

THE REGULATION OF IMMUNOGLOBULIN E CLASS-SWITCH RECOMBINATION

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Immunoglobulin E (IgE) isotype antibodies are associated with atopic disease, namely allergic rhinitis, asthma and atopic dermatitis, but are also involved in host immune defence mechanisms against parasitic infection. The commitment of a B cell to isotype class switch to an IgE-producing cell is a tightly regulated process, and our understanding of the regulation of IgE-antibody production is central to the prevention and treatment of atopic disease. Both those that are presently in use and potential future therapies to prevent IgE-mediated disease take advantage of our existing knowledge of the specific mechanisms that are required for IgE class switching.

Immunoglobulin E (IgE) isotype antibodies are normally present at low levels in the plasma and are mainly produced by plasma cells in the mucosal-associated lymphoid tissue. Patients suffering from atopic conditions, such as asthma, allergic rhinitis and atopic dermatitis, have elevated serum levels of both total IgE and of IgE that is specific for the antigens that drive these diseases. Most of the IgE that is produced is bound by its high-affinity Fc receptor, FcεRI, expressed by mast cells and basophils. Crosslinking of IgE on tissue mast cells by specific antigens results in the local release of inflammatory mediators (for example, histamine and leukotrienes), enzymes and cytokines that mediate the clinical manifestations of atopy (FIG. 1). The low-affinity IgE receptor, FcεRII (CD23), is expressed by several immune cell types, including B cells. IgE that is bound to CD23 facilitates allergen uptake by B cells, enhancing presentation to T cells and augmenting secondary immune responses. IgE levels are also increased in parasitic diseases, in which they have a role in immunity, and in rare genetic disorders that affect the immune system (for example, Wiskott–Aldrich syndrome (WAS) and hyper-IgE syndrome). However, the level of serum IgE in these conditions remains several thousand-fold lower than the level of serum IgG, indicating that IgE synthesis is

tightly regulated. In this review, we discuss new insights into the control of IgE production at the level of IgE isotype class switching and in the development of T helper 2 (T_H2) cells, which drive IgE responses. The implications of this understanding for the treatment of IgE-mediated diseases are also discussed.

Mechanisms of class-switch recombination

A two-step process of DNA excision and ligation is required for the assembly of a functional IgE gene¹. These two steps are common to all immunoglobulin isotypes that are encoded downstream of IgM and IgD. The first step occurs during the pre-B-cell stage, in which individual heavy-chain variable (V_H), diversity (D) and joining (J_H) exons randomly combine with precise joins to generate a V_H(D)J_H cassette that encodes an antigen-specific V_H domain. This V_H(D)J_H cassette, which is situated just upstream of the constant (C) μ exons, allows for production of the μ-heavy-chain protein. The second step, known as class-switch recombination (CSR), allows appropriately stimulated B cells to alter the isotype of the antibodies that they produce while retaining their antigen specificity. This step involves tightly regulated and irreversible exchange of the C_H cassettes of the various isotypes to construct different heavy chains.

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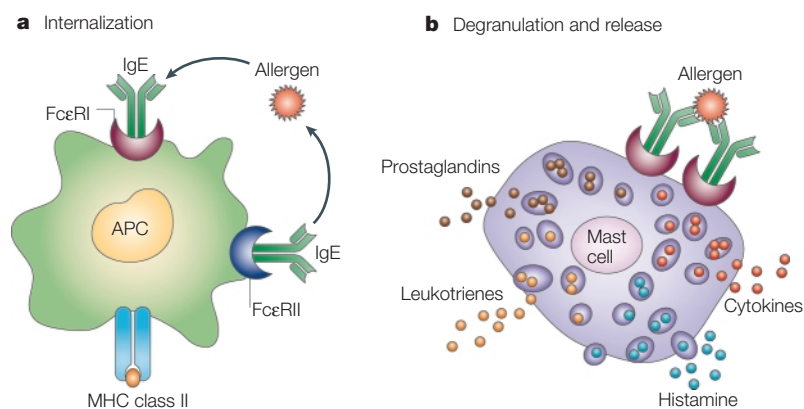


Figure 1 | Biological effects of IgE binding. **a** | The low-affinity IgE receptor, FcεRII (CD23), is expressed by a wide variety of immune cell types, including B cells and dendritic cells. IgE that is bound to FcεRI or FcεRII can facilitate allergen uptake by antigen-presenting cells (APCs) and augment secondary immune responses. **b** | Most IgE is bound by its high-affinity receptor, FcεRI, expressed by mast cells and basophils. Crosslinking of IgE bound to FcεRI on tissue mast cells by specific antigen results in the local release of inflammatory mediators (for example, histamine and leukotrienes), enzymes and cytokines that mediate the clinical manifestations of atopy.

The architecture of the Cε locus is shared with the other C_H genes. The 5' intronic region of each heavy-chain isotype gene, except Cδ, includes a switch region (S), which contains repeated tandem pentamers (predominantly GAGCT, GGGGT or GGGCT), or a 49 base-pair sequence that is flanked immediately upstream by a region that encodes a short I exon and its promoter. The switch region in individual C_H genes (Sε in the case of IgE) is the site that undergoes physical recombination to form a DNA hybrid molecule with the μ switch region (Sμ) during CSR. Sε is excised downstream of V_H(D)J_H and upstream of the Cε locus (FIG. 2). The contiguous joining of V_H(D)J_H and Cε sequences, resulting from imprecise and heterogeneous Sμ–Sε ligation, generates a functional gene encoding IgE. As discussed, germline transcription from the promoter of the Iε exon, which is located immediately upstream of Sε, is a prerequisite of CSR to IgE^{2,3}.

The crucial role of activation-induced cytidine deaminase (AID) in CSR has been established by the observation that CSR is markedly impaired in both AID-deficient mice⁴ and in a subset of patients with autosomal-recessive hyper-IgM syndrome type 2 (HIGM2), who have mutations in the AID gene⁵. AID has homology with APOBEC1, an RNA-editing enzyme, and has been shown to have cytidine-deaminating activity⁶. Recently, it was shown that AID deaminates cytidines on single-stranded DNA (ssDNA) substrates, but not double-stranded DNA (dsDNA) substrates⁷. However, dsDNA can be deaminated by AID when the reaction is coupled to transcription. It has been proposed that AID targets ssDNA that is produced during transcription and substitutes U for C. A role for RNA deamination by AID and its requirement in CSR has not been eliminated. Base-excision repair results in dsDNA breaks, and repair of these breaks in the Sμ and Sε regions by the joining of non-homologous ends results in Sμ to Sε deletional switch recombination.

Regulation of CSR to IgE

Specific signals from cytokines and cell-surface receptors collaborate to regulate CSR in B cells (FIG. 3). The levels of regulation that are common for switching to all isotypes are the germline transcription of C_H genes and the induction of AID expression. The specificity of the C_H switch is at the level of C_H germline transcription. The classic pathway to IgE switching involves the cytokines interleukin-4 (IL-4) and IL-13 and the tumour-necrosis factor receptor (TNFR)-superfamily member CD40.

Induction of Cε germline transcription. The Iε promoter is activated before the initiation of CSR in B cells and results in the production of 'sterile' Cε germline transcripts (GLTs), which lack the VDJ region. The Iε promoter contains binding sites for several known transcription factors, including signal transducer and activator of transcription 6 (STAT6), nuclear factor-κB (NF-κB), PU.1 (REF. 8), B-cell-specific activator protein (BSAP; also known as paired-box protein 5, PAX5)⁹, CCAAT/enhancer binding protein (C/EBP)¹⁰ and activator protein 1 (AP1)¹¹, all of which promote Cε germline transcription (FIG. 4). In addition, the Iε promoter contains the E-box binding sites, E1 and E2, for the transcription factor E2A, which also promotes Cε germline transcription¹².

The T_H2-type cytokines IL-4 and IL-13 are potent inducers of Cε germline transcription in B cells¹³ (BOX 1). They function through the activation of STAT6, which can synergize with NF-κB to induce the production of Cε GLTs (FIG. 4). The TNF-superfamily member CD40 ligand (CD40L, also known as CD154) is transiently expressed by antigen-stimulated T cells and promotes Cε germline transcription through its activation of NF-κB¹⁴ (FIG. 4). The PU.1 element overlaps the distal NF-κB site in the Iε promoter and, similar to NF-κB, PU.1 synergizes with STAT6 in activating germline transcription from the Iε promoter⁸. Although neither BSAP nor NF-κB nuclear-binding activities are altered by cytokine signalling, these promoter elements must be present for normal Iε promoter function¹⁵. Furthermore, IgE isotype switching is selectively impaired in NF-κB p50-deficient mice¹⁶ and is enhanced in mice that lack the NF-κB inhibitor IκBα¹⁷, whereas overexpression of BSAP can drive the transcription of Iε and promote IgE isotype switching¹⁸.

Regulation of AID induction. Ligation of CD40 has an essential role in CSR to IgE and all other isotypes. Ligation of CD40 synergizes with IL-4 to induce the expression of AID-encoding messenger RNA and AID protein⁷. In the case of initiating IgE CSR, IL-4 also induces the binding of STAT6 to a site in the 5' upstream region of the AID gene, whereas CD40 ligation induces the binding of NF-κB to two sites in that region of the AID gene. B cells from STAT6-deficient mice fail to upregulate the expression of AID in response to IL-4, whereas B cells from NF-κB p50-deficient mice are impaired in their ability to upregulate AID expression in response to CD40 ligation and IL-4 signals that are

LIPID RAFTS

Cholesterol and sphingolipid-enriched membrane detergent-resistant microdomains (DRMs), which function as aggregation points for membrane and cytosolic signalling complexes.

CORTICOSTEROIDS

Small lipophilic molecules that regulate physiological processes, including immune responses, by binding cytoplasmic receptors. They have broad therapeutic use as potent anti-inflammatory and immunosuppressive agents.

also required for C ϵ germline transcription (F. Dedeoglu, E. Castigli and R.S.G., unpublished observations). So, NF- κ B that is activated by CD40 ligation synergizes with STAT6, which is activated by IL-4, to induce *AID* gene expression. This indicates that synergy between IL-4 and CD40 might be required to achieve a threshold level of AID expression for CSR to IgE¹⁹.

CD40 signalling. CD40 is expressed by all B cells and its intracellular domain associates with, and signals through, four intracellular proteins that belong to the family of TNFR-associated factors (TRAFs) — that is, **TRAF2**, **TRAF3**, **TRAF5** and **TRAF6** (REFS 20–22). Ligation of CD40 by membrane-bound trimeric CD40L causes a higher degree of oligomerization, which promotes the association of CD40 with, and signalling through, TRAFs²³. It might also cause a conformational change that results in the translocation of CD40, together with associated TRAFs, to LIPID RAFTS in which signalling molecules are concentrated following receptor ligation²⁴. The association of TRAF2, TRAF5 and TRAF6 with CD40 promotes the dissociation of NF- κ B from its inhibitor, I κ B, allowing NF- κ B to translocate to the nucleus and synergize with STAT6 to activate the I ϵ promoter^{19,25}. TRAF3, which seems to function through mitogen-activated protein kinase (MAPK)/extracellular

signal-regulated kinase kinase 1 (MEK1)²⁶, has also been implicated in the CD40-mediated upregulation of IL-4-driven production of C ϵ GLTs. Upregulation of IL-4-driven production of C ϵ GLTs, as well as S μ to S ϵ switch recombination, is effectively blocked by the loss of binding of TRAF2 or TRAF3 to CD40 (REFS 27,28). In addition to inducing TRAF association and signalling, the aggregation of CD40 activates protein tyrosine kinases (PTKs) and p38 kinase (**MAPK14**), both of which are important in immunoglobulin class switching^{29–31} by inducing the production of C ϵ GLTs.

Alternative pathways of IgE class switching

Several T-cell-independent pathways for inducing IgE class switching, in the presence of IL-4, have been described.

Corticosteroids. The CORTICOSTEROID hydrocortisone induces the synthesis of IgE by IL-4-stimulated human B cells³², and upregulates the expression of mRNA encoding CD40L and the cell-surface expression of CD40L protein by T and B cells³³. There are two potential glucocorticoid-responsive elements (GREs) in the 5' upstream region of the *CD40L* gene that might be responsible for this activity. Hydrocortisone fails to induce the synthesis of IgE by B cells

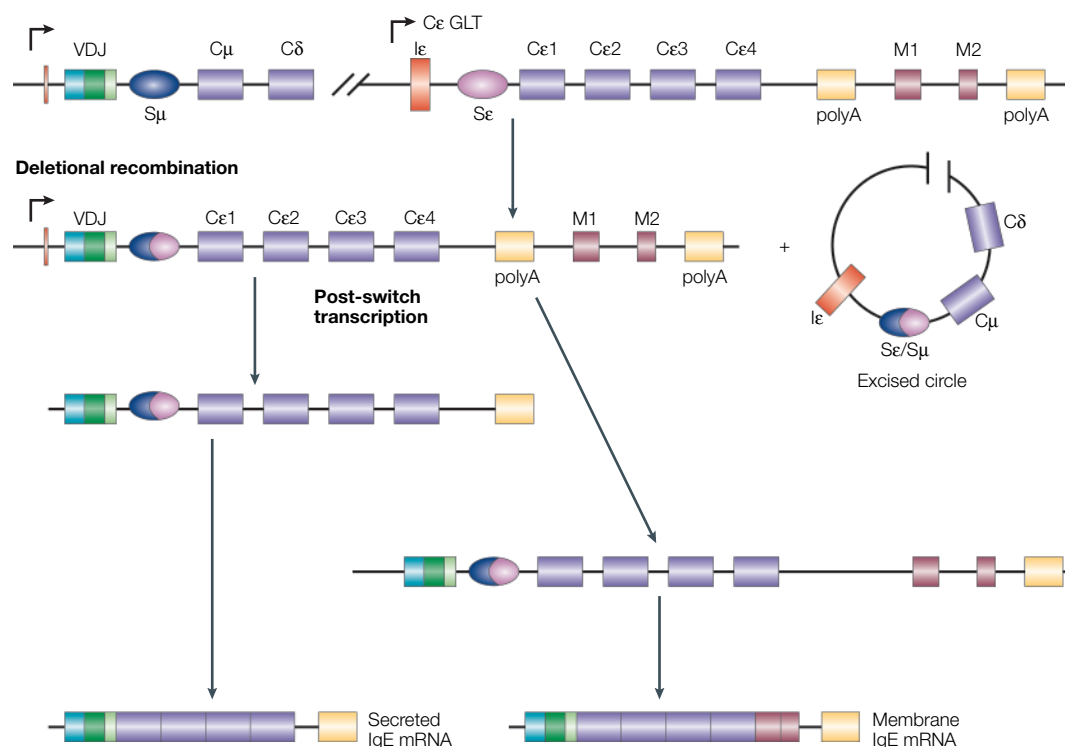


Figure 2 | Deletional class-switch recombination of the human immunoglobulin locus. The human immunoglobulin heavy-chain locus contains clusters of heavy chain variable (V_H), diversity (D_H) and joining (J_H) cassettes that are rearranged during B-cell development. This process results in the assembly of a complete VDJ exon that encodes an antigen-binding V_H domain capable of producing intact μ heavy chains. Following light-chain rearrangement, B cells can produce intact IgM antibodies. The production of other antibody isotypes that have the same antigen specificity requires an additional excision and repair process — known as deletional class-switch recombination. For IgE isotype switching, this process involves the excision of a large piece of genomic DNA spanning from μ switch (S μ) sequences to the S ϵ sequence. Ligation of the VDJ sequences to the constant (C) ϵ locus gives rise to an intact ϵ heavy-chain gene and a B cell that can produce intact IgE antibodies. RNA splice isoforms with the M1 and M2 exons encode membrane IgE. Similar to C ϵ , C μ and C δ genes are composed of multiple exons (not shown).

from CD40L-deficient patients, and disruption of CD40L–CD40 interactions by soluble CD40–immunoglobulin fusion proteins or CD40L-specific monoclonal antibodies blocks the capacity of hydrocortisone to induce IgE synthesis by normal B cells. So, hydrocortisone-inducible IgE class switching is CD40L dependent. The level of hydrocortisone that results in optimal expression of CD40L (10^{-6} M) is in the range of the cortisol levels that are found in the serum of humans under stress conditions³⁴. Rapid induction of CD40L expression by glucocorticoids that are secreted in response to stress might be advantageous for a host on first encounter with an infectious organism, when populations of antigen-specific T cells have not yet expanded. This is because, in addition to the induction of isotype switching in B cells, CD40L activates many CD40-expressing inflammatory cells, including macrophages, dendritic cells and eosinophils, which contributes to the elimination of pathogens. The effect of hydrocortisone on the class switching of other immunoglobulin isotypes has not yet been tested.

BAFF and APRIL. The recently described TNF-superfamily members B-cell-activating factor belonging to the TNF family (BAFF) and a proliferation-inducing ligand (APRIL) have also been reported to induce CSR³⁵, and, in particular, the induction of IgE-specific CSR.

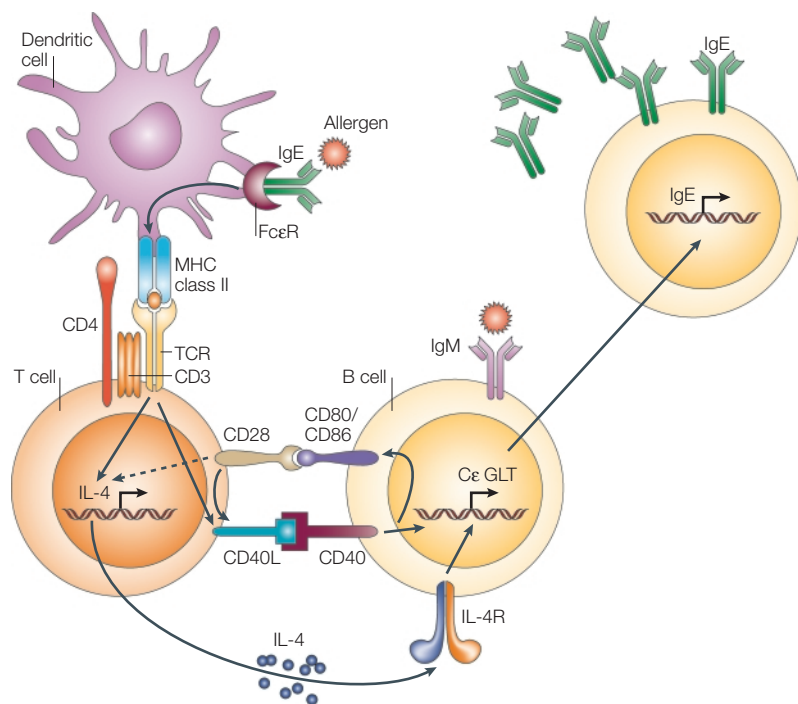


Figure 3 | Cellular interactions important for IgE class-switch recombination. Uptake of allergens by dendritic cells allows for the presentation of antigenic determinants to T cells. The subsequent stimulation of specific CD4⁺ T cells leads to the production of interleukin-4 (IL-4) and the upregulation of expression of CD40 ligand (CD40L) by T cells. CD40 stimulation of allergen-specific B cells upregulates the expression of the co-stimulatory molecules CD80 and CD86, which allows for more efficient T-cell expression of CD40L and enhanced stimulation of B cells through the induction of IL-4. CD40-mediated stimulation of B cells also synergizes with IL-4-receptor (IL-4R) signals to enhance the transcription of Cε germline transcripts (Cε GLTs) and activation-induced cytidine deaminase (AID), rearrangement of the IgE genomic locus and production of IgE antibodies. FcR, Fc receptor; TCR, T-cell receptor.

These factors also induce CSR to the IgA and IgG isotypes. BAFF and APRIL are mainly expressed by monocytes and dendritic cells. Interferon- α (IFN- α), IFN- γ , lipopolysaccharide (LPS) and CD40L induce their expression on the surface of dendritic cells. Engagement of the BAFF and APRIL receptors expressed by B cells, together with IL-4, induces the production of Cε GLTs, the expression of AID and IgE class switching³⁵. In addition, CSR that is induced by cytokine-stimulated dendritic cells is inhibited by antibodies specific for BAFF and APRIL.

At present, the receptors that mediate the activation of CSR by BAFF and APRIL are unknown. APRIL and BAFF both bind to two receptors that are expressed by B cells—BCMA (B-cell maturation antigen) and TACI (transmembrane activator and calcium-modulator and cytophilin ligand interactor), which are members of the TNFR superfamily³⁶. A third receptor, BAFFR (also known as BR3), that is unique for BAFF is expressed mainly by B cells³⁷. BCMA binds TRAF1, TRAF2, TRAF3, TRAF5 and TRAF6 and activates NF- κ B, JUN N-terminal kinase (JNK) and p38 kinase³⁸. TACI recruits TRAF2, TRAF5 and TRAF6, and has been found to activate NF- κ B, JNK and nuclear factor of activated T cells (NFAT)^{39,40}. BAFFR binds TRAF3, but the signalling pathways that are activated by the ligation of BAFFR remain unknown. Unlike CD40, BAFF and APRIL receptors stimulate NF- κ B activity through an alternative pathway that involves the NF- κ B-inducing kinase (NIK) and p52 activation pathway⁴¹. Isotype switching induced by BAFF and APRIL could be important for the response to T-cell-independent antigens, as mice that are deficient for TACI have virtually no antibody responses to type II T-cell-independent antigens, such as pneumococcal polysaccharides and trinitrophenyl (TNP)–Ficoll⁴².

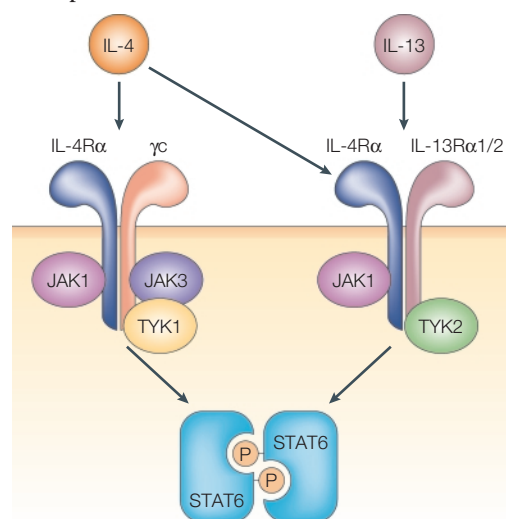
Epstein–Barr virus infection. Infection with Epstein–Barr virus (EBV) of freshly isolated peripheral-blood mononuclear cells (PBMCs) in the presence of IL-4 has long been reported to induce CSR to IgE⁴³. This effect is probably not specific for IgE CSR, because it has been shown that introduction of a transgene encoding the EBV-encoded protein latent membrane protein 1 (LMP1) in B cells from CD40-deficient mice restores the ability to switch to IgG1 (REF. 44). The ability of LMP1 to recruit TRAFs⁴⁵, activate NF- κ B⁴⁵ and promote cell survival^{46,47} might explain its capacity to mimic the effect of CD40 ligation *in vivo* and the ability of EBV to synergize with IL-4 in inducing CSR *in vitro*. The induction of isotype switching in B cells by EBV might simply be a secondary effect of CD40 mimicry by virus-encoded LMP1 in the activation of B cells to ensure replication of the virus.

C4BP. C4-binding protein (C4BP) is a circulating regulatory component of the classical complement pathway that is synthesized by liver cells and activated monocytes^{48,49} and is upregulated by glucocorticoids and inflammatory cytokines (IFN- γ , IL-1, IL-6 and TNF)^{50,51}. C4BP is a functional cofactor for factor-I-dependent degradation of C4B and C3B⁵², and it accelerates the decay of C3-convertase. Recently, C4BP was found to

Box 1 | **IL-4 and IL-13**

Interleukin-4 (IL-4) and IL-13 are mainly produced by T helper 2 (T_H2) cells and have overlapping activities¹³. Mice with targeted deletions of the gene encoding IL-4 or IL-13 have impaired T_H2 -cell responses and decreased production of IgE, but *Il4*^{-/-}, *Il13*^{-/-} double-mutant mice have a more marked T_H2 -cell impairment and virtual absence of IgE-antibody responses¹³¹.

IL-4 binds the IL-4 receptor α -chain (IL-4R α) that is contained in both IL-13R and IL-4R. IL-13R has a unique IL-13-binding chain (IL-13R α 1 or IL-13R α 2). IL-4R also contains the common cytokine receptor γ -chain (γ c). IL-4R triggers the activation of the Janus family tyrosine kinases JAK1 (through IL-4R α), JAK3, IRS1 and TYK1 (through γ c). IL-13R activates JAK1 (through IL-4R α) and TYK2. The activated JAKs phosphorylate tyrosine residues in the intracellular domains of IL-4R α , which act as signal transducer and activator of transcription 6 (STAT6)-binding sites. STAT6 is phosphorylated, dimerizes and translocates to the nucleus, where it can activate transcription of the I ϵ promoter.



bind CD40 and to mimic CD40L in the stimulation of B cells through CD40 (REF. 53). C4BP synergizes with IL-4 to induce the transcription of C ϵ GLTs and the expression of AID, and to cause S μ to S ϵ CSR, and the synthesis and secretion of IgE by purified B cells. The effect of C4BP on the class switching of other immunoglobulin isotypes has not yet been tested. C4BP co-localizes with B cells in the germinal centres of human tonsils, which indicates that sustained CD40 signalling by C4BP in B cells after their activation by CD40L-positive T cells in extrafollicular areas might be important for B-cell survival and activation in germinal centres⁵³.

Negative regulation of class switching

In non-allergic individuals, the level of IgE in the plasma is 10,000 to 50,000-fold less than that of plasma IgG. Even in highly atopic individuals, the level of plasma IgE remains less than 1,000-fold that of plasma IgG. Only a

relatively small part of this difference is due to the shorter half-life of circulating IgE. The main factor in the maintenance of low levels of plasma IgE is a tight control of IgE class switching. This is not surprising given the potent, and potentially destructive and life threatening, biological effects of antigen-specific IgE¹. The tight control of IgE class switching is exerted by several molecules, the action of which converges on the regulation of C ϵ germline transcription that is induced by T_H2 -type cytokines (FIG. 5).

Inhibition by cytokines. Several cytokines regulate IgE class switching, either by regulating the production of T_H2 -type cytokines and/or regulating the effect of these cytokines on C ϵ germline transcription.

IFN- γ inhibits the development of T_H2 cells and therefore inhibits the production of IL-4 and IL-13. IFN- γ also acts directly on B cells to repress C ϵ germline transcription and subsequently to downregulate CSR to C ϵ ⁵⁴. The effect of IFN- γ on isotype class switching is specific to the IgE and IgG1 isotypes.

IL-21 induces T- and B-cell proliferation and the differentiation of natural killer cells⁵⁵. *In vivo* injection of IL-21 specifically blocks antigen-induced IgE production, but not IgG2a production, after immunization⁵⁶. T_H2 -cell differentiation and IL-4 production are not altered. IL-21 blocks IgE production by inhibiting the transcription of C ϵ GLTs that is stimulated by IL-4 without altering STAT6 activation⁵⁷, although, the exact mechanism is unknown.

By contrast, IL-18 — an IL-1-like cytokine that requires cleavage by caspase-1 for activation — promotes IgE class switching⁵⁸. *In vivo* administration of IL-18 alone increased serum IgE levels in an IL-4-dependent manner. This is achieved by the activation of T cells by IL-18, which leads to the upregulation of expression of CD40L and the release of IL-4, IL-10, IL-13 and IFN- γ ⁵⁹. Overexpression of IL-18 by transgenic mice results in higher production of both T_H1 - and T_H2 -type cytokines by splenic T cells, and a marked increase in the levels of serum IL-4 and IFN- γ . The overall results, however, were that the levels of serum IgE and IgG1 were higher, indicating a bias to a T_H2 -type response by IL-18 overproduction⁶⁰.

Inhibition by B-cell surface receptors. Although CD40 promotes switching to IgE, several other B-cell surface receptors, including the B-cell receptor (BCR), CD45, cytotoxic T lymphocyte antigen 4 (CTLA4) and the low-affinity IgE receptor CD23, seem to inhibit this process.

Crosslinking of the BCR with immunoglobulin-specific monoclonal antibodies inhibits the CD40/IL-4-driven isotype switching to IgG1 and IgE. This is regardless of their effect on proliferation, which can range from enhancement to inhibition. This indicates that the ligation of the BCR by antigen can modify the rates of B-cell clonal expansion and class switching independently and might be important in the selection of immunoglobulin isotypes during the T-cell-dependent humoral response⁶¹.

HUMANIZED SEVERE COMBINED IMMUNODEFICIENCY (SCID) MICE

Mice that have the *Scid* mutation, which leads to an absence of T and B cells, that have been reconstituted with T and B cells from human peripheral blood.

The transmembrane phosphatase CD45 can dephosphorylate Janus-activated kinase 1 (JAK1) and JAK3, blocking IL-4-mediated stimulation of STAT6 and, therefore, acting as a negative regulator of IgE CSR and C ϵ GLT production⁶². Recently, it has also been shown that crosslinking of CD45 can block IL-4-induced expression of AID⁶³. CD45 might also have a role in the inhibition of CD40-induced activation of JNK and p38 kinase are involved in the production of C ϵ GLTs. The activities of these enzymes are enhanced following ligation of CD40 in CD45-deficient B cells⁶⁴, and CD45–CD40 crosslinking inhibits IgE class switching⁶⁵. The effect of CD45 on switching to other immunoglobulin isotypes has not yet been tested.

The expression of the inhibitory co-stimulatory molecule CTLA4 is induced by either CD40 or LPS on IL-4-stimulated B cells⁶⁶. Engagement of CTLA4 after upregulation of expression by B cell, inhibits the activation of NF- κ B and STAT6 and blocks the induction of IgE and IgG1 isotype switching by abrogating the transcription of C ϵ and C γ GLTs. Interaction of CTLA4 with its B7 ligands CD80 and CD86 expressed by activated B cells might provide a negative-feedback mechanism for the inhibition of isotype switching.

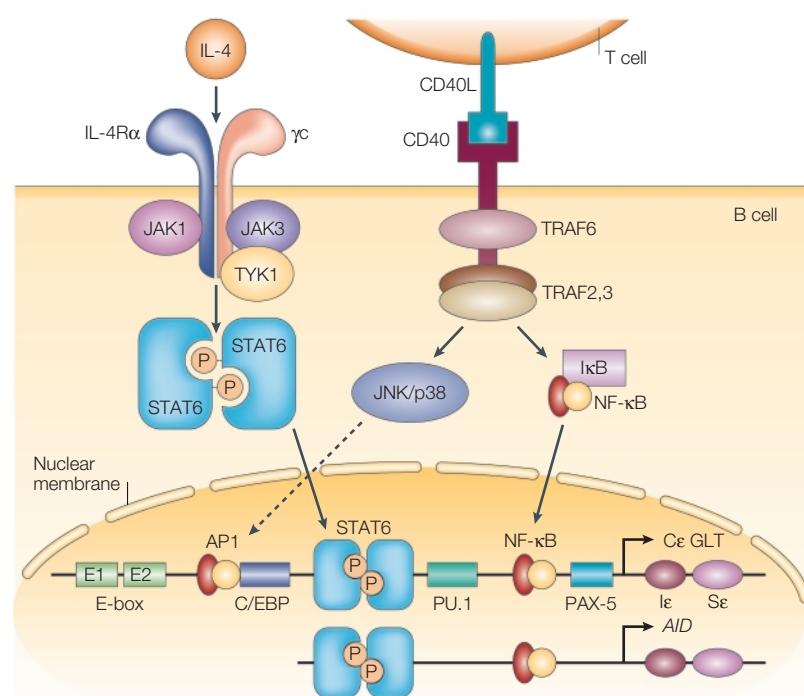
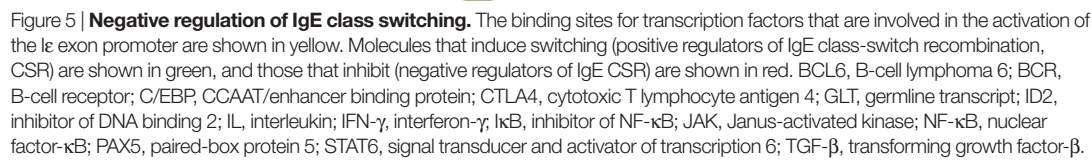


Figure 4 | IL-4 and CD40 activation signals lead to C ϵ germline and AID transcription. Class-switch recombination is preceded by RNA transcription at the heavy-chain constant (C ϵ) locus, which is induced by specific cytokine signals. C ϵ germline transcripts (C ϵ GLTs) originate at a promoter upstream of the I ϵ exon and do not encode functional proteins. The binding of interleukin-4 (IL-4) to the IL-4 receptor (IL-4R) leads to the recruitment and activation of the tyrosine kinases Janus-activated kinase 1 (JAK1), JAK3 and TYK1, which activate signal transducer and activator of transcription 6 (STAT6). Interaction of T-cell-expressed CD40 ligand (CD40L) with CD40 expressed by B cells allows for the recruitment and activation of tumour-necrosis factor receptor-associated factors (TRAFs) and nuclear translocation of the transcription factors nuclear factor- κ B (NF- κ B) and activator protein 1 (AP1). The stimulation of IL-4R and CD40 promotes the synergistic enhancement of transcription of both the C ϵ GLTs and activation-induced cytidine deaminase (AID) by NF- κ B and STAT6 (F. Dedeoglu, E. Castigli and R.S.G., unpublished observations). C/EBP, CCAAT/enhancer binding protein; I κ B, inhibitor of NF- κ B; JNK, JUN N-terminal kinase; PAX5, paired-box protein 5.

In addition to its role in allergen uptake, CD23 seems to have specific regulatory influences on IgE synthesis and allergic inflammation. Human and animal studies indicate that ligation of membrane-bound CD23 expressed by B cells suppresses the production of IgE^{67,68}, and transgenic mice that overexpress CD23 have suppressed IgE responses⁶⁹. Conversely, mice that are rendered CD23 deficient by targeted gene disruption have increased and sustained specific IgE titres following immunization, consistent with a suppressive effect of membrane-bound CD23 (REF. 69). Soluble CD23 fragments, which are generated by proteolytic cleavage, enhance the production of IgE by binding to IgE and thereby blocking its interaction with membrane-bound CD23, or possibly also by directly interacting with B cells through CD21 (REF. 70). Metalloproteinase inhibitors block the shedding of soluble CD23 in cultures of tonsillar B cells or PBMCs, and this is accompanied by decreased IgE production after stimulation with IL-4 (REF. 71). Recent data implicate a role for allergens, some of which are proteases, as effectors of CD23 cleavage⁷². Two possible consequences of such allergen-mediated cleavage would be decreased suppressive signalling to the B cell through CD23 and an increased production of activating soluble CD23 fragments, both promoting the production of IgE. Inhibition of proteolytic activity of the mite allergen Derp1 blocks its ability to induce IgE responses *in vivo* both in normal and HUMANIZED SEVERE COMBINED IMMUNODEFICIENCY (SCID) MICE^{73,74}. Similar effects are also observed in culture systems.

Inhibition by transcription factors. B-cell lymphoma 6 (BCL6) — a Poxvirus and zinc-finger-domain transcription factor that is expressed by B cells — is an important negative regulator of C ϵ germline transcription. BCL6 represses the IL-4-mediated induction of C ϵ GLTs by binding to STAT6 sites in the I ϵ promoter⁷⁵. Consistent with this model, Bcl6-deficient mice have enhanced IgE isotype switching, whereas Stat6-deficient animals are severely impaired in IgE production⁷⁶, and Bcl6 and Stat6 double-deficient animals do not produce IgE (TABLE 1). Loss of Stat6 expression also markedly impairs the production of IgG1 (REF. 76). Bcl6-deficient mice also overexpress T_H2-type cytokines and have increased eosinophilia⁷⁷.

Inhibitor of DNA binding 2 (ID2) is a dominant-negative regulator of helix–loop–helix (HLH) transcription factors. ID2 is constitutively expressed by resting peripheral-blood lymphocytes (PBLs)⁷⁸, in immature B-cell populations^{79,80} and during dendritic-cell development. Transforming growth factor- β (TGF- β) — a cytokine that is involved in the suppression of IgE CSR — induces the expression of ID2. ID2 binds to the transcription factor E2A and inhibits its interaction with DNA⁸¹. Recently, it was shown that Id2-deficient mice have increased serum levels of the T_H2-dependent IgG1 and IgE antibodies⁸². There was marked skewing to T_H2-cell phenotypes and a reduction of T_H1-inducing dendritic-cell populations. ID2 seems to have a dual effect on IgE CSR in B cells: first, it functions as a regulator of the T_H1/T_H2 balance through the regulation of



In humans, there are strong links between total IgE levels and markers that flank the cytokine cluster on chromosome 5 (5q31). This locus contains the genes encoding IL-13 and IL-4 receptor (IL-4R)⁸⁹. In particular, links have been reported between total IgE levels and polymorphisms in the 5' region, and between intron 3 of the *IL4* gene^{90,91} and the promoter region and coding sequence of the *IL13* gene⁹². High IgE levels have been associated with the polymorphism Arg551Gln in IL-4R α , which results in decreased binding of the cytosolic phosphatase SRC-homology-2-domain-containing protein tyrosine phosphatase 1 (SHP1), giving rise to a potential gain-of-function mutation with delayed termination of the IL-4 signal⁹³. High IgE levels have also been associated with polymorphisms in the 5' untranslated and coding regions of IL-13R α 1 (for example, Ala1398Gly)⁹⁴. Recently, linkage of IgE levels with asthma at the 5q21–5q33 region showed positive association with the D5S673 marker that includes the hepatitis A virus cellular receptor (**HAVCR1**) — a member of the T-cell immunoglobulin mucin (TIM) family — and **IL-12B**, a pro-inflammatory cytokine and inducer of B-cell proliferation⁹². Chromosome 13q14 also shows

Table 1 | **Mouse models of selective dysregulated IgE production**

Mouse deficiency	Pathway disrupted	IgE levels	References
Bcl6	Inhibition of Stat6 activation of C ϵ germline transcription	↑	76,77
Swap70	Unknown (potential DNA recombination mechanism)	↓	116
IL-4 IL-13 IL-4R	IL-4 or IL-13 signalling cascade	↓	131
Id2	Inhibition of E2A/C ϵ germline transcription	↑	12

Bcl6, B-cell lymphoma 6; IL, interleukin; R, receptor; Stat6, signal transducer and activator of transcription 6.

consistent linkage to atopy and total serum IgE levels, and fine mapping of this site recently attributed the linkage to several alleles in a single gene, *PHF11* — a PHD zinc-finger protein that might regulate transcription and is expressed by immune tissues and cells^{95,96}. A restriction fragment length polymorphism (RFLP) in the first intron of the *BCL6* gene has been associated with high IgE levels and atopy, indicating that it might interfere with the ability of BCL6 to compete with STAT6 for binding to the I ϵ promoter⁹⁷. Finally, linker for activation of T cells (**LAT**) knock-in mice with the mutation Tyr136Phe develop a lymphoproliferative disorder with a T_H2-cell bias and high levels of serum IgE. The LAT knock-in data indicate that point mutations in signal-transducing molecules, in addition to transcription factors, cytokines and cytokine receptors, could underlie atopy in some patients⁹⁸.

Allergy and the hygiene hypothesis. In the past few years, several epidemiological studies have documented an association between increased incidence of allergic diseases and improved hygiene. Early childhood exposure to antibiotics is associated with increased incidence of allergy. By contrast, large family size, early placement in day care, animal exposure early in life, exposure to hepatitis A virus and other infectious agents are associated with decreased incidence of allergic disease. The *TIM1* gene — the prototype of a new gene family that affects T_H1-cell development — functions as a receptor for hepatitis A virus⁹⁹. TIM1 is expressed by T_H2 cells and polymorphisms in TIM1 are associated with the development of T_H2-cell responses. These data, together with the observation that infection with hepatitis A virus protects against atopy¹⁰⁰, indicate that the effect of environmental agents and genetics in the development of atopy are related.

The hygiene hypothesis proposes that infection with viruses and bacteria and exposure to bacterial products, such as LPS, early in life promote the development of T_H1 cells which, in turn, downregulate the development of T_H2 cells and an IgE antibody response^{101,102}. However, this is inconsistent with the concomitant rise in T_H1-mediated diseases (for example, type 1 diabetes) with better hygiene, and with the observation that T_H1 cells exacerbate allergic inflammation in animal models of allergic disease. Recent data indicate that the protective effects of early infection lie in stimulating the emergence of regulatory T cells that include CD4⁺CD25⁺ T cells, which downregulate both T_H2- and T_H1-cell responses¹⁰³.

Parasitic diseases. Infestation with several parasites gives rise to a robust T_H2-cell response and an increased serum IgE level¹⁰⁴. T_H2-type cytokines and the induction of IgE synthesis are thought to be important in parasite immunity¹⁰⁵. Most of the IgE in parasitic disease is polyclonal and only a small fraction of it is directed against the invading pathogen. IgE could have a role in parasite immunity by arming mast cells and eosinophils to release the contents of their toxic granules after recognition of the parasite. The role of T_H2-type cytokines in parasitic diseases is illustrated by the case of infection with *Schistoma mansoni*, in which administration of IL-13-specific antibody inhibited the rise in serum IgE levels through the loss of C ϵ germline transcription¹⁰⁶. Interestingly, at least one parasite antigen derived from *Dirofilaria immitis* also acts directly on B cells as a CD40 agonist that synergizes with IL-4 to induce IgE class switching¹⁰⁷.

Despite their propensity to trigger T_H2-cell responses and IgE production, parasitic infections protect against the development of allergic diseases in humans. In an animal model, helminth-dependent blockade of allergen-specific IgE production and protection against allergic disease were shown to involve immunoregulatory mechanisms that include IL-10, because the inhibitory effects of helminth infection were reversed by IL-10-specific antibody (REFS 108,109). It has been suggested that parasitic infections, such as infection with bacteria and viruses, stimulate the production of regulatory T cells that exert their effect, in part, through IL-10, to block the production of allergen-specific IgE.

IgE immunodeficiencies

Several primary immunodeficiencies, which result from a single gene mutation, and several mice with targeted mutations of genes that are expressed by immune cells have abnormalities in IgE serum levels. These observations shed further light on the physiological mechanisms of regulation of IgE synthesis. Some of these mutations affect CSR to all immunoglobulin isotypes, whereas others selectively affect CSR to IgE.

Gene mutations that affect CSR to all classes. Several gene mutations result in a generalized decrease in all serum isotypes, other than IgM. These defects are often associated with a rise in the level of serum IgM owing to the inability of soluble IgM-positive B cells to undergo class switching to downstream isotypes¹¹⁰. These conditions are known as HIGM syndromes and they include mutations that affect CD40L (as in HIGM1) and CD40 (as in HIGM3). In addition, mutations of various elements of the molecular machinery that are involved in CSR — namely, AID (as in HIGM2) and uracil-DNA glycosylase (UNG) — have been classified as HIGM syndromes. Inactivation of members of the Ku70–Ku80–DNA-dependent protein kinase (DNA-PK) complex and DNA mismatch repair enzymes, for example, mutS homology 2 (MSH2)¹¹¹, also results in defective production of all immunoglobulins. Furthermore, mutations that affect the NF- κ B regulator inhibitor of NF- κ B kinase (**IKK γ** , also known

CPG MOTIFS

Hypomethylated DNA sequences that typically contain a purine-purine-C-G-pyrimidine-pyrimidine core hexamer. These motifs are suppressed in mammalian DNA, but are enriched in bacterial active DNA. The mammalian innate immune system is stimulated by CpG motifs through Toll-like receptor 9.

as NF- κ B essential modulator, NEMO)^{112,113}, and the NF- κ B-family members REL and p50 (REFS 16,114) also result in defective CSR, possibly because these gene products are crucial for C_H germline transcription and expression of AID.

Gene mutations that selectively affect CSR to IgE. As we have discussed, inactivation of the *Il4*, *Il13* and *Il4RA* genes in mice results in a selective decrease of CSR to IgE and, to a lesser extent, to IgG1. Polymorphisms in these genes in humans have been associated with selective increase of serum IgE levels in allergic individuals. Mice that are deficient in Swap70 — a component of the multiprotein complex Swap that is involved in CSR¹¹⁵ — have reduced total IgE serum levels, reduced IgE responses to immunization and impaired CD40-dependent IgE isotype switching *in vitro*¹¹⁶. A specific mechanism for the action of SWAP70 in IgE CSR has not been elucidated.

Human genetic disorders that result in elevated IgE levels include WAS, IPEX (immune dysregulation, polyendocrine enteropathy, X linked syndrome), Netherton's syndrome and hyper-IgE syndrome (TABLE 2). Human hyper-IgE syndrome is characterized by elevated IgE levels, bone and teeth abnormalities and susceptibility to infections, particularly with *Staphylococcus aureus* and *Aspergillus*¹¹⁷; however, the defective gene is not known. The gene mutated in patients with IPEX (and its mouse equivalent, the *scurfy* mutant) is the transcription factor **FOXP3**, which is essential for the development of CD4⁺CD25⁺ regulatory T cells¹¹⁸. The allergic manifestations in this syndrome indicate that CD4⁺CD25⁺ T cells regulate the production of T_H2-type cytokines and the synthesis of IgE. WAS is an X-linked immunodeficiency syndrome that is characterized by eczema, thrombocytopenia and T-cell immunodeficiency¹¹⁹. It is caused by mutations in the WAS protein (**WASP**). The fact that patients with WAS develop eczema and Wasp-deficient mice develop a colitis that is characterized by the predominance of T_H2 cells, indicate T_H2-cell predominance in this disease. This could be related to

the fact that T-cell receptor (TCR) signalling and immune-synapse formation are attenuated in T cells that lack WASP, as decreased intensity of TCR signalling promotes T_H2-cell development¹²⁰.

Loss of integrity of the epidermal layer results in epicutaneous sensitization to antigens and has also been shown to induce a strong T_H2-cell response. This might underlie the elevated IgE levels that are seen in Netherton's syndrome and Nga/c mice. Netherton's syndrome is due to deficiency of the skin-specific serine protease inhibitor Kazal type 5 (**SPINK5**) and results in ichthyosis of the skin¹²¹. The role of SPINK5 in the induction of increased IgE levels and the development of skin lesions is unknown at present. The specific gene defect in Nga/c mice is unknown, but these mice also develop severe eczema when housed under conventional conditions¹²².

Therapeutic approaches

The rapid progress in understanding the mechanisms of regulation of IgE synthesis has been brought to the clinic through the development of therapeutic interventions that are aimed at inhibiting the development of T_H2 cells and promoting the development of T_H1 cells to block IgE synthesis by B cells. So far, the only proven disease-modifying strategy in allergic disease is specific immunotherapy with whole antigens. This therapy is limited due to the associated risk of systemic adverse effects.

Newer approaches in the treatment of allergic disease have focused on altering the T_H1-cell versus T_H2-cell balance and these are briefly introduced. The use of bacteria-derived oligonucleotides containing CPG MOTIFS or immunostimulatory oligodeoxynucleotides to enhance T_H1-cell responses and suppress T_H2-cell immunity¹²³, or the use of fusion proteins of allergen and T_H1-promoting cytokines^{124,125} are two of these new approaches. The combined approach of immunization by traditional (whole antigen) or peptide immunotherapy with immunostimulatory oligodeoxynucleotides shows promise in treating allergic disease¹²⁶. Inhibition

Table 2 | Human genetic disorders affecting IgE regulation

Syndrome	Gene	Phenotype	IgE levels	References
Diseases that affect class-switch recombination to all immunoglobulin isotypes				
X-linked hyper-IgM type 1 syndrome	<i>CD40L</i>	B-cell immunodeficiency, susceptibility to opportunistic infections, low serum immunoglobulin levels, except IgM	↓	110
Hyper-IgM type 3 syndrome	<i>CD40</i>	Same as CD40L deficiency and bone abnormalities	↓	110
X-linked ectodermal dysplasia with immune deficiency	<i>NEMO</i>	Hypogammaglobulinaemia, deficient NK-cell and innate immunity, tooth abnormalities and reduced sweating	↓	112
UNG deficiency	<i>UNG</i>	Low serum immunoglobulin levels, except IgM	↓	110
Diseases that selectively affect class-switch recombination to IgE				
Netherton's disease	<i>SPINK5</i>	Ichthyosis	↑	121
Wiskott-Aldrich syndrome	<i>WASP</i>	Eczema, thrombocytopenia, immune deficiency	↑	119,120
Hyper-IgE syndrome	?	Susceptibility to infection with <i>Staphylococcus aureus</i> and fungus, bone and teeth abnormalities	↑	117
IPEX	<i>FOXP3</i>	Defective development of CD4 ⁺ CD25 ⁺ regulatory T cells	↑	118

IPEX, immune dysregulation, polyendocrine enteropathy, X linked syndrome; L, ligand; NEMO, nuclear factor- κ B essential modulator; NK, natural killer; UNG, uracil-DNA glycosylase; SPINK5, serine protease inhibitor, Kazal type 5; WASP, Wiskott-Aldrich syndrome protein.

of IgE class switching has also been explored through the administration of soluble IL-4R¹²⁷ and IFN- γ ¹²⁸, but these methods fail to block the increase in serum IgE levels due to the presence of existing IgE-secreting B cells. Clinical trials of CD23-specific antibody to inhibit C ϵ germline transcription in atopic patients are being initiated at present¹²⁹. As illustrated with soluble IL-4R and IFN- γ , therapies that inhibit IgE class switching might need to be combined with therapies that are aimed towards the elimination of already existing IgE-secreting B cells and/or the neutralization of IgE antibody. Treatment with humanized IgE-specific monoclonal antibody was recently shown to decrease allergic sensitivity to oral peanut challenge¹³⁰.

Conclusion

The increasing incidence of allergic diseases in societies with a high degree of socio-economic development is a pressing public-health problem. This has prompted physicians to advise parents to institute

major changes in the feeding of infants (for example, the avoidance of shellfish and peanuts, and has even prompted communities and airlines to ban the serving of peanuts in schools and airplanes). The marked increase in our understanding of IgE and its role in allergic diseases have led to therapies that selectively target allergen-specific IgE. New therapies with IgE-specific antibodies, which have been recently approved by the Food and Drug Administration, or immunostimulatory oligodeoxynucleotides are the beginning of what promises to be a series of therapeutic interventions that aim to eliminate IgE. However, IgE-specific antibody therapy is expensive, cumbersome and has potential long-term, undesirable effects. The ideal therapeutic agent will block IgE isotype switching in B cells without adverse effects, and will be inexpensive and easy to administer. Given the high incidence of morbidity and the cost of treatment of allergic diseases, there is a great imperative to develop such an agent.

1. Oettgen, H. C. & Geha, R. S. IgE regulation and roles in asthma pathogenesis. *J. Allergy Clin. Immunol.* **107**, 429–440 (2001).
2. Gauchat, J. F., Leberman, D. A., Coffman, R. L., Gascan, H. & de Vries, J. E. Structure and expression of germline ϵ transcripts in human B cells induced by interleukin 4 to switch to IgE production. *J. Exp. Med.* **172**, 463–473 (1990).
3. Jung, S., Rajewsky, K. & Radbruch, A. Shutdown of class switch recombination by deletion of a switch region control element. *Science* **259**, 984–987 (1993).
4. Muramatsu, M. *et al.* Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* **102**, 553–563 (2000).
- The authors report the first activation-induced cytidine deaminase (AID)-deficient mice and show the importance of AID in class-switch recombination (CSR) and somatic hypermutation of immunoglobulin variable regions.**
5. Revy, P. *et al.* Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2). *Cell* **102**, 565–575 (2000).
6. Muramatsu, M. *et al.* Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. *J. Biol. Chem.* **274**, 18470–18476 (1999).
7. Chaudhuri, J. *et al.* Transcription-targeted DNA deamination by the AID antibody diversification enzyme. *Nature* **422**, 726–730 (2003).
- The first evidence to show that, *in vitro*, AID deaminates single-stranded DNA but not double-stranded DNA (dsDNA), and that dsDNA can only be deaminated if the reaction is coupled with transcription.**
8. Stutz, A. M. & Woisetschlager, M. Functional synergism of STAT6 with either NF- κ B or PU.1 to mediate IL-4-induced activation of IgE germline gene transcription. *J. Immunol.* **163**, 4383–4391 (1999).
9. Thienes, C. P. *et al.* The transcription factor B cell-specific activator protein (BSAP) enhances both IL-4- and CD40-mediated activation of the human ϵ germline promoter. *J. Immunol.* **158**, 5874–5882 (1997).
10. Delphin, S. & Stavnezer, J. Characterization of an interleukin 4 (IL-4) responsive region in the immunoglobulin heavy chain germline ϵ promoter: regulation by NF-IL-4, a C/EBP family member and NF- κ B/p50. *J. Exp. Med.* **181**, 181–192 (1995).
11. Mao, C. S. & Stavnezer, J. Differential regulation of mouse germline Igy1 and ϵ promoters by IL-4 and CD40. *J. Immunol.* **167**, 1522–1534 (2001).
12. Sugai, M. *et al.* Essential role of Id2 in negative regulation of IgE class switching. *Nature Immunol.* **4**, 25–30 (2003).
- The first paper to show that transforming growth factor- β 1 (TGF- β 1)-mediated induction of inhibitor of DNA binding 2 (ID2) is responsible for suppressing the production of IgE.**
13. de Vries, J. E., Punnonen, J., Cocks, B. G., de Waal Malefyt, R. & Aversa, G. Regulation of the human IgE response by IL-4 and IL-13. *Res. Immunol.* **144**, 597–601 (1993).
14. Grewal, I. S. & Flavell, R. A. The role of CD40 ligand in costimulation and T-cell activation. *Immunol. Rev.* **153**, 85–106 (1996).
15. Monticelli, S., De Monte, L. & Vercelli, D. Molecular regulation of IgE switching: let's walk hand in hand. *Allergy* **53**, 6–8 (1998).
16. Sha, W. C., Liou, H. C., Tuomanen, E. I. & Baltimore, D. Targeted disruption of the p50 subunit of NF- κ B leads to multifocal defects in immune responses. *Cell* **80**, 321–330 (1995).
17. Chen, C. L. *et al.* RAG2^{-/-}, I κ B- α ^{-/-} chimeras display a psoriasisform skin disease. *J. Invest. Dermatol.* **115**, 1124–1133 (2000).
18. Qiu, G. & Stavnezer, J. Overexpression of BSAP/Pax-5 inhibits switching to IgA and enhances switching to IgE in the I.29 μ B cell line. *J. Immunol.* **161**, 2906–2918 (1998).
19. Messner, B., Stutz, A. M., Albrecht, B., Peiritsch, S. & Woisetschlager, M. Cooperation of binding sites for STAT6 and NF- κ B/rel in the IL-4-induced up-regulation of the human IgE germline promoter. *J. Immunol.* **159**, 3330–3337 (1997).
- The authors show that ligation of CD40 increases the production of C ϵ germline transcripts through the binding of nuclear factor- κ B (NF- κ B)-REL to two tandem NF- κ B sites in the C ϵ germline promoter.**
20. Cheng, G. *et al.* Involvement of CRAF1, a relative of TRAF, in CD40 signaling. *Science* **267**, 1494–1498 (1995).
21. Ishida, T. *et al.* Identification of TRAF6, a novel tumor necrosis factor receptor-associated factor protein that mediates signaling from an amino-terminal domain of the CD40 cytoplasmic region. *J. Biol. Chem.* **271**, 28745–28748 (1996).
22. Ishida, T. K. *et al.* TRAF5, a novel tumor necrosis factor receptor-associated factor family protein, mediates CD40 signaling. *Proc. Natl. Acad. Sci. USA* **93**, 9437–9442 (1996).
23. Pullen, S. S. *et al.* High-affinity interactions of tumor necrosis factor receptor-associated factors (TRAFs) and CD40 require TRAF trimerization and CD40 multimerization. *Biochemistry* **38**, 10168–10177 (1999).
24. Hostager, B. S., Catlett, I. M. & Bishop, G. A. Recruitment of CD40 and tumor necrosis factor receptor-associated factors 2 and 3 to membrane microdomains during CD40 signaling. *J. Biol. Chem.* **275**, 15392–1538 (2000).
25. Iciek, L. A., Delphin, S. A. & Stavnezer, J. CD40 cross-linking induces IgE germline transcripts in B cells via activation of NF- κ B: synergy with IL-4 induction. *J. Immunol.* **158**, 4769–4779 (1997).
26. Basaki, Y., Ikizawa, K., Kajiwara, K. & Yanagihara, Y. CD40-mediated tumor necrosis factor receptor-associated factor 3 signaling upregulates IL-4-induced germline C ϵ transcription in a human B cell line. *Arch. Biochem. Biophys.* **405**, 199–204 (2002).
27. Ahonen, C. *et al.* The CD40–TRAF6 axis controls affinity maturation and the generation of long-lived plasma cells. *Nature Immunol.* **3**, 451–456 (2002).
28. Jabara, H. *et al.* The binding site for TRAF2 and TRAF3 but not for TRAF6 is essential for CD40-mediated immunoglobulin class switching. *Immunity* **17**, 265–276 (2002).
- This paper shows that disruption of the binding of tumour-necrosis factor (TNF)-receptor-associated factor 2 (TRAF2) and TRAF3 to CD40 blocks CD40-mediated isotype switching and activation of primary mouse B cells. See also reference 27.**
29. Faris, M., Gaskin, F., Geha, R. S. & Fu, S. M. Tyrosine phosphorylation defines a unique transduction pathway in human B cells mediated via CD40. *Trans. Assoc. Am. Physicians* **106**, 187–195 (1993).
30. Ren, C. L., Morio, T., Fu, S. M. & Geha, R. S. Signal transduction via CD40 involves activation of lyn kinase and phosphatidylinositol-3-kinase, and phosphorylation of phospholipase C γ 2. *J. Exp. Med.* **179**, 673–680 (1994).
31. Zhang, K. *et al.* CD40-mediated p38 mitogen-activated protein kinase activation is required for immunoglobulin class switch recombination to IgE. *J. Allergy Clin. Immunol.* **110**, 421–428 (2002).
32. Jabara, H. H., Ahern, D. J., Vercelli, D. & Geha, R. S. Hydrocortisone and IL-4 induce IgE isotype switching in human B cells. *J. Immunol.* **147**, 1557–1560 (1991).
33. Jabara, H. H., Brodeur, S. R. & Geha, R. S. Glucocorticoids upregulate CD40 ligand expression and induce CD40L-dependent immunoglobulin isotype switching. *J. Clin. Invest.* **107**, 371–378 (2001).
34. Beato, M., Herrlich, P. & Schutz, G. Steroid hormone receptors: many actors in search of a plot. *Cell* **83**, 851–857 (1995).
35. Litinskiy, M. B. *et al.* DCs induce CD40-independent immunoglobulin class switching through BLYS and APRIL. *Nature Immunol.* **3**, 822–829 (2002).
- This paper shows a new CD40-independent pathway for IgE CSR that is induced by BLYS (B-lymphocyte stimulator) and APRIL — new members of the TNF family — in interleukin-4 (IL-4)-stimulated B cells.**
36. Masters, S. A. *et al.* Interaction of the TNF homologues BLYS and APRIL with the TNF receptor homologues BCMA and TACI. *Curr. Biol.* **10**, 785–788 (2000).
37. Yan, M. *et al.* Identification of a novel receptor for B lymphocyte stimulator that is mutated in a mouse strain with severe B cell deficiency. *Curr. Biol.* **11**, 1547–1552 (2001).
38. Hatzoglou, A. *et al.* TNF receptor family member BCMA (B cell maturation) associates with TNF receptor-associated factor (TRAF) 1, TRAF2, and TRAF3 and activates NF- κ B, elk-1, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase. *J. Immunol.* **165**, 1322–1330 (2000).
39. von Bulow, G. U. *et al.* Molecular cloning and functional characterization of murine transmembrane activator and CAML interactor (TACI) with chromosomal localization in human and mouse. *Mamm. Genome* **11**, 628–632 (2000).

40. Xia, X. Z. *et al.* TACI is a TRAF-interacting receptor for TALL-1, a tumor necrosis factor family member involved in B cell regulation. *J. Exp. Med.* **192**, 137–143 (2000).
41. Claudio, E., Brown, K., Park, S., Wang, H. & Siebenlist, U. BAFF-induced NEMO-independent processing of NF- κ B2 in maturing B cells. *Nature Immunol.* **3**, 958–965 (2002).
42. Stein, J. V. *et al.* APRIL modulates B and T cell immunity. *J. Clin. Invest.* **109**, 1587–1598 (2002).
43. Jabara, H. H. *et al.* Induction of germ-line and mature Ce transcripts in human B cells stimulated with rIL-4 and EBV. *J. Immunol.* **145**, 3468–3473 (1990).
44. Uchida, J. *et al.* Mimicry of CD40 signals by Epstein–Barr virus LMP1 in B lymphocyte responses. *Science* **286**, 300–303 (1999).
45. Brodeur, S. R., Cheng, G., Baltimore, D. & Thorley-Lawson, D. A. Localization of the major NF- κ B-activating site and the sole TRAF3 binding site of LMP-1 defines two distinct signaling motifs. *J. Biol. Chem.* **272**, 19777–19784 (1997).
46. Fries, K. L., Miller, W. E. & Raab-Traub, N. The A20 protein interacts with the Epstein–Barr virus latent membrane protein 1 (LMP1) and alters the LMP1–TRAF1–TRADD complex. *Virology* **264**, 159–166 (1999).
47. Kieser, A., Kaiser, C. & Hammerschmidt, W. LMP1 signal transduction differs substantially from TNF receptor 1 signaling in the molecular functions of TRADD and TRAF2. *EMBO J.* **18**, 2511–2521 (1999).
48. Kusada-Funakoshi, M., Sasaki, J., Takada, Y., Soji, T. & Arakawa, K. Evidence that C4b-binding protein (proline-rich protein) is synthesized by hepatocytes. *Biochem. Med. Metab. Biol.* **45**, 350–354 (1991).
49. Lappin, D. F., Birnie, G. D. & Whaley, K. Modulation by interferons of the expression of monocyte complement genes. *Biochem. J.* **268**, 387–392 (1990).
50. Moffat, G. J., Vik, D. P., Noack, D. & Tack, B. F. Complete structure of the murine C4b-binding protein gene and regulation of its expression by dexamethasone. *J. Biol. Chem.* **267**, 20400–20406 (1992).
51. Moffat, G. J. & Tack, B. F. Regulation of C4b-binding protein gene expression by the acute-phase mediators tumor necrosis factor- α , interleukin-6, and interleukin-1. *Biochemistry* **31**, 12376–12384 (1992).
52. Blom, A. M., Kask, L. & Dahlback, B. CCP1–4 of the C4b-binding protein α -chain are required for factor I mediated cleavage of complement factor C3b. *Mol. Immunol.* **39**, 547–556 (2003).
53. Brodeur, S. R. *et al.* C4b-binding protein (C4BP) activates B cells through the CD40 receptor. *Immunity* **18**, 837–848 (2003).
- The first paper showing complement component 4 binding protein (C4BP) to be an inducer of B-cell activation and IL-4-dependent IgE class switching.**
54. Xu, L. & Rothman, P. IFN- γ represses ϵ germline transcription and subsequently down-regulates switch recombination to ϵ . *Int. Immunol.* **6**, 515–521 (1994).
55. Parrish-Novak, J., Foster, D. C., Holly, R. D. & Clegg, C. H. Interleukin-21 and the IL-21 receptor: novel effectors of NK and T cell responses. *J. Leukoc. Biol.* **72**, 856–863 (2002).
56. Ozaki, K. *et al.* A critical role for IL-21 in regulating immunoglobulin production. *Science* **298**, 1630–1634 (2002).
57. Suto, A. *et al.* Interleukin 21 prevents antigen-induced IgE production by inhibiting germ line Ce transcription of IL-4-stimulated B cells. *Blood* **100**, 4565–4573 (2002).
58. Gracie, J. A., Robertson, S. E. & McInnes, I. B. Interleukin-18. *J. Leukoc. Biol.* **73**, 213–224 (2003).
59. Hoshino, T., Yagita, H., Ortaldo, J. R., Witrout, R. H. & Young, H. A. *In vivo* administration of IL-18 can induce IgE production through T_H2 cytokine induction and up-regulation of CD40 ligand (CD154) expression on CD4⁺ T cells. *Eur. J. Immunol.* **30**, 1998–2006 (2000).
60. Hoshino, T. *et al.* Cutting edge: IL-18-transgenic mice: *in vivo* evidence of a broad role for IL-18 in modulating immune function. *J. Immunol.* **166**, 7014–7018 (2001).
61. Rush, J. S., Hasbold, J. & Hodgkin, P. D. Cross-linking surface Ig delays CD40 ligand- and IL-4-induced B cell Ig class switching and reveals evidence for independent regulation of B cell proliferation and differentiation. *J. Immunol.* **168**, 2676–2682 (2002).
62. Yamada, T., Zhu, D., Saxon, A. & Zhang, K. CD45 controls interleukin-4-mediated IgE class switch recombination in human B cells through its function as a Janus kinase phosphatase. *J. Biol. Chem.* **277**, 28830–28835 (2002).
63. Zhou, C., Saxon, A. & Zhang, K. Human activation-induced cytidine deaminase is induced by IL-4 and negatively regulated by CD45: implication of CD45 as a Janus kinase phosphatase in antibody diversification. *J. Immunol.* **170**, 1887–1893 (2003).
64. Arimura, Y. *et al.* CD45 is required for CD40-induced inhibition of DNA synthesis and regulation of c-Jun NH2-terminal kinase and p38 in BAL-17 B cells. *J. Biol. Chem.* **276**, 8550–8556 (2001).
65. Loh, R. K. *et al.* Role of protein tyrosine kinases and phosphatases in isotype switching: crosslinking CD45 to CD40 inhibits IgE isotype switching in human B cells. *Immunol. Lett.* **45**, 99–106 (1995).
66. Pioli, C., Gatta, L., Ubaldi, V. & Doria, G. Inhibition of IgG1 and IgE production by stimulation of the B cell CTLA-4 receptor. *J. Immunol.* **165**, 5530–5536 (2000).
67. Sherr, E., Macy, E., Kimata, H., Gilly, M. & Saxon, A. Binding the low affinity Fc ϵ R on B cells suppresses ongoing human IgE synthesis. *J. Immunol.* **142**, 481–489 (1989).
68. Yu, P., Kosco-Vilbois, M., Richards, M., Kohler, G. & Lamers, M. C. Negative feedback regulation of IgE synthesis by murine CD23. *Nature* **369**, 753–756 (1994).
69. Payet, M. & Conrad, D. H. IgE regulation in CD23 knockout and transgenic mice. *Allergy* **54**, 1125–1129 (1999).
70. Saxon, A., Ke, Z., Bahati, L. & Stevens, R. H. Soluble CD23 containing B cell supernatants induce IgE from peripheral blood B-lymphocytes and costimulate with interleukin-4 in induction of IgE. *J. Allergy Clin. Immunol.* **86**, 333–344 (1990).
71. Christie, G. *et al.* IgE secretion is attenuated by an inhibitor of proteolytic processing of CD23 (Fc ϵ RII). *Eur. J. Immunol.* **27**, 3228–3235 (1997).
72. Schulz, O. *et al.* Cleavage of the low-affinity receptor for human IgE (CD23) by a mite cysteine protease: nature of the cleaved fragment in relation to the structure and function of CD23. *Eur. J. Immunol.* **27**, 584–588 (1997).
73. Gough, L., Schulz, O., Sewell, H. F. & Shakib, F. The cysteine protease activity of the major dust mite allergen Derp1 selectively enhances the immunoglobulin E antibody response. *J. Exp. Med.* **190**, 1897–1902 (1999).
74. Mayer, R. J. *et al.* Inhibition of CD23 processing correlates with inhibition of IL-4-stimulated IgE production in human PBL and hu-PBL-reconstituted SCID mice. *Clin. Exp. Allergy* **30**, 719–727 (2000).
75. Harris, M. B. *et al.* Transcriptional repression of Stat6-dependent interleukin-4-induced genes by BCL-6: specific regulation of *le* transcription and immunoglobulin E switching. *Mol. Cell. Biol.* **19**, 7264–7275 (1999).
- This paper shows that the B-cell lymphoma 6 (BCL6) inhibitory effect on IL-4-induced IgE production is due to its binding to the signal transducer and activator of transcription 6 (STAT6) site in the *le* promoter.**
76. Takeda, K. *et al.* Essential role of Stat6 in IL-4 signalling. *Nature* **380**, 627–630 (1996).
77. Dent, A. L., Shaffer, A. L., Yu, X., Allman, D. & Staudt, L. M. Control of inflammation, cytokine expression, and germinal center formation by BCL-6. *Science* **276**, 589–592 (1997).
78. Ishiguro, A. *et al.* Expression of Id2 and Id3 mRNA in human lymphocytes. *Leuk. Res.* **19**, 989–996 (1995).
79. Becker-Herman, S., Lanther, F. & Shachar, I. Id2 negatively regulates B cell differentiation in the spleen. *J. Immunol.* **168**, 5507–5513 (2002).
80. Wilson, R. B. *et al.* Repression of immunoglobulin enhancers by the helix-loop-helix protein Id: implications for B-lymphoid-cell development. *Mol. Cell. Biol.* **11**, 6185–6191 (1991).
81. Sun, X. H., Copeland, N. G., Jenkins, N. A. & Baltimore, D. Id proteins Id1 and Id2 selectively inhibit DNA binding by one class of helix-loop-helix proteins. *Mol. Cell. Biol.* **11**, 5603–5611 (1991).
82. Kusunoki, T. *et al.* T_H2 dominance and defective development of a CD8⁺ dendritic cell subset in Id2-deficient mice. *J. Allergy Clin. Immunol.* **111**, 136–142 (2003).
83. Ouyang, W. *et al.* Stat6-independent GATA-3 autoactivation directs IL-4-independent T_H2 development and commitment. *Immunity* **12**, 27–37 (2000).
84. Usui, T., Nishikomori, R., Kitani, A. & Strober, W. GATA-3 suppresses T_H1 development by downregulation of Stat4 and not through effects on IL-12R β 2 chain or T-bet. *Immunity* **18**, 415–428 (2003).
85. Robinson, D. S. & Lloyd, C. M. Asthma: T-bet — a master controller? *Curr. Biol.* **12**, R322–R324 (2002).
86. Szabo, S. J. *et al.* A novel transcription factor, T-bet, directs T_H1 lineage commitment. *Cell* **100**, 655–669 (2000).
87. Ranger, A. M., Oukka, M., Rengarajan, J. & Glimcher, L. H. Inhibitory function of two NFAT family members in lymphoid homeostasis and T_H2 development. *Immunity* **9**, 627–635 (1998).
88. Viola, J. P., Kiani, A., Bozza, P. T. & Rao, A. Regulation of allergic inflammation and eosinophil recruitment in mice lacking the transcription factor NFAT1: role of interleukin-4 (IL-4) and IL-5. *Blood* **91**, 2223–2230 (1998).
89. Shirakawa, I. *et al.* Atopy and asthma: genetic variants of IL-4 and IL-13 signalling. *Immunol. Today* **21**, 60–64 (2000).
90. Marsh, D. G. *et al.* Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* **264**, 1152–1156 (1994).
91. Rosenwasser, L. J. Genetics of atopy and asthma: promoter-based candidate gene studies for IL-4. *Int. Arch. Allergy Immunol.* **113**, 61–64 (1997).
92. Graves, P. E. *et al.* A cluster of seven tightly linked polymorphisms in the IL-13 gene is associated with total serum IgE levels in three populations of white children. *J. Allergy Clin. Immunol.* **105**, 506–513 (2000).
93. Hershey, G. K., Friedrich, M. F., Esswein, L. A., Thomas, M. L. & Chatila, T. A. The association of atopy with a gain-of-function mutation in the α subunit of the interleukin-4 receptor. *N. Engl. J. Med.* **337**, 1720–1725 (1997).
94. Heinzmann, A. *et al.* Genetic variants of IL-13 signalling and human asthma and atopy. *Hum. Mol. Genet.* **9**, 549–559 (2000).
95. Ahmadi, K. R. *et al.* Novel association suggests multiple independent QTLs within chromosome 5q21–33 region control variation in total humans IgE levels. *Genes Immun.* **4**, 289–297 (2003).
96. Zhang, Y. *et al.* Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. *Nature Genet.* **34**, 181–186 (2003).
97. Adra, C. N. *et al.* Variants of B cell lymphoma 6 (BCL6) and marked atopy. *Clin. Genet.* **54**, 362–364 (1998).
98. Zhang, W., Sloan-Lancaster, J., Kitchen, J., Triple, R. P. & Samelson, L. E. LAT: the ZAP-70 tyrosine kinase substrate that links T cell receptor to cellular activation. *Cell* **92**, 83–92 (1998).
99. McIntire, J. J. *et al.* Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. *Nature Immunol.* **2**, 1109–1116 (2001).
- The authors identified in mice, Tapr, a gene that controls the development of airway hyperreactivity, and cloned the T-cell immunoglobulin mucin (Tim) family — a new gene family and homologue of the human hepatitis A virus receptor that co-segregated with Tapr.**
100. Locarnini, S. A virological perspective on the need for vaccination. *J. Viral Hepat.* **7**, S5–S6 (2000).
101. Holla, A. D., Roy, S. R. & Liu, A. H. Endotoxin, atopy and asthma. *Curr. Opin. Allergy Clin. Immunol.* **2**, 141–145 (2002).
102. Kemp, A. & Bjorksten, B. Immune deviation and the hygiene hypothesis: a review of the epidemiological evidence. *Pediatr. Allergy Immunol.* **14**, 74–80 (2003).
103. Wills-Karp, M., Santeliz, J. & Karp, C. L. The germless theory of allergic disease: revisiting the hygiene hypothesis. *Nature Rev. Immunol.* **1**, 69–75 (2001).
104. Svetic, A. *et al.* A primary intestinal helminth infection rapidly induces a gut-associated elevation of T_H2-associated cytokines and IL-3. *J. Immunol.* **150**, 3434–3441 (1993).
105. Yazdanbakhsh, M., Krensmeyer, P. G. & van Ree, R. Allergy, parasites, and the hygiene hypothesis. *Science* **296**, 490–494 (2002).
106. Chiaramonte, M. G. *et al.* IL-13 is a key regulatory cytokine for T_H2 cell-mediated pulmonary granuloma formation and IgE responses induced by *Schistosoma mansoni* eggs. *J. Immunol.* **162**, 920–930 (1999).
107. Imai, S., Tezuka, H., Furuhashi, Y., Muto, R. & Fujita, K. A factor of inducing IgE from a filarial parasite is an agonist of human CD40. *J. Biol. Chem.* **276**, 46118–46124 (2001).
108. Ferreira, M. B., da Silva, S. L. & Carlos, A. G. Atopy and helminths. *Allerg. Immunol. (Paris)* **34**, 10–12 (2002).
109. Tezuka, H., Imai, S., Muto, R., Furuhashi, Y. & Fujita, K. Recombinant *Dirofilaria immitis* polypeptide that stimulates murine B cells to produce nonspecific polyclonal immunoglobulin E antibody. *Infect. Immun.* **70**, 1235–1244 (2002).
110. Durandy, A. Hyper-IgM syndromes: a model for studying the regulation of class switch recombination and somatic hypermutation generation. *Biochem. Soc. Trans.* **30**, 815–818 (2002).
111. Manis, J. P., Tian, M. & Alt, F. W. Mechanism and control of class-switch recombination. *Trends Immunol.* **23**, 31–39 (2002).
112. Jain, A. *et al.* Specific missense mutations in NEMO result in hyper-IgM syndrome with hypohydrotic ectodermal dysplasia. *Nature Immunol.* **2**, 223–228 (2001).
113. Orange, J. S. *et al.* Deficient natural killer cell cytotoxicity in patients with IKK- γ /NEMO mutations. *J. Clin. Invest.* **109**, 1501–1509 (2002).
114. Hilliard, B. A. *et al.* Critical roles of c-Rel in autoimmune inflammation and helper T cell differentiation. *J. Clin. Invest.* **110**, 843–850 (2002).
115. Borggrete, T., Wabl, M., Akhmedov, A. T. & Jessberger, R. A B-cell-specific DNA recombination complex. *J. Biol. Chem.* **273**, 17025–17035 (1998).
116. Borggrete, T., Keshavarzi, S., Gross, B., Wabl, M. & Jessberger, R. Impaired IgE response in SWAP-70-deficient mice. *Eur. J. Immunol.* **31**, 2467–2475 (2001).
117. Buckley, R. H. The hyper-IgE syndrome. *Clin. Rev. Allergy Immunol.* **20**, 139–154 (2001).
118. Bennett, C. L. *et al.* A rare polyadenylation signal mutation of the FOXP3 gene (AAUAAA→AAUGAA) leads to the IPEX syndrome. *Immunogenetics* **53**, 435–439 (2001).

119. Ramesh, N., Anton, I. M., Martinez-Quiles, N. & Geha, R. S. Waltzing with WASP. *Trends Cell Biol.* **9**, 15–19 (1999).
120. Anton, I. M. *et al.* WIP deficiency reveals a differential role for WIP and the actin cytoskeleton in T and B cell activation. *Immunity* **16**, 193–204 (2002).
121. Chavanas, S. *et al.* Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nature Genet.* **25**, 141–142 (2000).
122. Matsuda, H. *et al.* Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *Int. Immunol.* **9**, 461–466 (1997).
123. Sur, S. *et al.* Long term prevention of allergic lung inflammation in a mouse model of asthma by CpG oligodeoxynucleotides. *J. Immunol.* **162**, 6284–6293 (1999).
124. Muller, U. *et al.* Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J. Allergy Clin. Immunol.* **101**, 747–754 (1998).
125. Rolland, J. M., Douglass, J. & O'Hehir, R. E. Allergen immunotherapy: current and new therapeutic strategies. *Expert Opin. Investig. Drugs* **9**, 515–527 (2000).
126. Horner, A. A. & Raz, E. Immunostimulatory sequence oligodeoxynucleotide-based vaccination and immunomodulation: two unique but complementary strategies for the treatment of allergic diseases. *J. Allergy Clin. Immunol.* **110**, 706–712 (2002).
127. Sato, T. A. *et al.* Recombinant soluble murine IL-4 receptor can inhibit or enhance IgE responses *in vivo*. *J. Immunol.* **150**, 2717–2723 (1993).
128. Boguniewicz, M. *et al.* Recombinant γ -interferon in treatment of patients with atopic dermatitis and elevated IgE levels. *Am. J. Med.* **88**, 365–370 (1990).
129. Yabuuchi, S., Nakamura, T., Kloetzer, W. S. & Reiff, M. E. Anti-CD23 monoclonal antibody inhibits germ-line C ϵ transcription in B cells. *Int. Immunopharmacol.* **2**, 453–461 (2002).
130. Leung, D. Y. *et al.* Effect of anti-IgE therapy in patients with peanut allergy. *N. Engl. J. Med.* **348**, 986–993 (2003).
This clinical trial of subcutaneous administration of IgE-specific monoclonal antibody (TNX-901) was shown to increase the threshold of sensitivity to oral peanut challenge.
131. Barner, M., Mohrs, M., Brombacher, F. & Kopf, M. Differences between IL-4R α -deficient and IL-4-deficient mice reveal a role for IL-13 in the regulation of T_H2 responses. *Curr. Biol.* **8**, 669–672 (1998).

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