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Review Article

Molecular regulation of human IgE synthesis

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Abstract

Human IgE synthesis is largely dependent on the production of interleukin (IL)-4 or IL-13 and the expression of CD40 ligand. Such B cell help is not only provided by $CD4^+$ T cells, but also by $CD8^+$ T cells, γδ T cells, mast cells, basophils and eosinophils. The IL-4 receptor α chain (IL-4Rα) expressed on B cells is shared by the functional IL-4R and IL-13R and is a crucial component required for signal transduction leading to germline Cε transcription, which is a prerequisite for IgE isotype switching. Interleukin-4 activates Janus kinase (JAK)1, JAK3 and phosphatidylinositol 3-kinase (PI3-K) and, subsequently, induces nuclear translocation of signal transducers and activators of transcription (STAT)6 and nuclear factor (NF)-κB, which interact at the level of the Iε promoter. The two variants of the IL-4Rα, which have been identified in association with atopy, are associated with enhanced responsiveness to IL-4. Ligation of CD40 on B cells up-regulates IL-4- or IL-13-driven germline Cε transcription and further induces deletional switch recombination that results in IgE isotype switching, mature Cε transcription and IgE synthesis. Signaling pathways mediated by CD40 include activation of Lyn, PI3-K, JAK3 and members of the mitogen-activated protein kinase subfamily, multimerization of tumor necrosis factor-α receptor-associated factor (TRAF)2, TRAF3, TRAF5 and TRAF6 and translocation of NF-κB and STAT3. In addition, Ku70/86, DNA-dependent protein kinase and rad51/54 may be involved in switch recombination. Taken together, activation of kinases, induction of second messengers, nuclear expression of transcription factors and localization of DNA-binding proteins are integrated to produce the terminal differentiation of a B cell into an IgE-secreting plasma cell. Elucidation of the detailed mechanisms of IgE isotype switching will contribute to the development of potential new therapeutic procedures for the regulation of the IgE response in atopic patients.

Key words: atopy, CD40 ligand, IgE, interleukin-4, switch recombination.

Introduction

Immunoglobulin E plays a central role in the pathogenesis of allergic diseases, including asthma, rhinitis and urticaria. The production of IgE is initiated by the interaction of B cells with T cells and is regulated by soluble and cell surface molecules provided by activated T cells. During the development of the IgE response, B cells can differentiate into IgE-secreting plasma cells. This differentiation process involves isotype switching, which allows expression of IgE. Although a complete set of switch recombination events is still unknown, considerable progress has been made in the past decade in elucidating the class-specific mechanisms of IgE regulation. The aim of the present article is to provide a current overview of the cellular, biochemical and molecular events underlying human IgE synthesis.

Cell types supporting IgE production by B cells

Mature B cells, which express IgM and IgD antigen receptors, respond to protein antigens and T cell help by undergoing proliferation, Ig class switching and plasma cell differentiation. T cell help is mediated through cytokines and cell surface molecules provided by activated $CD4^+$ T cells. Two cytokines, interleukin (IL)-4 and IL-13, are functionally related and direct IgE synthesis in B cells.

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Cell interaction molecules important for the antibody responses include CD40 on B cells and CD40 ligand (CD40L, CD154) expressed on activated $CD4^+$ T cells.[1,2] Both the transmembrane form of tumor necrosis factor (TNF)-α on activated $CD4^+$ T cells and CD2 on T cells are also involved in the productive T–B cell interactions that are required for IgE synthesis.[3,4] Nevertheless, CD40L appears to be most important in providing a costimulatory signal for B cell activation, because T cell-dependent B cell responses, including IgE production, can be blocked by soluble forms of CD40. Actually, engagement of CD40 leads to B cell proliferation and differentiation in the presence of certain cytokines, which supports the notion that the CD40–CD40L interaction is central to the delivery of T cell help to B cells. The essential role of the CD40–CD40L interaction in Ig class switching has been shown in patients with X-linked hyper-IgM syndrome, who have a reduction or loss of isotype switching due to defective CD40L expression.[5] The CD40L is also expressed on $CD8^+$ T cells, γδ T cells, mast cells, basophils and eosinophils upon activation.[6–10] Thus, these cells can replace $CD4^+$ T cells in delivering the contact-mediated signal required for IgE synthesis.

In addition to CD40L, cytokines produced by several cell types, including T cells, play an important regulatory role in the immune response. $CD4^+$ helper T (Th) cells are classified into two distinct cytokine-secreting subsets, Th1 and Th2.[11] T helper 2 cells, the counterparts of Th1 cells (which secrete IL-2, interferon (IFN)-γ and lymphotoxin), produce IL-4, IL-5, IL-6, IL-10 and IL-13 and are associated with antibody and allergic responses. $CD8^+$ cytotoxic T cells are also similarly divided into two subsets, termed Tc1 and Tc2 cells.[12] Cells in the Tc1 subset produce Th1-type cytokines, while Tc2 cells, which have decreased cytolytic activity, secrete Th2-type cytokines. Interestingly, CD40L is preferentially expressed on Tc2 rather than Tc1 cells. Cytotoxic T cell 2-type $CD8^+$ T cells have been described in patients with lepromatous leprosy, acquired immune deficiency syndrome and atopy.[7,13–15] A recent study has shown that $CD8^+$ T cells with a Tc2 phenotype are also detectable in a patient with an adenosine deaminase deficiency who has received autologous T cell-directed gene therapy.[16,17] Interleukin-4 and/or IL-13 are secreted not only by Th2 cells, but also by Th0 cells, Tc2 cells, mast cells, basophils and eosinophils. Naïve $CD4^+$ T cells and Th1 cells, although lacking the ability to produce IL-4, secrete IL-13, thereby contributing to IgE production by B cells.[18] These findings indicate that, like Th2 cells, other cell types also participate in a form of the immunological synapse by producing IL-4 or IL-13 and expressing CD40L. This is supported by the observation that, in severe combined immunodeficiency mice reconstituted with peripheral blood mononuclear cells from atopic patients, IL-4 production and CD40L expression can be detected not only in $CD4^+$ T cells but also in $CD8^+$ T cells, γδ T cells and high-affinity IgE receptor (FcεRI)-bearing cells (probably basophils).[17] By itself, IgE has the capacity to enhance expression of FcεRI on mast cells,[19] to stabilize CD23 on B cells[20] and to facilitate cholinergic neurotransmission in airways.[21] Thus, exposure to monomeric IgE also contributes to the pathogenesis of allergic diseases. In this regard, Tc2 cells, γδ T cells, mast cells, basophils and eosinophils, in addition to allergen-specific $CD4^+$ T cells, may be important targets for achieving the down-regulation of IgE synthesis by B cells (Fig. 1).

Molecular events in IgE isotype switching

In antibody responses, IgM^+ IgD^+ B cells undergo isotype switching that results in the production of other Ig classes (IgG, IgA or IgE). Isotype switching of the constant region of the heavy chain (C_H) allows a new C_H gene to be transcribed with the same variable gene, thus keeping the antigen-binding specificity unchanged. The mechanisms by which different isotypes are produced may be explained by a model of alignment of two switch (S) region sequences

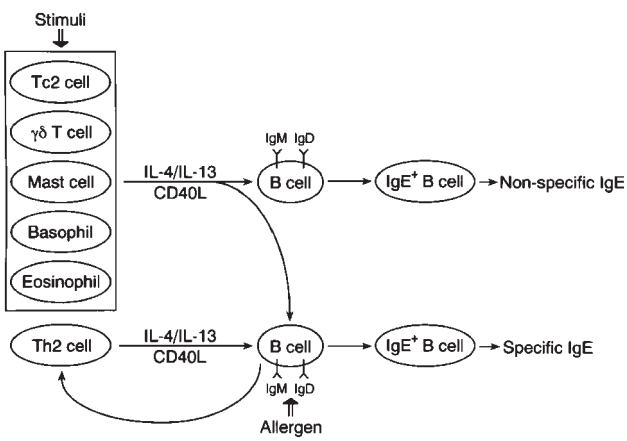

Fig. 1 Cell types that support IgE production by B cells. After appropriate stimulation, cytotoxic T cell (Tc)2 cells, γδ T cells, mast cells, basophils and eosinophils secrete interleukin (IL)-4 and/or IL-13 and express CD40L. Such cellular responses provide help to adjacent B cells, resulting in the induction of IgE production. This may also involve up-regulation of IgE synthesis induced by allergen-specific T helper 2 (Th2) cells.

with the deletion of the intervening DNA region.[22] The S regions located upstream of each CH gene, except Cδ, include Sμ, Sγ, Sα and Sε (Fig. 2a). In addition, the germline I exon is located upstream of the Sγ, Sα or Sε region. The specificity of isotype switching from Cμ to Cγ, Cα or Cε is regulated by cytokines that direct switching by altering the accessibility of CH genes and their S regions to a common recombinase system.

In the course of the IgE response, B cells differentiate into plasma cells or memory cells that produce IgE. This differentiation process allows a single B cell to switch from the production of IgM to IgE at the molecular level. As shown in Fig. 2b, IgE isotype switching occurs by a DNA rearrangement that is accompanied by the looping out and deletion of the intervening DNA between the Sμ and Sε regions.[23,24] Thus, activation of transcription through the Sε region is an essential step in the IgE switching process. Induction of IgE isotype switching is preceded by transcription of Cε RNA, which initiates from the Iε promoter containing an IL-4 response element. This promoter contains binding sites for transcription factors, including signal transducers and activators of transcription (STAT)6, nuclear factor (NF)-κB and B cell-specific activator protein.[25–27] Nuclear expression of these transcription factors results in transcription of the Iε exon, the Sε region and the Cε exons (Cε1–Cε4). The germline Cε transcript is composed of the Iε and Cε exons, because the Sε region is spliced out. Transcripts of the Sε region, but not germline Cε transcripts, may play a role in Sμ/Sε switch recombination through DNA complex formation.[28] Both IL-4 and IL-13 induce germline Cε transcription in B cells, with the subsequent ability to induce IgE switching. Although regulation of germline Cε transcription by cytokines, such as IFN-γ, TNF-α and transforming growth factor-β, correlates well with levels of IgE production, additional costimulatory molecules, including CD40L, are required for the induction of IgE isotype switching. CD40 ligation enhances IL-4- or IL-13-driven germline Cε transcription and further induces the deletional switch recombination that results in IgE switching. This DNA switch recombination induces the substitution of the Cμ gene for the Cε gene and subsequently brings a specific variable region adjacent to a rearranged Cε gene, allowing mature Cε transcription and IgE synthesis. Therefore,

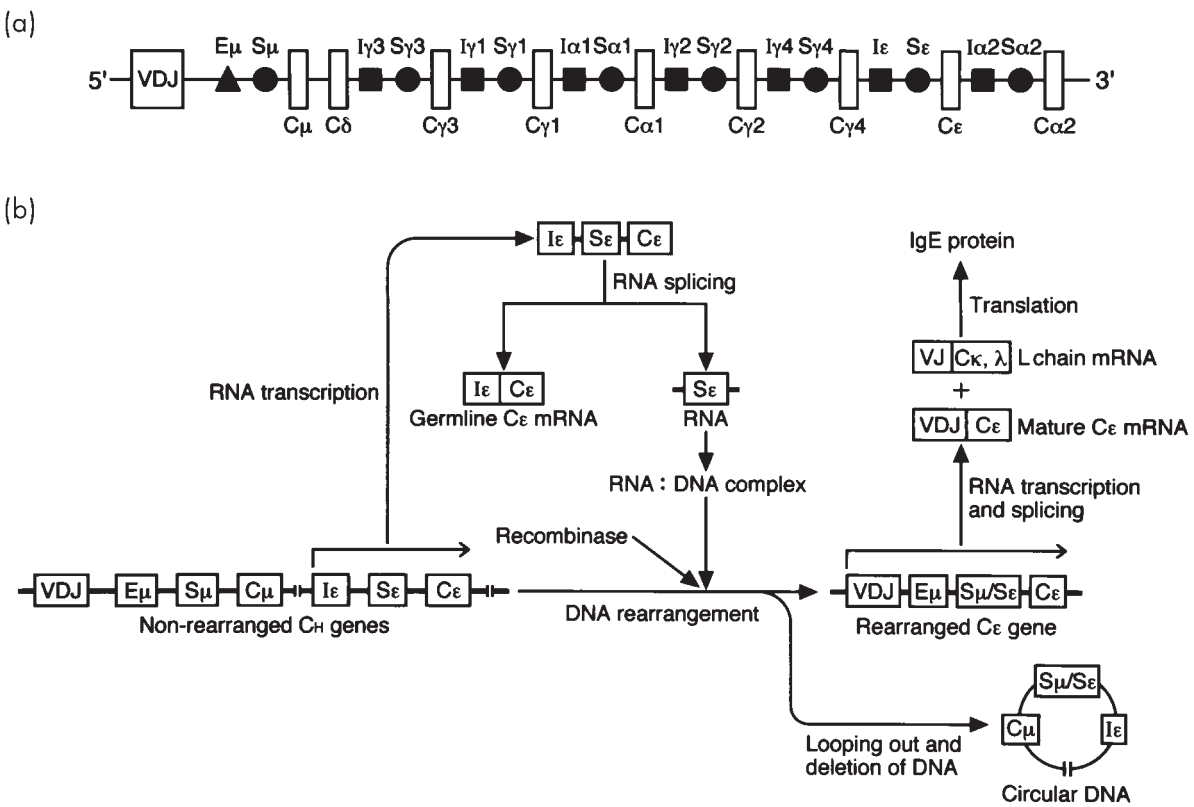

Fig. 2 Schematic diagram of the molecular events in IgE isotype switching. (a) Genes of CH isotypes and S regions in a B cell expressing IgM and IgD. (b) DNA switch recombination that results in IgE isotype switching. See text for details.

both membrane and secreted forms of IgE are expressed by the same cell at different stages of differentiation. Although the transition from the membrane to the secreted form of IgE involves alternative mRNA splicing, the extracellular, transmembrane and cytoplasmic domains of IgE regulate IgE secretion or IgE responses.[29–31]

Interleukin-4 signal transduction events leading to germline Cε transcription

Both IL-4 and IL-13 contribute to the induction of germline Cε transcription, the production of IgE and the expression of CD23 by B cells. These overlapping effects of IL-4 and IL-13 on B cell function are mediated by the binding of cytokines to their specific receptors on B cells. The functional IL-4 receptor (IL-4R) consists of at least two components, the IL-4R α chain (IL-4Rα) and the IL-2R γ chain, termed the common γ chain (γc).[32,33] Although the γc is a shared element of the receptors for IL-2, IL-4, IL-7, IL-9 and IL-15, the IL-4Rα may be shared by IL-13R. Several studies have demonstrated that an IL-13-binding chain (IL-13Rα) associates with the IL-4Rα[34,35] and that B cells of X-linked severe combined immunodeficiency patients with mutations in the γc gene are induced to express germline Cε transcripts by IL-4 and IL-13 and to produce IgE by costimulation with IL-4 or IL-13 and an agonistic anti-CD40 monoclonal antibody (mAb).[36,37] Thus, the IL-4Rα is a common component of the functional IL-4R and IL-13R in B cells.

Many cytokine receptors, including IL-4Rα, γc and IL-13Rα, do not have any unique sequences for signal transduction, such as protein tyrosine kinase (PTK) sites, in their intracytoplasmic domain. However, cytokine binding induces tyrosine phosphorylation and activation of members of the Janus kinase (JAK) family,[38,39] indicating that the intracellular portions of cytokine receptors function as binding sites for cytoplasmic JAK. Activated JAK phosphorylate tyrosine residues in the cytoplasmic tails of the oligomerized cytokine receptor, thereby resulting in association with the Src-homology (SH) 2 domains of members of the STAT family. The JAK further phosphorylate STAT proteins recruited to the phosphorylated cytokine receptor. The receptor chains IL-4Rα, γc and IL-13Rα associate with JAK1, JAK3, and JAK2 and TYK2, respectively. Interleukin-4 and IL-13 induce phosphorylation of JAK1, which, in turn, activates STAT6. When the phosphorylated STAT6 forms a homodimer via the SH2 domain, this dimer is dissociated from the IL-4Rα, translocates to the nucleus and binds to the consensus sequence in the Iε promoter. Thus, STAT6 is an important transcription factor that regulates the expression of various genes by IL-4 and IL-13. For example, transfection of decoy oligodeoxynucleotides containing STAT6 binding sites into a human B cell line, as well as pretreatment with herbimycin A, a PTK inhibitor, leads to a marked decrease in germline Cε transcription induced by IL-4.[40,41] The essential role of STAT6 in IgE synthesis has been shown in STAT6-deficient mice.[42,43]

Nuclear factor-κB, known to play a broad role in gene regulation, is also activated by IL-4. Inactive NF-κB, which is present in a non-DNA binding form in the cytoplasm, is a heterotrimer composed of three subunits. Phosphorylation and dissociation of the IκB subunit results in the translocation of the active heterodimer (p50/p65 or p50/rel) to the nucleus, in which active NF-κB binds to its consensus sequence in the promoters of various genes, including the Iε exon. It has been shown that targeted disruption of the p50 subunit of NF-κB in mice results in a substantial decrease in germline Cε transcription in splenic B cells activated by appropriate stimulation.[44] Phosphorylation of IκB is inducible by at least the ζ isoform of protein kinase C (PKCζ), which is activated by the lipid products of phosphatidylinositol (PI) 3-kinase (PI3-K), such as PI 3,4, 5-triphosphate. Activation of PI3-K may be mediated through the tyrosine phosphorylation of docking proteins, such as insulin receptor substrate (IRS)-1, IRS-2 and the *c-fes* proto-oncogene product (FES).[45,46] Previous work has shown that PI3-K inhibitors, such as wortmannin and LY294002, inhibit IL-4-induced nuclear expression of NF-κB by abrogating the translocation of PKCζ from the cytosol to the membrane fraction in a B cell line.[47,48] Dominant-negative PKCζ also decreases the activation of the Iε promoter by IL-4. In addition, *N*-acetyl-L-cysteine (NAC), a NF-κB inhibitor, partly inhibits IL-4-induced germline Cε transcription. Transfection of the two decoy oligodeoxynucleotides containing NF-κB and STAT6 binding sites into a B cell line results in almost complete abrogation of IL-4-driven germline Cε transcription.[41] These data suggest that a coordinated action of NF-κB and STAT6 may be required for sufficient induction of germline Cε transcripts by IL-4. This suggestion is supported by a recent study indicating that STAT6 and NF-κB cooperatively bind their cognate DNA binding sites and synergistically activate transcription.[49] Interleukin-4 also induces the generation of 1,2-diacylglycerol (DAG) without the initial accumulation of inositol 1,4,5-triphosphate, through the activation of PI-specific phospholipase C (PLC)-γI associated with tyrosine-phosphorylated Shc,

thereby resulting in translocation of PKCδ (K Ikizawa and Y Yanagihara, unpubl. obs., 1998). It should be noted that PKCδ is dependent on DAG, while PKCζ is dependent on PI3-K products and that neither isoform requires Ca^{2+} for activation. However, the precise roles of PKCδ and its substrate in regulating germline Cε transcription are not known. In contrast with FES, Shc, IRS-1 and IRS-2 share a novel phosphotyrosine-binding domain capable of binding to the phosphorylated NPXY motifs that are present in the IL-4Rα as well as in the insulin receptor. Thus, Shc also functions as an adaptor molecule in IL-4 signaling.

The receptor chain IL-4Rα is a crucial component, required for IL-4 binding, signal transduction and kinase binding, leading to the cellular responses. An extracellular variant of human IL-4Rα, containing a valine (Val) for isoleucine (Ile) substitution at amino acid 50 (numbering for mature peptide), has recently been shown to associate with atopic asthma.[50] Indeed, a high frequency of Ile50 homozygotes was observed in the atopic asthma group, compared with the normal and non-atopic groups. In B cell lines transfected with complete cDNA for the Ile50 and Val50 variants, greater activation of STAT6 and the Iε promoter was induced in Ile50-transfected cells than in Val50-transfected cells after IL-4 stimulation.[50] However, the two variants did not alter the ligand-binding affinity of IL-4Rα, indicating that the Ile50 variant of IL-4Rα up-regulates the receptor response to IL-4. In addition to the Ile50Val substitution, a cytoplasmic variant of IL-4Rα, containing an arginine (Arg) 576 to glutamine (Gln) substitution, has been described in patients with hyper-IgE syndrome and atopic dermatitis.[51] This mutation also enhances the IL-4 signaling that leads to CD23 expression. Although activation of STAT6 by IL-4 is not up-regulated in the presence of the Arg576Gln substitution, decreased binding of the SH2-containing tyrosine phosphatase 1 to the tyrosine-phosphorylated IL-4Rα can be detected in the mutation. This may lead to exaggerated IL-4 signaling. These findings demonstrate that both extracellular and cytoplasmic variants of IL-4Rα are associated with enhanced responsiveness to IL-4.

CD40-MEDIATED SIGNAL TRANSDUCTION EVENTS LEADING TO DELETIONAL SWITCH RECOMBINATION

CD40, a member of the TNF receptor superfamily, plays a key role in T cell-dependent B cell responses.[52] CD40 signaling, which is initiated by the binding of CD40 to CD40L, a non-covalent trimer, activates multiple pathways to produce B cell maturation. Despite the absence of characteristic PTK sequences within the cytoplasmic domain of CD40, antibody ligation of CD40 on B cells has been shown to induce tyrosine phosphorylation and activation of Lyn and, to a lesser extent, Fyn, which are coupled with increased activity of PI3-K and tyrosine phosphorylation of PLC-γ2.[53,54] Thus, members of the Src family are involved in CD40 signaling. In addition, the cytoplasmic region of CD40 is associated with four members of the TNF receptor-associated factor (TRAF) family, TRAF2, TRAF3, TRAF5 and TRAF6,[55–58] and with JAK3.[59] Although the TRAF proteins have no enzymatic activity, they function as second messengers in CD40 signaling. Engagement of CD40 also induces activation of NF-κB and members of the mitogen-activated protein kinase (MAPK) subfamily.[56,57,60] Nuclear factor-κB activation is mediated by the PI3-K pathway and by TRAF2, TRAF5 and TRAF6. Furthermore, TRAF2 activates c-Jun NH_2-terminal kinase (JNK) and NF-κB-inducing kinase (NIK) and TRAF6 activates extracellular signal-regulated kinase (ERK). Expression of c-Jun is up-regulated by JNK, whereas ERK induces serine/threonine phosphorylation of Elk-1.[60] However, the downstream events in the TRAF3-dependent signaling pathway are not known. Activated JAK3 induces tyrosine phosphorylation of STAT3, which leads to complex assembly, nuclear translocation and STAT3-dependent gene transcription.[59] These data demonstrate that multiple CD40 signaling pathways are integrated at the level of transcriptional activation to regulate B cell responses.

The Iε promoter contains a CD40 response element that is distinct from the IL-4 response element.[61] The CD40 response element has binding sites for NF-κB-related factor and activator protein-1. Nuclear factor-κB inhibitors, such as NAC, have been shown to be effective in inhibiting CD40-mediated enhancement of IL-4-driven germline Cε transcription in a B cell line.[48] Thus, NF-κB induced by CD40 ligation may play a role in up-regulating IL-4-driven germline Cε transcription. In contrast, NAC has also been shown to inhibit mature Cε transcription and IgE synthesis in normal B cells costimulated with IL-4 and anti-CD40 mAb.[62] Because mature Cε transcription results from IgE isotype switching, we examined whether NAC could influence CD40-mediated switch recombination. As expected, NAC could inhibit the generation of Sμ/Sε switch fragments in normal B cells costimulated with IL-4 and anti-CD40 mAb.[41] This result suggests that NF-κB-dependent steps may be important in activating the switch recombination machinery, as well as activating expression from the

Iε promoter. Although the two signal transduction pathways for IL-4 and CD40 ligation operating through NF-κB interact at the transcriptional level, NF-κB activation triggered either by IL-4 or by CD40 ligation fails to cause isotype switching to IgE. This implies that NF-κB activated via the PI3-K pathway, which is commonly transduced through IL-4Rα and CD40, does not participate in IgE isotype switching. Therefore, it is likely that NF-κB activation triggered by TRAF multimerization may contribute, at least in part, to switch recombination. However, the details of members of the TRAF and NF-κB/Rel families that play a role in regulating the switch machinery are, at present, unknown. In addition to NF-κB, other DNA-binding proteins, such as Ku70/86, which is capable of forming complexes with the DNA-dependent protein kinase (DNA-PK), and rad51/54, expressed via switch recombination, are involved in isotype switching.[63–66] Thus, DNA-PK also functions as part of the switch recombinase. These findings suggest that localization of DNA-binding proteins may be critically important in prompting switch recombination.

CONCLUDING REMARKS

Recent advances in elucidating the signaling pathways to produce B cell maturation have led to new insights into the molecular mechanisms underlying human IgE synthesis. Terminal differentiation of a single B cell into an IgE-secreting plasma cell is regulated at the level of transcriptional activation by multiple pathways of B cell triggering (Fig. 3). Although a combination of signals delivered by IL-4Rα and CD40 regulate isotype switching to IgE, the detailed mechanisms of DNA switch recom-

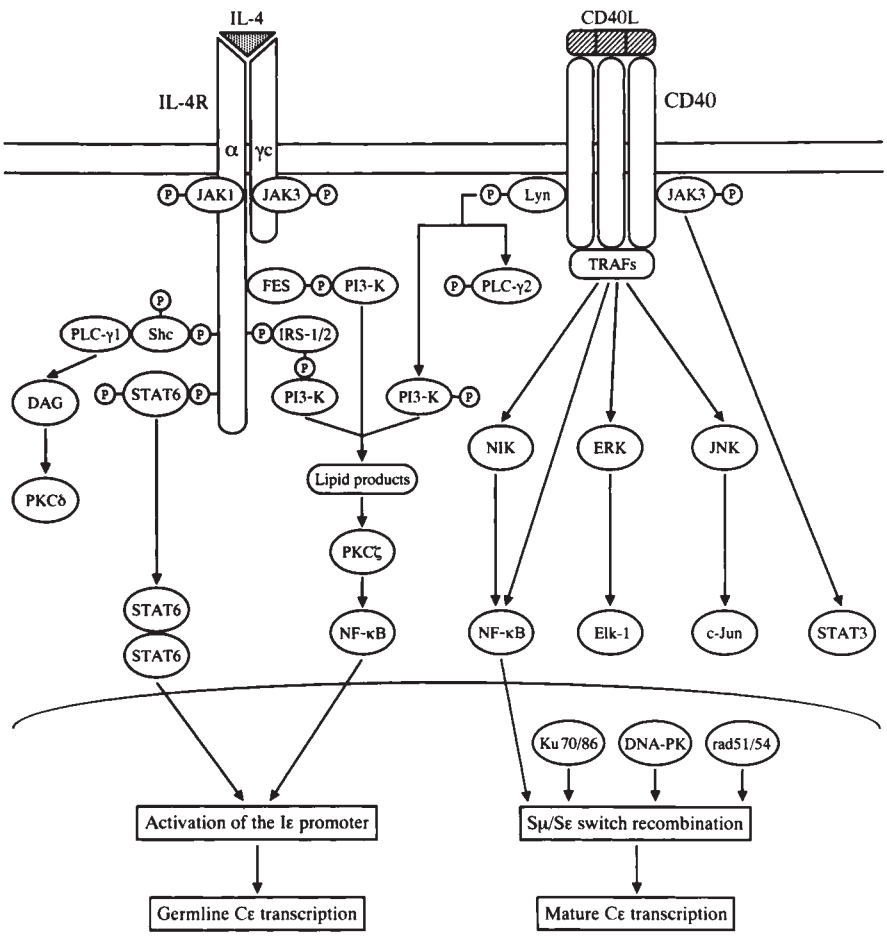

Fig. 3 B cell transduction events leading to IgE isotype switching. Interleukin (IL)-4 activates STAT6 and nuclear factor (NF)-κB, which interact at the level of the Iε promoter, thereby resulting in sufficient induction of germline Cε transcription. CD40 ligation, in conjunction with IL-4 stimulation, induces deletional switch recombination that leads to IgE switching and mature Cε transcription. Multiple pathways, including protein tyrosine kinase (PTK), tumor necrosis factor receptor-associated factor (TRAF) proteins and members of the mitogen-activated protein kinase subfamily, are involved in CD40 signaling. DNA-binding proteins, such as NF-κB, Ku70/86, DNA-dependent protein kinase (DNA-PK) and rad51/54, are also implicated in switch recombination. However, the detailed mechanisms of Sμ/Sε switch recombination are presently unclear. IL-4R, IL-4 receptor; JAK, Janus kinase; PLC, phospholipase C; DAG, diacylglycerol; PKC, protein kinase C; STAT, signal transducers and activators of transcription; FES, *c-fes* proto-oncogene product; IRS, insulin receptor substrate; PI3-K, phosphatidylinositol 3-kinase; ERK, extracellular signal-regulated kinase.

bination are, at present, unknown. Identification and characterization of the key molecules essential for IgE isotype switching will provide important strategies for the manipulation of the IgE response in atopic patients.

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