that involves the generation of an extensive repertoire of Ig specificities. Such a repertoire is acquired by a series of gene rearrangements of the variable regions of the Ig heavy and light chains.[1,2] Although pro-B cells start to synthesize cytoplasmic μ heavy chains, but not the conventional light chains of the κ or λ type necessary for the formation of a complete IgM molecule, the cells constitutively express recombination-activating gene-1 and -2, the products of which (RAG-1 and RAG-2, respectively) initiate V (variable)–D (diversity)–J (joining) recombination at the μ chain locus. Subsequently, these two gene products expressed in pre-B cells, which express both μ chains and surrogate light chains, initiate V-J recombination at the light chain loci, thereby leading to the formation of completed IgM molecules and pre-B cell transition into immature B cells bearing surface IgM. These cells further differentiate into mature B cells that coexpress IgD, having identical binding specificity.

Mature B cells respond to antigens by undergoing plasma cell differentiation. This terminal differentiation process involves isotype switching, which allows expression of IgG, IgA or IgE.[3] The generation of switched B cells and their differentiation are dependent on T cell help, which is mediated through cytokines and cell surface molecules provided by activated T cells. Re-expression of RAG-1 and RAG-2 is also inducible in mature B cells under suitable conditions before isotype switching, which is predicted to allow secondary rearrangements leading to Ig receptor revision.[4] Isotype switching results from Ig gene rearrangement that involves the deletion of constant region genes upstream of the one to be expressed. Thus, individual plasma cells produce antibodies of a single isotype.

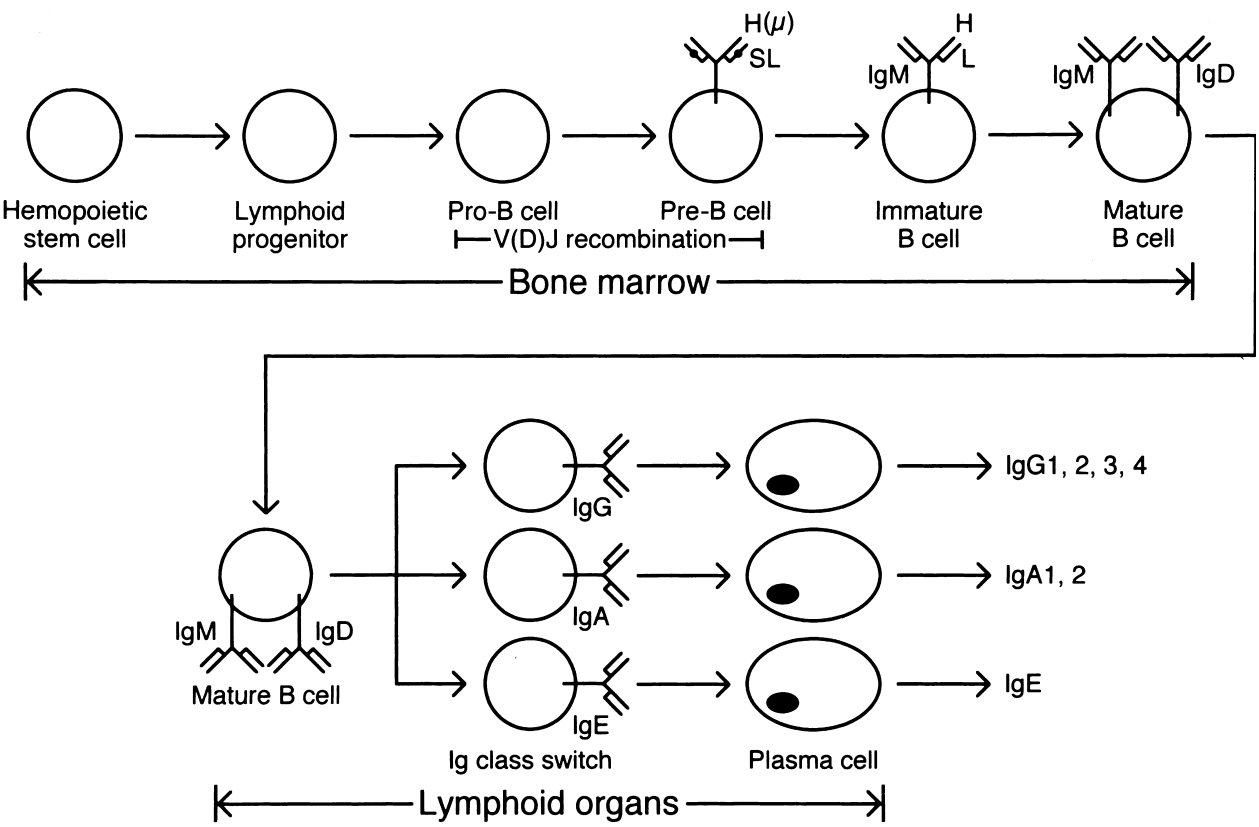

Fig. 1 B cell differentiation pathway and immunoglobulin gene rearrangements. Cell surface molecules indicate immunoglobulins expressed during B cell differentiation. H, heavy chain; L, light chain; SL, surrogate light chain; V(D)J, variable (diversity) joining.

Cellular events in the induction of IgE synthesis

Cognate interactions between B and T cells are required to induce allergen-specific IgE synthesis (Fig. 2). When a mature B cell recognizes a particular allergen via B cell receptor, the cell processes the allergen and presents its T cell epitope, together with major histocompatibility complex (MHC) class II molecules, to an allergen-specific $CD4^+$ T cell with a Th2 phenotype. Subsequently, the T cell is activated through engagement of the $\alpha\beta$ T cell receptor–CD3 complex and is induced to produce Th2-type cytokines, such as interleukin (IL)-4 and IL-13, and to express CD40 ligand (CD40L, CD154), which belongs to the tumor necrosis factor superfamily. In the presence of T cell-derived IL-4 or IL-13, the B cell undergoes germline Cε transcription, which is a critical initiating step for switching from IgM to IgE. This cytokine-dependent transcription directs switching to the corresponding isotype.[5–7]

Signaling through the IL-4 receptor α chain (IL-4Rα, CD124), which is a component of both the heterodimeric IL-4R consisting of the IL-4Rα and the common γ chain (γc, CD132) and the heterodimeric IL-13R consisting of the IL-4Rα and IL-13Rα1 (CD213a1), plays a key role in the induction of germline Cε transcription.[8–10] In support of this is the finding that B cells of X-linked severe combined immunodeficiency patients with mutations in the γc gene can express germline Cε transcripts in response to IL-4 or IL-13.[11,12] CD40L, a non-covalent trimer expressed on the activated T cell, in conjunction with IL-4 or IL-13, not only enhances cytokine-dependent germline Cε transcription, but also induces IgE isotype switching through cross-linking of CD40 constitutively expressed on the B cell. Engagement of CD40 by CD40L also mediates rescue from apoptosis, proliferation and terminal differentiation into IgE antibody secreting plasma cells.[13,14] Thus, IL-4Rα and CD40 signaling pathways are integrated to induce IgE isotype switching and IgE synthesis.

The central role of the interaction of CD40 with CD40L in switching to T cell-dependent isotypes has been shown in patients with X-linked hyper-IgM syndrome who have mutations in the CD40L gene that result in defective isotype switching.[15] Although CD40 on B cells prompts endocytosis of surface CD40L expressed on activated T cells, engagement of CD40L by CD40 is able to increase IL-4 production by T cells, thereby leading to enhanced germline Cε transcription.[16,17] This may

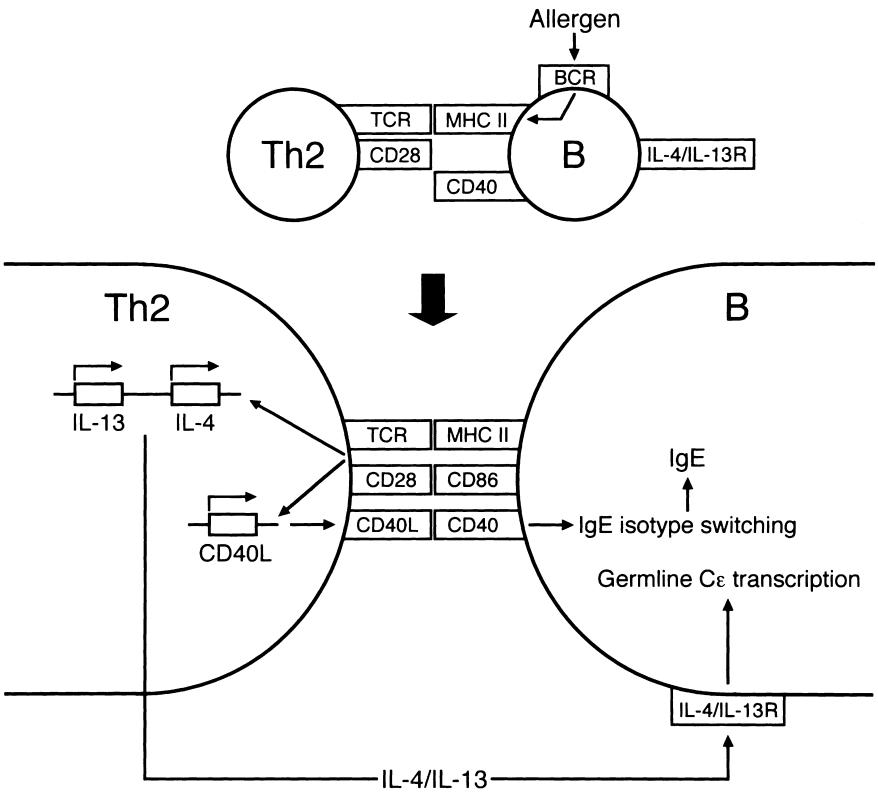

Fig. 2 Induction of allergen-specific IgE synthesis by cognate interactions between B and T cells. BCR, B cell receptor; CD40L, CD40 ligand; IL, interleukin; IL-4/IL-13R, receptor for IL-4 or IL-13; MHC II, major hisotocompatibility complex class II molecules; TCR, α/β T cell receptor.

contribute to upregulation of IgE isotype switching and IgE synthesis.

In addition to $CD4^+$ T cells with a Th2 phenotype, $CD8^+$ T cells with a Tc2 phenotype, $CD3^+$ T cells bearing the γδ T cell receptor, mast cells, basophils and eosinophils produce IL-4 and/or IL-13 and express CD40L after immunologic or non-immunologic stimulation.[18–22] Such cellular responses allow adjacent B cells to induce IgE isotype switching and differentiation into IgE-secreting plasma cells.

More recently, glucocorticoids have been shown to upregulate CD40L expression both in T and B cells.[23] Thus, several cell types are involved in the induction of IgE synthesis, although the production of specific IgE antibody by a given B cell clone is critically dependent on the interaction with an allergen-specific $CD4^+$ T cell. In contrast, other lineage cells may participate mainly in polyclonal IgE production by different B cells.

Molecular mechanisms of IgE isotype switching

A mature B cell can switch the Ig class while retaining the same antigen specificity. This event results from isotype switching that occurs by a DNA rearrangement in the CH (constant region of the heavy chain) gene locus.[3,24] The human *CH* gene family consists of nine functional genes and two pseudogenes. The organization of the CH locus located at the 3′ side of a given VH segment, a D segment and a JH segment that complete a VH region sequence is as follows: 5′-JH-Cμ-Cδ-Cγ3-Cγ1-Cψε-Cα1-Cψγ-Cγ2-Cγ4-Cε-Cα2-3′ (Fig. 3a). A DNA recombination involved in isotype switching takes place between two switch (S) regions located at the 5′ side of each *CH* gene, except Cδ and Cψγ.[25] The S regions include Sμ, Sγ, Sα and Sε, each of which is composed of tandem repeats of short unit sequences. Although the Sε region is also present before the *Cψε* gene, this region is not involved in recombination because of the defect in a part of the exon. Furthermore, the germline IH exons (Iμ, Iγ, Iα and Iε) are located 5′ to each functional S region. With the exception of constitutive activation of the Iμ promoter, the other IH promoter is activated in response to appropriate cytokines, resulting in transcription of the IH exon, the S region and the CH exons. Because transcripts of the S region are spliced out by splicing factors, the resultant IH and CH transcripts are germline CH transcripts, expression of which directs

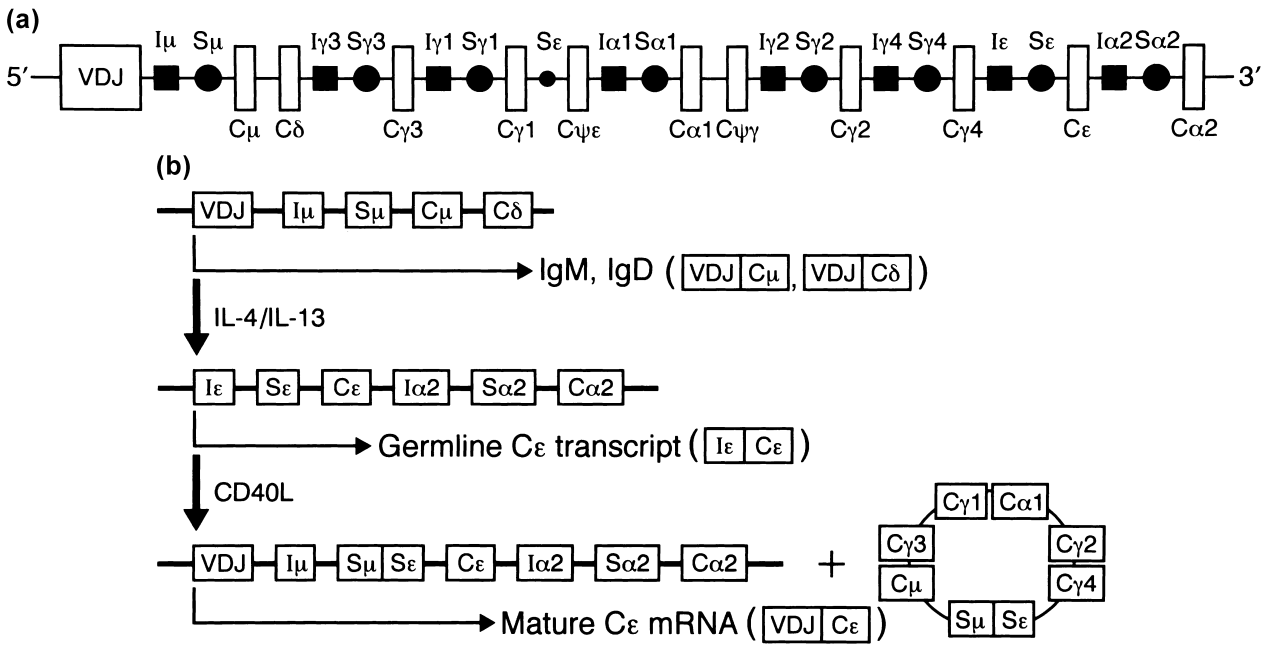

Fig. 3 Molecular events involved in IgE isotype switching. (a) Organization of nine functional genes and two pseudogenes of CH isotypes. The functional *Cε* gene is located between the *Cγ4* gene and the *Cα2* gene. (b) Interleukin (IL)-4- or IL-13-dependent germline Cε transcription and CD40 ligand (CD40L)-dependent switching from IgM to IgE. See text for details.

isotype switching by regulating the accessibility of a particular S region to a putative common recombinase system.[5,26] This type of recombination involves the Sμ region as one of the pair and the Sγ, Sα or Sε region is involved as the other partner, resulting in the activation of switch recombination responsible for the induction of switching from Cμ to Cγ, Cα or Cε.

Engagement of CD40 by CD40L in the presence of a particular cytokine plays a crucial role in a given switch recombination.[3,14,27] During recombination, the DNA segment between the expressed *VH* and *CH* genes is looped out as a circle and deleted from the chromosome. Although the switch recombinase has not as yet been identified, activation-induced cytidine deaminase (AID), a B cell-specific RNA-editing enzyme, has recently been reported to be expressed after cytokine and CD40L stimulation and to be involved in regulation or catalysis of the DNA modification step of isotype switching.[28–30] Actually, AID deficiency causes the autosomal recessive form of the hyper-IgM syndrome[30] characterized by defective DNA switch recombination.

During an IgE response, IL-4- or IL-13-dependent induction of germline Cε transcription precedes CD40-mediated Sμ–Sε recombination (Fig. 3b). Activation of the Iε promoter, which contains an IL-4 or IL-13 response element, initiates transcription of the Iε exon, the Sε region and the Cε1–4 exons. Subsequently, splicing cuts the transcript of the Sε region, thereby allowing expression of germline Cε transcripts. Several studies have shown that spliced switch transcripts bind the DNA of the corresponding S region and induce stable RNA/DNA hybrids that are a target for both a ribonuclease and a switch recombinase.[5,6,26] Thus, processing of germline Cε transcripts may be of importance in directing Sμ–Sε recombination.

CD40 ligation not only upregulates IL-4- or IL-13-driven germline Cε transcription due to full activation of the Iε promoter, but also induces expression of AID, which plays a role downstream of the germline Cε transcription. Activation-induced cytidine deaminase expression is followed by activation of the recombination machinery that allows the deletion of the intervening DNA between the Sμ region and the targeted Sε region. This deletional recombination results in the juxtaposition of the *Cε* gene to the expressed gene of the variable region and in the subsequent induction of mature Cε transcription and IgE synthesis. In addition, alternative splicing is involved in the transition from the membrane to the secreted form of IgE.

Signal transduction of IL-4 and IL-13

The pleiotropic activities of IL-4 and IL-13 in B cells are ascribed to the ability of these cytokines to mediate a diverse array of functions, including induction of germline Cε transcription and enhanced expression of CD23, CD86 and MHC class II molecules.[9–11] Such overlapping activities arise from using the IL-4Rα that forms a heterodimeric complex with either the γc or the IL-13Rα1. Although these three receptor chains lack the intrinsic tyrosine kinase domain, IL-4 and IL-13 induce tyrosine phosphorylation of several cellular proteins. Many cytokines activate members of the Janus kinase (JAK) family, resulting in activation of members of the signal transducer and activator of transcription (STAT) family.[31] The IL-4Rα, γc and IL-13Rα1 associate with JAK1, JAK3 and TYK2, respectively. Ligand binding activates these JAK, which, in turn, induces phosphorylation of STAT-6 recruited to the IL-4Rα. Furthermore, the phosphorylated STAT-6 forms a homodimer via its Src homology (SH) 2 domain, translocates to the nucleus and binds to the consensus sequence present in the promoter regions of the IL-4- or IL-13-responsive genes. These regions include the Iε promoter, activation of which leads to induction of germline Cε transcription. Extensive studies have shown that the Iε promoter contains binding elements for STAT-6, CCAAT/enhancer-binding protein (C/EBP), nuclear factor (NF)-κB and B cell-specific activator protein (BSAP, Pax-5).[32–36] In addition, the upstream NF-κB site overlaps with a binding element for PU.1, a product of the *ets* proto-oncogene family.[36] The essential role of STAT-6 in germline Cε transcription and IgE isotype switching is well established. Interestingly, a genetic variant of IL-4Rα, namely Ile50Val, has been identified in relation to atopic asthma, associates with IL-4 or IL-13 activity and upregulates STAT-6 activation.[37,38]

In addition to the JAK–STAT pathway, other pathways are involved in the activation of the Iε promoter. The IL-4Rα associates with adaptor molecules, such as insulin recepor substrate (IRS)-1, IRS-2, Src homologous and collagen (Shc) and IL-4 receptor-interacting protein (FRIP) and the products of the *fes* proto-oncogene family (FES and FER),[39–42] and these molecules transmit the downstream signaling (Fig. 4). Although FRIP is not expressed in B cells, other molecules are constitutively expressed in many cell types, including B and T cells. Both IRS-1/2 and Shc bind to the insulin/IL-4R (I4R) region of the IL-4Rα. This region contains an Asn-Pro-X-Tyr (NPXY)

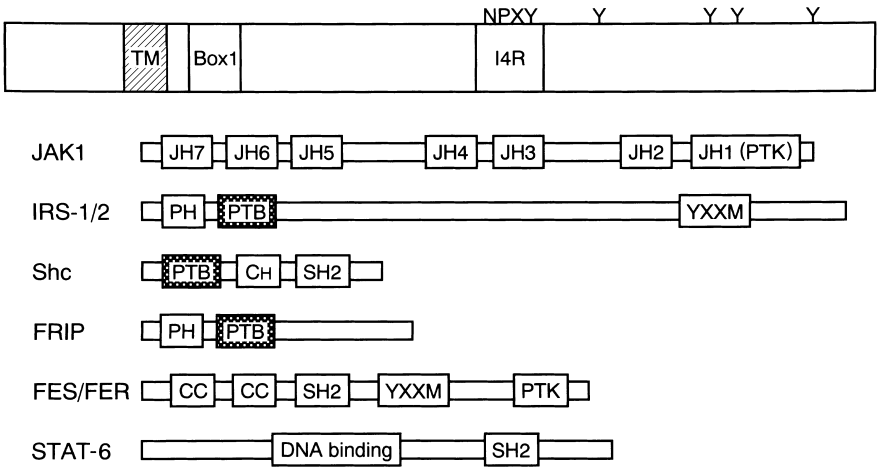

Fig. 4 Association of several molecules with the intracellular domain of the interleukin (IL)-4 receptor α chain. Of these molecules, insulin receptor substrate (IRS), Src homologous and collagen (Shc), IL-4 receptor interaction protein (FRIP) and the products of the *fes* proto-oncogene family (FES/FER) are adaptor molecules containing the phosphotyrosine-binding (PTB) domain that binds to an Asn-Pro-X-Tyr (NPXY) motif present in the insulin/IL-4 receptor (I4R) region. Only FRIP is not expressed in B cells. Both IRS-1/2 and FES/FER contain the conserved Tyr-X-X-M (YXXM) motif to which the Src-homology (SH) 2 domain of phosphatidylinositol 3-kinase binds. The Box1 region is critical for the constitutive association with Janus kinase (JAK) 1 containing the JAK homology (JH) domains and a protein tyrosine kinase (PTK) sequence. CC, coiled-coil region; CH, collagen homology domain; TM, transmembrane region; PH, pleckstrin homology (PH) domain.

motif that specifically interacts with the phosphotyrosine-binding (PTB) domain of IRS-1/2 or Shc.[39,44] In contrast, FES/FER contains the coiled-coil regions that are able to interact with the region located between the Box1 and the I4R.[41] Among these adaptor molecules, IRS-1/2 and FES/FER contain the conserved Tyr-X-X-Met (YXXM) motif to which the SH2 domain of phosphatidylinositol 3-kinase (PI3K) binds. Furthermore, FER, but not FES, is selectively expressed in mature B cells.[41] Although IRS-1/2-dependent activation of PI3K has been most extensively studied in many cell types, FER can mediate PI3K activation in mature B cells, independently of IRS-1/2.[43] Therefore, it is possible that, in B cells, Shc rather than IRS-1/2 preferentially binds to the NPXY motif of the I4R region. This possibility is supported by the finding that recombinant Shc can bind to a phosphopeptide identical to the I4R region, despite the negligible sequence homology between the PTB domain of IRS-1/2 and that of Shc.[44,45] Although Shc mediates activation of phospholipase Cγ1 (PLCγ1) through direct association, the initial production of inositol 1,4,5-trisphosphate is marginal, thus resulting in no significant change in intracellular Ca^{2+} levels.[43] This contrasts with the high and prolonged production of 1,2-diacylglycerol (DAG). Both the lipid product of PI3K activated through FER and DAG generated through Shc-dependent PLCγ1 activate isozymes of protein kinase C (PKC), the substrates of which include transcription factors that cooperate functionally with STAT-6 (Fig. 5).

Isozymes of PKC can be classified into four groups according to endogeneous and exogeneous activators: (i) conventional PKC (α, β1, β2 and γ), which depend on both Ca^{2+} and DAG; (ii) novel PKC (δ, ε, θ and η), which are Ca^{2+} independent and regulated by DAG; (iii) atypical PKC (ζ and ι/λ), which require neither Ca^{2+} nor DAG; and (iv) PKCμ that has a putative transmembrane domain.[46] Moreover, both novel and atypical PKC isozymes are activated by the lipid products of PI3K. Among these, the Ca^{2+}-independent isozymes PKCδ and PKCζ are specifically activated in response to IL-4 or IL-13 and can mediate threonine phosphorylation of PU.1.[24,47] Although binding elements for C/EBP, NF-κB and BSAP are also present in the Iε promotor, none of these transcription factors is susceptible to PKCδ and PKCζ. Thus, two such PKC isozymes appear to regulate transactivation by PU.1. Indeed, activation of the Iε promotor by IL-4 and IL-13 can be blocked not only by dominant negative mutants of PKCδ and PKCζ, but also by isozyme-specific inhibitors rottlerin and PKCζ pseudosubstrate peptide.[47] However, these mutants and inhibitors do not affect tyrosine phosphorylation and DNA

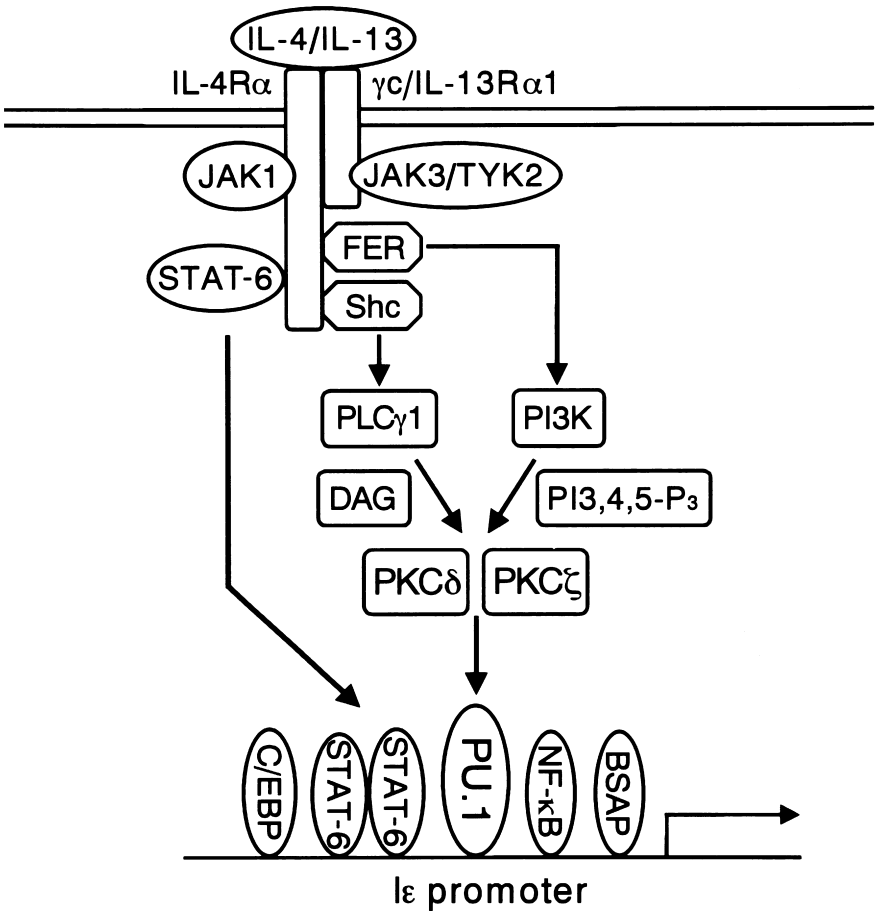

Fig. 5 Signal transduction pathways of interleukin (IL)-4 and IL-13 for the activation of the Iε promoter that results in germline Cε transcription. Ligand binding activates not only the Janus kinase (JAK)-dependent signal transducer and activator of transcription (STAT) pathway, but also the adaptor molecule-dependent pathway. BSAP, B cell-specific activator protein; C/EBP, CCAAT/enhancer-binding protein; DAG, 1,2-diacylglycerol; FER, a product of the *fes* proto-oncogene family; IL-4Rα, IL-4 receptor α chain; IL-13Rα1, IL-13 receptor α1 chain; NF-κB, nuclear factor-κB; PI3,4,5-P_3, phosphatidylinositol 3,4,5-triphosphate; PI3K, phosphatidylinositol 3-kinase; PLCγ1, phospholipase Cγ1; PKC, protein kinase C; PU.1, a product of the *ets* proto-oncogene family; γc, common γ chain; Shc, Src homologous and collagen; TYK2, tyrosine kinase 2.

binding activity of STAT-6. Several lines of evidence support the notion that PU.1, as well as NF-κB, cooparates with STAT-6 for the synergistic activation of the Iε promotor.[34,36,48,49]

Signal transduction through CD40

Ligation of CD40 on B cells upregulates IL-4- or IL-13-driven germline Cε transcription and activates DNA switch recombination that leads to IgE isotype switching, mature Cε transcription and IgE synthesis. The cytoplasmic domain of CD40, which lacks any motifs for transducing signals into B cells, associates not only with two tyrosine kinases (Lyn and JAK3), but also with four members of the six known tumor necrosis factor receptor-associated factor (TRAF) family proteins, namely TRAF2, TRAF3, TRAF5 and TRAF6.[50–53] These molecules can mediate activation of transcription factors, such as STAT-3 and NF-κB. In particular, NF-κB cooperates with STAT-6 and thereby contributes to the increased activity of the Iε promotor. However, none of the known transcription factors activated through CD40 ligation is critical for isotype switching that results from loop-out and deletional recombination. Isotype switching requires AID, expression of which is dependent on a combination of cytokine stimulation and CD40 ligation.[29,30] This novel enzyme appears to play a role upstream of the putative switch recombinase (Fig. 6). Furthermore, Ku70 and Ku80, which form a heterodimer and are associated with CD40, are required to perform switch recombination.[54–56] This heterodimer is dissociated from the CD40 following cytokine stimulation and CD40 ligation, translocates into the nucleus and binds to the DNA-dependent protein kinase. Such a heterotrimeric complex, as well as DNA ligase IV, is involved in the repair of double-strand breaks. Thus, CD40 signaling activates multiple pathways that are important for both the enhancement of germline Cε transcription and the induction of IgE switching.

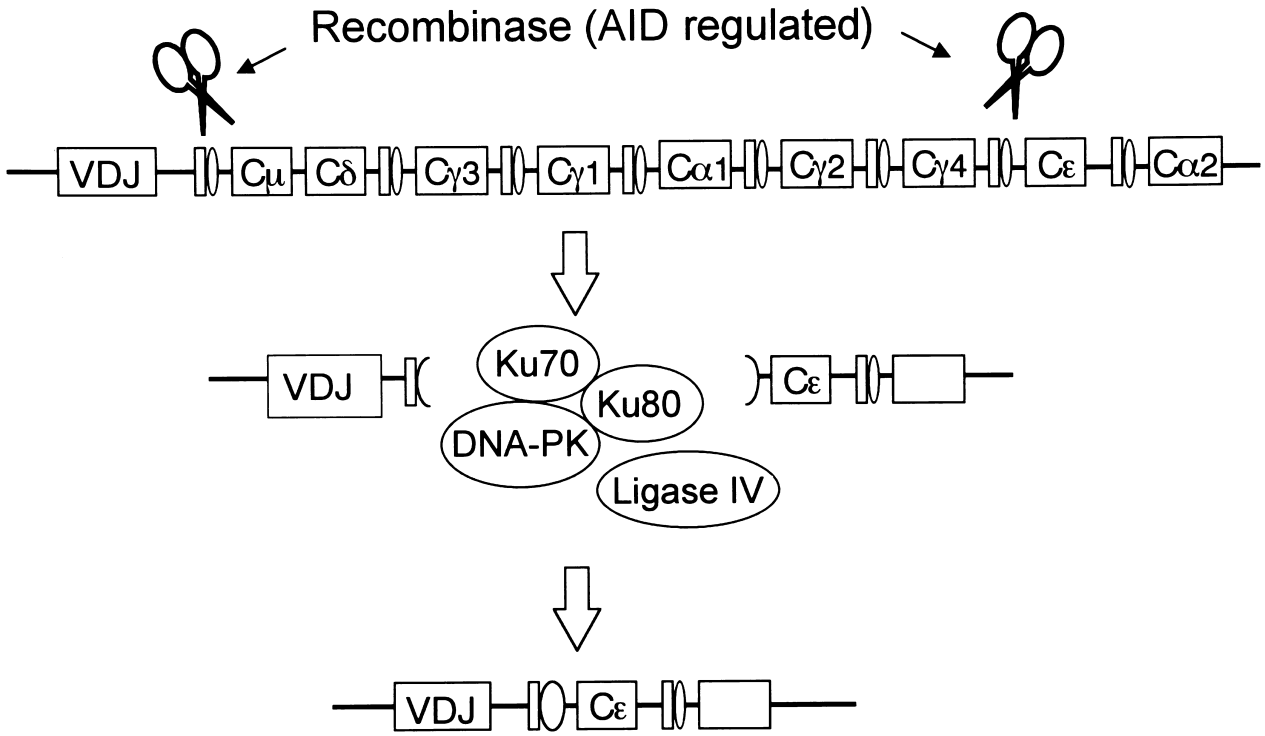

Fig. 6 Involvement of activation-induced cytidine deaminase (AID) in IgE isotype switching. Expression of AID induced by stimulation with interleukin (IL)-4/IL-13 and CD40 ligand is a critical step for the activation of the putative switch recombinase. The same stimulation also induces formation of a heterotrimeric complex composed of Ku70, Ku80 and DNA-dependent protein kinase (PK). This heterotrimer, as well as DNA ligase IV, is involved in the repair of double strand breaks, which is required to perform switch recombination. VDJ, variable–diversity–joining segment.

CD40 ligation-derived signals enhance IL-4- or IL-13-driven germline Cε transcription. One such signal is NF-κB, which synergizes with STAT-6 on the Iε promoter for enhanced DNA-binding affinity.[34,36,48] Among the TRAF proteins associated with CD40, TRAF2, TRAF5 and TRAF6 mediate activation of NF-κB through their ability to bind activators of the IκB kinase complex.[53,57] However, TRAF-dependent NF-κB activation appears to be cell type specific. In B cells, TRAF6 is of importance in activating NF-κB and exerts an enhancing effect on germline Cε transcription.[53] Furthermore, TRAF3 is involved in upregulating germline Cε transcription in a manner that is independent of NF-κB activation. This may be mediated through TRAF3-dependent activation of mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase 1 (MEK1).[58] Actually, like PD98059, a specific inhibitor of MEK1, selective abrogation of constitutive expression of TRAF3 protein by antisense oligodeoxynucleotide for TRAF3 inhibits CD40-mediated ERK activation, resulting in decreased germline Cε transcription.[58] However, the downstream events that arise from TRAF3-dependent ERK activation remain unclear.

Although at least TRAF3 and TRAF6 are important for CD40-mediated enhancement of germline Cε transcription, none of the TRAF proteins is involved directly in CD40-mediated IgE switching. CD40 ligation also mediates activation of tyrosine kinases, including Lyn and JAK3. The finding that tyrosine kinase inhibitors, such as genistein and herbimycin A, inhibit CD40-mediated isotype switching suggests that tyrosine kinase activity may contribute to the activation of the switch recombination machinery system.[59] However, neither Lyn nor JAK3 is critical in isotype switching, because B cells of JAK3-deficient patients have the ability to switch to IgE and because Lyn, as well as JAK3, is also associated with other receptors.[59] Despite great efforts, the nature of the tyrosine kinase(s) involved in CD40-mediated switch recombination has not yet been defined. It is also currently unclear whether CD40 signaling pathway for the

upregulation of germline Cε transcription and that for the induction of AID expression and IgE switching are overlapping or totally different.

Pharmacologic regulation of B cell differentiation into IgE-secreting plasma cells

Several attempts have been made to regulate germline Cε transcription and IgE synthesis. Cytokine-dependent induction of germline Cε transcription is inhibited by neutralizing antibodies against IL-4 or IL-13, a soluble form of the IL-4Rα or IL-13Rα1/α2 and a single or double mutant of IL-4.[60,61] In addition to these antibodies and antagonists, agents that prevent IL-4Rα signaling inhibit germline Cε transcription. Such agents include not only interferon (IFN)-γ itself or inducers of IFN-γ production, but also inhibitors of PKCδ and PKCζ that are activated through adaptor molecules associatd with the IL-4Rα.[47,62] Activation of IFN-γR induces expression of a negative regulator of JAK-dependent STAT-6 activation.[62] As for IL-12- or IL-18-dependent IFN-γ production, the predominant expression of a 91 base deletion of the IL-12Rβ2 cDNA or a three base deletion of the IL-18Rα cDNA is associated with reduced IFN-γ production in some allergic patients with high serum IgE levels.[63,64] Thus, therapy with inducers of IFN-γ production will limit their usefulness in allergic patients without such a deletion of the cytokine receptor cDNA. Although abrogation of CD40 signaling can be targeted by inhibiting switch recombination, this abrogation leads to non-specific suppression of isotype switching. Thus, strategies that target the merging point of IL-4Rα and CD40 signaling pathways would be desirable. Because allergic individuals have some B cells that have already switched to IgE *in vivo*, therapy directed towards IgE-expressing B cells also needs to regulate the terminal differentiation into IgE-secreting plasma cells. In this respect, a therapeutic approach using potent IgE-binding agents, such as a soluble form of the high-affinity IgE receptor α subunit (soluble FcεRIα) and anti-IgE antibodies, may be useful in inactivating or eliminating IgE-expressing B cells.

Both soluble FcεRIα and anti-IgE antibodies can selectively modulate IgE synthesis by binding to IgE-expressing B cells.[65,66] Although the membrane-bound form of IgE is a common target for these agents, regulation of IgE synthesis by soluble FcεRIα differs entirely from that by anti-IgE antibodies. For instance, soluble FcεRIα inhibits IgE synthesis via monovalent recognition of membrane IgE, whereas $F(ab')_2$ but not Fab fragments of anti-IgE antibodies have an inhibitory effect.[65,66] The latter finding indicates that inhibition of IgE synthesis by anti-IgE antibodies requires divalent recognition of membrane IgE. Furthermore, there is a marked difference between mechanisms for soluble FcεRIα- or anti-IgE antibody-induced inhibition of IgE synthesis.[67] Binding of soluble FcεRIα to IgE-expressing B cells leads to a decrease in the autocrine production of IL-6, which provides a late amplification signal for IgE synthesis.[67] In contrast, anti-IgE antibodies or their $F(ab')_2$ fragments induce apoptosis in IgE-expressing B cells, although neither their Fab fragments nor soluble FcεRIα have such apoptotic activity. Thus, cross-linking of membrane IgE is able to induce apoptosis, which accords with a report describing that anti-IgE antibodies were effective in downregulating expression of Bcl-2, known to inhibit apoptotic cell death.[68] These data suggest that both soluble FcεRIα and anti-IgE antibodies may be useful in inhibiting the terminal differentiation of IgE-expressing B cells, including memory cells, into IgE-secreting plasma cells. In particular, non-anaphylactogenic humanized or chimeric anti-IgE monoclonal antibodies have been produced that bind to free IgE and membrane IgE but not to IgE bound to the cell surfact FcεRI.[69–72] These properties are similar to those of soluble FcεRIα, which traps IgE via its Cε3 domain responsible for receptor binding.

Conclusions

Allergen-specific IgE synthesis contributes to the induction and maintenance of allergic symptoms. The expotential increase in the understanding of the cellular mechanisms of IgE synthesis has led to the development and clinical trials of agents capable of regulating the differentiation of B cells into IgE-secreting plasma cells. However, the molecular mechanisms involved in IgE isotype switching are incompletely understood. Although AID appears to be an essential part of the switch recombination machinery, the switch recombinase has not, as yet, been identified. Elucidation of the merging point of IL-4Rα and CD40 signaling pathways that are required for AID expression and IgE switching, as well as identification and characterization of the switch recombinase, are the next challenge for future studies and should provide potential new strategies for the isotype-specific regulation of IgE synthesis.

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