



Dossier: Allergy

Regulation of IgE homeostasis, and the identification of potential targets for therapeutic intervention

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Abstract

Atopic allergies have increased during the past 20–30 years in frequency quite dramatically and in many countries have reached almost epidemic proportions. Allergies have thereby become one of the major medical issues of the western world. Immunoglobulin E (IgE) is here a central player. IgE is the Ig class that is present in the lowest concentration in human plasma. IgG is, for example, 10 000 to 1 million times more abundant than IgE. However, despite of its low plasma levels IgE is a very important inducer of inflammation, due to its interaction with high-affinity receptors on mast cell and basophils. IgE has been conserved as a single active gene in all placental mammals studied, and the expression of this gene is under a very stringent control, most likely due to its very potent inflammatory characteristics. IgE expression is being regulated at many levels: by cytokines, switch region length, positive and negatively acting transcription factors and suppressors of cytokine signaling (SOCS). In addition, the plasma half-life differs markedly for IgG and IgE, with 21 and 2.5 days, respectively. This review summarizes the rapid progress in our understanding of the complex network of regulatory mechanisms acting on IgE and also how this new information may help us in our efforts to control IgE-mediated inflammatory conditions.

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1. Introduction

Placental mammals generally express five immunoglobulin classes, IgM, IgD, IgG, IgE and IgA. The only known exception is rabbit, which appears to lack a functional gene for IgD [1]. Of these five Ig classes IgG and IgE were the last two to appear as separate immunoglobulin classes during vertebrate evolution. Both of them originate from a gene duplication that most likely occurred during early mammalian evolution, 215–350 million years ago [2–5]. The duplicated genes diversified and gave rise to two separate Ig classes with very different biological characteristics and regulation, IgG and IgE. IgG did become the dominant plasma Ig class and is in placental mammals frequently present as several isotypes.

IgG concentrations in human plasma are normally in the range of 8–16 mg/ml. IgE has, in contrast, been conserved as a single active gene [3]. The expression of this gene is under a very stringent control, most likely due to its very potent inflammatory characteristics. IgE is usually present in very low concentrations, in the range of 1–400 ng/ml in non-atopic human. IgE was therefore the last Ig class to be isolated and characterized [6–9].

Similar to humans, most laboratory strains of rat and mice have very low plasma levels of IgE, below 200 ng/ml. There are, however, exceptions. The rat strain Brown Norway has levels from 200 ng to several µg/ml. We have actually observed animals with up to 100 µg of IgE/ml serum [10]. Among mice, the Balb/c strain is also a high IgE responder with IgE levels in the µg range. However, the absolute majority of laboratory strains have very low levels of IgE, and some strains, like the mouse SJA/9 strain, have almost no detectable IgE [11–13]. IgE levels in the range of 1–400 ng were

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therefore considered the general rule for domestic animal populations. However, recent studies on both dogs and horse have shown that some domestic animals may have very high plasma IgE levels. In a panel of almost 100 adult dogs the levels of IgE were found to range between 1 and 41 $\mu\text{g/ml}$ serum, which is almost 100 times the levels seen in non-atopic humans [14]. Horses have even higher IgE levels, a mean of approximately 100 $\mu\text{g/ml}$ has been observed in 26 Icelandic horses [15]. However, in humans IgE levels in the range of 5–100 $\mu\text{g/ml}$ are almost only seen in heavily parasite-infested persons and in humans with genetic disorders like Wiskott–Aldrich syndrome (WAS) and hyper-IgE syndrome [16–18].

Infections and other environmental factors as well as gene variants within the population seem to very strongly affect plasma IgE levels. IgE levels therefore vary markedly between individuals and also in the same individual over time. Plasma IgE levels are largely set by the regulation of gene expression and protein stability, which has been shown to depend on a large number of regulatory factors.

Following the V–D–J and V–J rearrangements of the heavy chain and the light chains, respectively, all B cells initially express IgM. While retaining their antigen specificity the B cell can later switch to a different isotype, dependent on appropriate stimulus. This second step, which is tightly regulated by cytokines, is known as class switch recombination (CSR). The cytokines determine the switching frequency by binding to specific receptors on the surface of the B cell. Signaling through the various cytokine receptors leads in turn to the activation of positively acting transcription factors, which relocate into the nucleus and activate promoters upstream of the switch region. Switch regions are highly repetitive sequences, which depending on the isotype they control, vary from approximately 1–12 kb in length. They are found at 5' (upstream) of all the different constant regions that are involved in CSR, i.e. all isotypes except IgD (Fig. 1). A number

of transcription factors that interact sequence specifically with and activate the epsilon switch promoter have been identified, namely STAT6, NF κ B, Pax5, Pu1, C/EBP, Ap1 and E2A. Two transcriptional repressors that are of importance in determining total IgE levels have also been described, namely Id2 and Bcl6. Different cytokines activate these different switch promoters and thereby determine the amount of the respective isotype being produced. For IgE, the important cytokines are IL-4 and IL-13.

However, there are also additional levels of regulation involved. The much shorter half-life of IgE, compared to IgG, also results in a much more rapid turnover of IgE and thereby in reduced plasma levels. In addition, cytokines also regulates the type of immune response mounted upon environmental challenge. IL-18, IL-25, IL-33, IL-15, TSLP and possibly also IL-3 may here be of key importance for setting the cytokine environment promoting a so-called TH2 response. The TH2 response results then in elevated IL-4 and IL-13 levels and increased IgE production.

In the following sections, I will describe more in detail various levels of regulation, the factors involved and how this information may affect our chances to control IgE-mediated inflammatory conditions.

2. Switch regions and the DNA rearrangement

Switch regions (S) are long simple repetitive sequences of 1–12 kb in length, located 1–5 kb immediately upstream of each constant region (CH) gene. The S regions are built from short 5–7 nt G-rich repeats, predominantly GAGCT, GGGGT and GGGCT, which are organized in larger blocks of 20–80 bp in length [19]. These blocks are in turn repeated a number of times to build the entire switch region [20]. The importance of the switch region has been well-documented in experimental systems. For example, the deletion of the μ

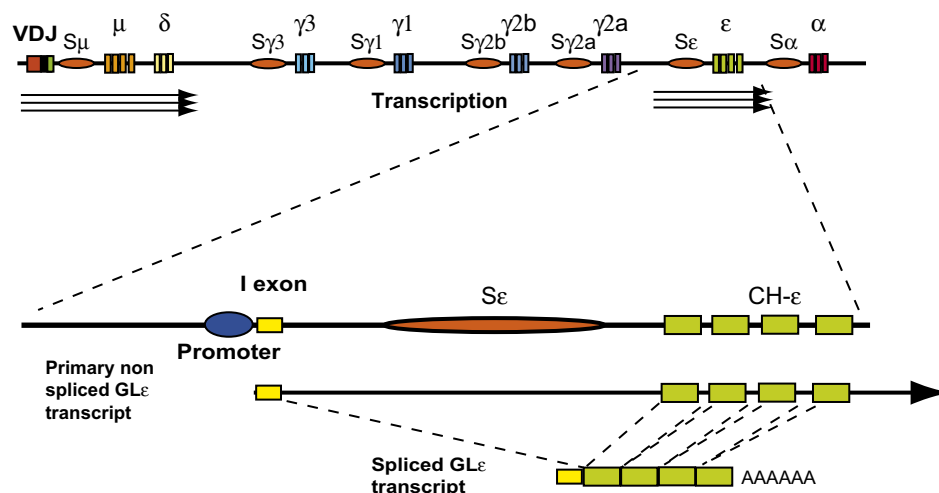


Fig. 1. The mouse immunoglobulin heavy chain locus. The top of the figure shows a schematic figure of the rearranged heavy chain locus with a functional VDJ joining. The individual heavy chain gene is depicted as individual block in different colors, the μ chain gene in orange, the different IgG isotypes in different shades of blue, the IgE ϵ chain gene in green and the IgA α chain gene in red. In front of all constant regions genes, except IgD, is a switch region positioned, shown in orange. Transcription preceding a CSR to IgE is also shown in the top panel. The lower panel of the figure shows an enlarged picture of the ϵ chain locus including the ϵ GL promoter and the ϵ I exon, as well as the primary and the spliced transcripts originating from this promoter.

switch region leads to a decrease in switch recombination, although not to its total absence [21,22]. This latter finding indicates that regions outside the switch region also are of importance for CSR. The DNA rearrangement during CSR is not site specific but can occur at any site over the entire length of the switch region, and no clear consensus sites for CSRs have been identified. This is marked in contrast to V–D–J rearrangements and RNA splicing, which occur at site-specific consensus sites, within a few nucleotides or at a specific nucleotide, respectively.

How is then CSR regulated? Transcription of the un-rearranged un-switched gene through so-called germ line transcripts (GL), which lack a VDJ region, has here been shown to be essential for CSR [23,24]. The ϵ GL-transcript originates from a promoter located approximately 2 kb upstream of the S region. The transcription starts with a short 240–245 bp exon (or 140 bp for the human ϵ gene), the I exon (Fig. 1). This exon becomes the 5'-end of the ϵ GL transcript that also contains four exons of the IgE CH region gene (Fig. 1) [23,25].

It has been proposed that transcription promotes CSR by opening the chromatin and making it more accessible for the enzymes carrying out the DNA rearrangement [26]. However, GL-transcripts stimulate recombination not only by the opening of chromatin, but also the specific transcript or splicing appears to be of importance. Namely, 10 to 100-fold higher switch frequency has been observed with transcripts including the I exon than with transcripts lacking this exon [27]. The reason for the importance of this exon is, however, not yet known. The resulting transcripts probably do not encode a protein, but may instead act primarily through the transcript itself or by a splicing dependent effect [28].

One enzyme has been shown to be particularly important for CSR, i.e. the activation induced cytidine deaminase (AID) [29,30]. AID is a B cell-specific enzyme with DNA editing activity. Deficiency in this enzyme causes complete defect in both class switching and somatic hypermutation, and a hyper-IgM phenotype [31]. A number of other enzymes are also of importance for the CSR, among them the DNA

repair proteins Ku70, Ku80 and DNA-PK [20]. Deficiencies in these enzymes greatly decrease CSR [32–34].

In the mouse CSR to both IgG1 and IgE is activated by IL-4, however, it is well-known that CSR to IgG1 occurs much more frequent than CSR to IgE [35]. One factor that may have an important effect on switch frequency is the size of the switch region and thereby the target region for the DNA recombination event. Accordingly, the IgE (ϵ) and IgG1 (γ 1) switch regions in the mouse are the shortest and the longest, with a size of 1 kb and 12 kb, respectively. Recently, an almost linear correlation between S region size and CSR frequency was observed in genetically modified mice with varying sizes of the IgG1 switch region [36]. These data strongly support the role of the S region size in determining the frequency of CSR and indicate that the small size of the epsilon S region is one factor contributing to its low expression levels.

For an evolutionary perspective on CSR see an excellent recent review by Stavnezer and Amemiya [37].

3. Activators of the epsilon switch region promoter

One of the most important events in regulating CSR is the initiation of transcription from the switch promoters. Transcription from these promoters is regulated by a large number of different positively and negatively acting transcription factors. Germ line (GL) transcription from the epsilon switch promoter is induced by the coordinated action of several positively acting transcription factors. Depending on their expression pattern some of them seem, however, to be more important than others for the initiation of CSR. Pax 5 (BSAP), Pu1, C/EBP, and E2A, all bind to sites within the epsilon switch promoter and promote C ϵ germ line transcripts (Fig. 2) [38–41]. These transcription factors are, however, already present from relatively early B cell development and are therefore not the key factors which open the promoter for transcription. The master regulator of IgE CSR appears instead to be STAT6, which is located in the cytoplasm as a dormant inactive transcription factor and becomes activated

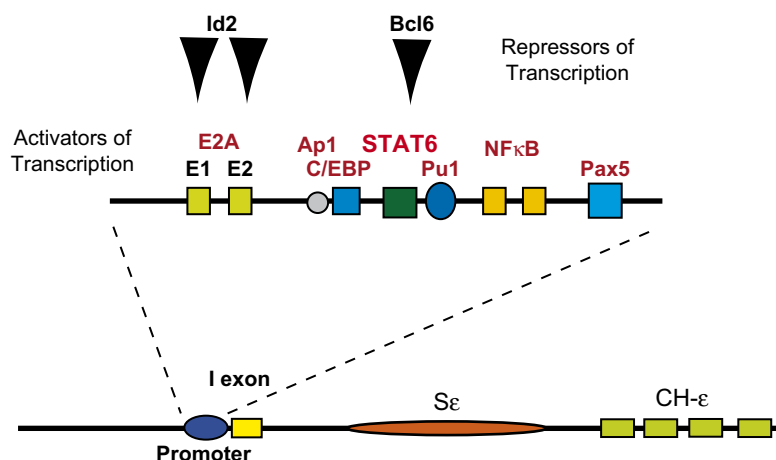


Fig. 2. A schematic figure of the ϵ GL promoter. Activating transcription factors are shown in red and repressors of transcription in black.

through phosphorylation. This phosphorylation, which is induced upon binding of IL-4 or IL-13 to their respective receptors leads to dimerization of STAT6 and subsequent translocation into the nucleus. Well inside the nucleus STAT6 dimers bind to specific sites in the epsilon switch promoter (Figs. 2 and 3). The importance of STAT6 in IgE regulation is clearly documented by the almost total absence of IgE in STAT6 knock out mice [42]. However, STAT6 also synergizes with NF κ B and Ap1, to induce epsilon GL transcription. The importance of NF κ B, which has two binding sites in the epsilon switch promoter (Fig. 2), has been shown by the selectively impaired epsilon CSR in NF κ B p50 deficient mice [43] and its enhancement in mice that lack the NF κ B inhibitor I κ Ba [44]. Notably, NF κ B is activated by its release from its inhibitors I κ Ba, which is induced by CD40–CD40L ligation (Fig. 3) [45,46]. An Ap1-binding site has also been found within the ϵ GL promoter and this site has been shown to be absolutely essential for promoter activity. Low basal levels of Ap1 transcription factors, specifically JunD and FosB, are present before activation. However, upon engagement of B cell surface receptors by antigen (experimentally anti-Ig), CD40L or bacterial lipopolysaccharide (LPS), the Ap1 transcription factors are markedly upregulated [47]. STAT6 and NF κ B also bind to a site in the upstream region of the AID gene and thereby contribute to an increase in available AID enzyme during the DNA rearrangement process (Fig. 3) [48].

Both the ϵ GL promoter and the GL γ 1 promoter bind to STAT6, but it has been shown that the affinity of STAT6 to the ϵ GL promoter is 10 times higher than to the GL γ 1 promoter [47]. The ϵ GL promoter is therefore more dependent on IL-4 and also more inducible than the GL γ 1 promoter. This finding is consistent with a more stringent regulation of the GL ϵ promoter [47]. The GL γ 1 promoter is, however, generally more active, which is reflected in the higher expression levels of IgG1 [47].

4. Negative regulators of the epsilon switch region promoter

The epsilon switch region promoter is not only regulated by positive acting transcription factors, but also by negative regulators. Two such negative regulators have been characterized in relative detail, Id2 and Bcl6. These two factors act by competing for binding sites with E2A and STAT6, respectively (Figs. 2 and 3).

Bcl6 is a POZ/zinc finger transcription factor that can bind with different affinities to several DNA elements recognized by STAT6 thereby acting as a sequence-specific repressor of transcription (Fig. 2). Bcl6 was recently been found to bind three sites within the epsilon promoter, of which at least two appear necessary for maximum repression [49]. Bcl6 can thereby repress IL-4 dependent induction of germ line ϵ transcripts. Mice lacking Bcl6 have been shown to display an

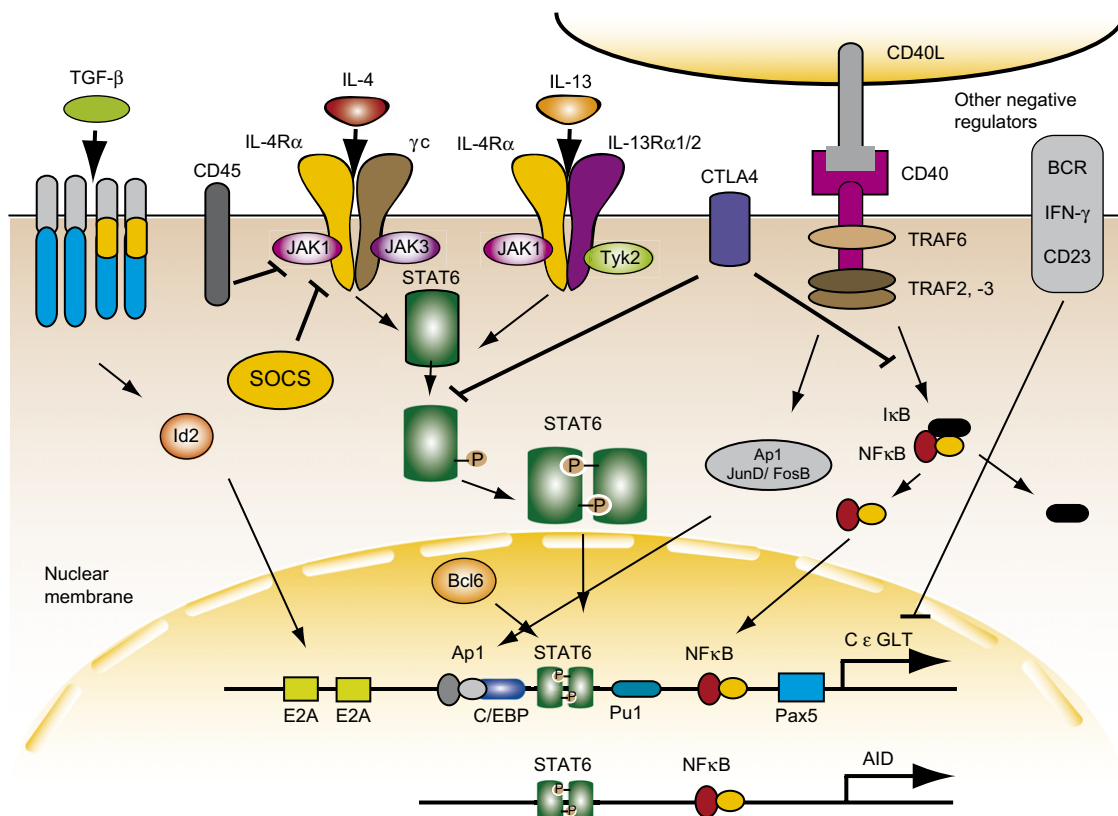


Fig. 3. Positive and negative regulations of IgE CSR. A schematic representation of the different cell surface receptors, signaling molecules and transcription factors regulating IgE CSR. All the different signaling molecules shown in the figure have been described in the text except the TNFR-associated factors (TRAFs).

increased ability to class switch to IgE in response to IL-4 *in vitro*. These mice also exhibit a multiorgan inflammatory disease characterized by the presence of a large number of IgE+ B cells [50].

The second important inhibitor of epsilon CSR, Id2, is a member of the Id family of negative regulators of basic helix-loop-helix (bHLH) transcription factors. The Id family negatively regulates the activity of transcription factors such as E2A proteins (E12 and E47) and MyoD. Knock out mice for Id2 lack lymph nodes and Payer's patches and exhibit a greatly reduced population of NK cells [51]. B cells in these mice preferentially switch to IgE and secrete increasing amounts of IgE in sera. Id2 inhibits IgE CSR by inhibiting the interaction between the E box-binding factors (E12 and E47) with E1 and E2 boxes of the GL ϵ promoter (Figs. 2 and 3). Interestingly, Id2 is induced by TGF- β 1, which offers an explanation as to why TGF- β 1 can negatively regulate CSR to IgE (Fig. 3) [41].

The presence of these negative regulators acting on the ϵ GL promoter appears to be one of the major differences between GL promoters of IgE and IgG. This seems to be the major contributing factor for the comparatively low levels of IgE in plasma [41]. Sugai et al. also proposed that Id2 might act as molecular safeguard to suppress IgE CSR, and thereby to prevent serious complications such as allergic hypersensitivity during normal immune responses [41].

For a more detailed description of some of these events I recommend a detailed and excellent review by Geha et al. [48].

5. Cytokines involved in regulating the CSR

Cytokines are important at almost all levels of immune regulation and in particular in the regulation of IgE. They both regulate the overall TH1 or TH2 environment and also more directly the CSR event. Interferon- γ (IFN- γ) activates CSR to IgG2a in mice and IgG2 in humans, and TGF- β is a central cytokine for the induction of isotype switch to IgA in both mice and man. The only cytokines that have been shown to positively promote CSR to IgE are IL-4 and IL-13 [52,53]. Their individual contribution to human IgE CSR *in vitro* is estimated to approximately 60 and 40% for IL-4 and IL-13, respectively [54].

Both IL-4 and IL-13 are typical TH2 type cytokines, i.e. growth and differentiation factors that primarily regulate humoral immunity. In addition to regulating IgE CSR, IL-4 also induces switching to IgG1 in mice and IgG4 in humans. However, these two cytokines also have numerous other functions. IL-4 is, for example, a key cytokine in the induction of TH2 type immunity involving enhanced antibody production and suppression of cell-mediated immunity. IL-4 has also been shown to display proliferative effects on human mast cells when administered together with SCF [55]. IL-13 upregulates MHC Class II expression on monocytes and is a potent mediator of tissue fibrosis in both schistosomiasis and asthma, which indicates that it is a key regulator of the extracellular matrix [56].

The activation of a cytokine receptor by binding of its ligand, the cytokine, is the first step in the CSR process. The receptor activation leads through a complex cascade of signaling molecules and phosphorylations to the activation of dormant transcription factors. These transcription factors bind then to promoters upstream of the switch regions. In more detail, the cascade initiated by binding of cytokines to their receptors results in receptor oligomerization and activation of Janus kinase (JAK) family of tyrosine kinases, i.e. JAK1, JAK 2, JAK3 and tyrosine kinase 2 (Tyk2) [57]. The cytoplasmic domain of the receptor then becomes phosphorylated by activated JAKs. This process creates recruitment sites for signaling molecules, like the signal transducers and activators of transcription (STATs). There are seven different STATs, STAT1, -2, -3, -4, -5a, -5b and -6, and one or a few of them are activated by a particular cytokine receptor [57]. The IL-4 receptor signals mainly via JAK1, JAK3, which activate the transcription factor STAT6 (Fig. 3) [57]. Although the IL-13 receptor signals via different JAKs, mainly JAK1 and TYK2, STAT6 is also activated upon IL-13 receptor engagement (Fig. 3) [57,58]. The activated STATs, together with other transcriptional activators, then activate promoters located 5' of each switch (S) region, and initiate germ line I-S-CH transcription (Figs. 1–3).

Although cytokines are the most important signal to govern isotype specificity, the B cell needs additional stimulatory interactions to initiate and complete the CSR (Fig. 3). The CD40-CD40-ligand (CD40L) interaction is the most important additional factor for T cell-dependent CSR [59–61]. CD40 and CD40L are members of the TNF receptor and TNF families, respectively. Cytokines and the CD40-CD40L interaction have been shown to be sufficient for completing a CSR *in vitro* [62]. In addition to the induction of GL transcription, IL-4 and CD40-CD40L interactions also induce AID expression, which further increases the activity of the recombination machinery [63].

CSR was previously thought to be an exclusively T cell-dependent process. However, T cell-independent CSR does also occur, although at lower frequency. Namely, viral glycoproteins and bacterial polysaccharides can stimulate IgG, IgA and IgE production in the absence of CD40L-expressing TH cells [64–67]. Two CD40-ligand homologues, APRIL and BAFF, seem here to be of major importance. These two TNF-superfamily members are expressed on myeloid dendritic cells and monocytes in response to type I interferons and upon exposure to CD40L and IFN- γ [68]. One very important source of type I interferons are plasmacytoid dendritic cells, which produce massive amounts of IFN- α and IFN- β in response to virus [68]. DCs thereby appear to be of major importance for T cell-independent CSR. In the presence of IL-10, TGF- β or IL-4 DCs have also been shown to induce CSR to IgG, IgA and IgE, respectively, in B cells [69]. Switched B cells then further differentiate into plasma cell-like cell, which produce secretory antibodies upon engagement of their B cell antigen receptor and exposure to IL-15. This clearly indicates that T cell-independent CSR also can contribute to plasma IgE levels [69].

6. Cytokines regulating TH2 development

Cytokines are not only involved in directly regulating the CSR, but also to affect the TH1 to TH2 balance. By setting the balance between humoral and cellular immunity these cytokines are therefore of major importance for the higher order of regulation. By doing so, they also regulate what cell types that are being activated and what Ig isotypes that are being produced. Although a large number of cytokines are involved in determining this balance, only a few are more directly responsible for strongly promoting TH2 development. Cytokines of this latter category are TSLP, IL-25, IL-18, IL-33 and IL-15. These five cytokines are therefore, in addition to IL-4 and IL-13, very interesting target candidates for the treatment of various TH2-mediated disorders and will be discussed here in more detail.

Thymic stromal lymphopoietin (TSLP) is a very interesting cytokine from an allergy viewpoint. This cytokine has even been described as the master switch of allergic inflammation [70]. TSLP is an IL-7 like cytokine that in humans is produced predominantly by epithelial cells and mast cells. Interestingly, the heterodimeric TSLP receptor appears to be exclusively expressed by myeloid dendritic cells (mDCs) [71]. This cytokine stimulates mDC to promote the differentiation of naïve CD4+ T cells into TH2 type effector cells [70]. These TH2 cells produce the allergy promoting cytokines IL-4, IL-5, IL-13 and TNF- α , but not IL-10 and IFN- γ [72]. Recent findings also indicate that TSLP activated DCs play important roles not only in TH2 priming, but also in the maintenance and further polarization of TH2 central memory cells in allergic diseases [73]. Keratinocytes in atopic dermatitis lesions have been shown to express high levels of TSLP and may thereby be a key cytokine in the development of atopic dermatitis [72]. Mice engineered to over-express TSLP in skin also develop atopic dermatitis and a dramatic increase in circulating TH2 cells and elevated serum IgE [74]. TSLP is, however, not only expressed in peripheral tissues, but also by Hassall's corpuscles within the human thymus. Here, TSLP is involved in instructing thymic DCs to convert high-affinity self-reactive T cells into CD4+CD25+ FoxP3+ regulatory T cells [75]. An excellent review on the role of TSLP in allergic inflammation has been published recently [70].

IL-25 is a novel member of the IL-17 family. By database searches five novel members of the IL-17 gene family with 20–30% identity to IL-17 were identified (IL-17B, IL-17C, IL-17D, IL-17E/IL-25, IL-17F). Of these novel cytokines IL-25 appears as the most interesting one from an allergy perspective. IL-25 induces elevated gene expression of the TH2 type cytokines IL-4, IL-5, IL-13, and of eotaxin [76,77]. The resulting TH2 responses lead to increases in serum IgE and pathological changes in lungs and digestive tract with eosinophilic infiltrates, increased mucus production and epithelial cell infiltrates. This latter finding clearly shows that IL-25 is capable of amplifying allergic inflammation [76,77]. From initial studies, IL-25 appeared to be exclusively expressed by TH2 type T cells. However, RT-PCR analysis showed that IL-25 mRNA is present in many tissues indicating that IL-

25 also is expressed by other cell types. Expression of IL-25 mRNA and protein is also induced in mast cells upon IgE receptor cross-linking. mRNA levels for IL-25 in bone marrow derived mast cells (BMMCs) are comparable with those of activated T cells [78].

Recent data on IL-25 have clearly documented its central role in parasite immunity. IL-25-deficient mice were shown to display inefficient expulsion of the worm parasite *Nippostrongylus brasiliensis* and delayed cytokine production by TH2 cells [79]. The authors here identify a novel non-B non-T cell population that is c-kit positive, but high-affinity IgE receptor (Fc ϵ RI) negative. These cells are most likely the source of the TH2 cytokines (IL-4, IL-5 and IL-13) produced in response to IL-25 [79]. In this context, it should also be mentioned that IL-13 has been shown to play a major role in the expulsion of several parasitic worms, nematodes, however, by yet unknown mechanisms [56].

Another very interesting cytokine is IL-18, a member of the IL-1 family. IL-18 has for several years been known as a potent inducer of TH1 responses by increasing IFN- γ and TNF- α production [80,81]. This TH1-inducing potential is, however, primarily seen in the presence of IL-12. Recently IL-18 was shown to also be a potent inducer of TH2-like responses. Namely, administration of IL-18 resulted in increases in total IgE and IgG1 levels. This increase was dependent on TH cells and a population of invariant chain (V α 14–J α 281) expressing CD1d-restricted NKT cells [81,82]. Moreover, in the presence of IL-3, IL-18 induces IL-13 production in both mast cells and basophils, and IL-4 in basophils. Interestingly, this effect was observed even in the presence of IL-12 [83]. In addition, transgenic expression of active IL-18 in mouse keratinocytes was found to induce severe atopic dermatitis [84]. This dermatitis is, however, not dependent on IgE as no difference in severity is seen in STAT6 $^{-/-}$ mice, which have undetectable levels of IgE [84]. The presence of both IgE dependent and independent pathways may have very interesting implications for the treatment of both severe asthma and atopic dermatitis. An excellent review on IL-18 and its role in allergic inflammation was published in Immunological reviews in 2004 [81].

IL-33 is another IL-1 like cytokine that shows strong TH2 potentiating effects. In vivo administration of this cytokine induces the expression of IL-4, IL-5 and IL-13, and leads to severe pathological changes in mucosal organs like the lung and digestive tract [85]. Intraperitoneal administration of IL-33 also resulted in increased numbers of eosinophils and lymphocytes and elevated levels of IgE and IgA [85]. IL-33 mediates its effects through the IL-1 orphan receptor ST2. This receptor has been shown to act as a negative regulator of Toll-like receptor and IL-1 receptor signalings [86]. The ST2 receptor is expressed primarily on mast cells and on TH2 cells and has in earlier studies also been linked to TH2 type effector functions [87–92]. Interestingly, administration of antagonistic antibodies against ST2 or a soluble ST2 receptor has inhibitory effects on TH2 associated allergic airway inflammation and promotes TH1 responses [88,92].

IL-15 is a cytokine known to promote activation and proliferation of T cells as well as their migration and cytokine

release [93,94]. This cytokine is produced by a wide range of cells including epithelial cells macrophages and dendritic cells (DC). DC-derived IL-15 has been shown to both induce the proliferation of naïve and memory CD8⁺ T cells and to enhance differentiation of TH2 cells when administered together with IL-4. Atopic or IgE-mediated allergies have classically been considered as primarily TH2 driven responses. However, the importance of CD8⁺ T cells in the induction of allergic sensitization has recently become more evident. In a murine model of allergic sensitization the airway hyperreactivity and eosinophil influx were correlated to CD8⁺ T cells [95]. In a recent study in a mouse ovalbumin sensitization model the blocking of endogenous IL-15 leads to a suppression of the induction of Ag-specific TH2 cells [96]. The blocking of IL-15 by soluble IL-15Ra chain, which was performed during the sensitization, resulted in reduced levels of antigen-specific IgE and IgG and prevented the induction of pulmonary inflammation [96].

Numerous other cytokines are probably also involved in positively regulating TH2 type immunity. One additional interesting candidate is IL-3. Apart from its role as a key cytokine in early hematopoiesis, IL-3 is also important for basophil activation and differentiation. Moreover, IL-3 enhances the effect of the complement component C5a, a potent inducer of cytokine production in basophils [97]. Basophils have recently been shown to be potent producers of both IL-4 and IL-13 [98–102]. *In vitro* they have been shown to produce approximately 20 times more IL-4 per cell than fully activated T cells [101]. In addition, basophils express the CD40-ligand and can induce IgE switch *in vitro* [103]. This shows that basophils and basophil-produced IL-4 and IL-13 most likely are important players in allergic sensitization. The importance of IL-3 is here that it is a key regulator of this cytokine production.

Apart from the above listed TH2 promoting cytokines there are also a number of cytokines that down-regulate TH2 responses. IL-12 is here one of the key cytokines. IL-12 negatively regulates TH2 responses by strongly promoting TH1 immunity. It is a potent inducer of IFN- γ , which in turn both promotes TH1 immunity and also directly inhibits IgE switch in B cells [104]. Type I interferons (IFN- α and IFN- β) are also important inducers of TH1-mediated immunity. One TH1 promoting effect of type I interferons is to inhibit the secretion of IL-4 and IL-13 by basophils [105]. TGF- β is a potent general anti-inflammatory cytokine. In addition, this cytokine has been shown to directly repress IgE production by upregulating the expression of Id2 in B cells [41].

IL-21 is a novel member of the type I cytokine family. Other members of this family are IL-2, IL-4, IL-7, IL-9 and IL-15 [106]. In one study, IL-21 expression was detected primarily from TH2 type T cells. The exposure of naïve T cells to IL-21 was here also shown to inhibit IFN- γ production by developing TH1 cells [107]. Moreover, IL-21 administration resulted in a decrease in IL-12 responsiveness in developing T cells and a reduced STAT4 expression. These results indicate that IL-21 is involved in amplifying TH2 responses [107]. In contrast Strengell et al. showed that IL-21 together with IL-15 and/or IL-18 is a potent inducer of IFN- γ from

NK and T cells and is thereby a potentiator of TH1 responses [108,109]. An IL-21 knock out also shows increased IgE production, however, without any increase in IL-4 levels [110]. The reason for this discrepancy in published effects of IL-21 administration is not known. Possibly, the large difference in response may depend on the absence or presence of other cytokines as has been observed for IL-18 [81]. Accordingly IL-21 serves as a potent amplifier of TH1 responses in the presence of IL-15 or/and IL-18, whereas is an inducer of TH2 in their absence. This does, however, not fully explain the increase in IgE in IL-21 knock out mice.

As outlined above, many cytokines have pleiotropic and sometimes synergistic effects. This implies that great care has to be taken when comparing results from different studies, and also when trying to modulate cytokine responses and effects *in vivo*. In spite of this, many of the cytokines listed above are very interesting potential targets for intervention. Numerous attempts to develop modifiers of their *in vivo* effects are also probably already ongoing.

7. Suppressors of cytokine signaling (SOCS)

In order to avoid excessive effects of cytokine stimulation, negative regulators tightly control the signals transmitted from the cell surface to the nucleus. At least three different types of such repressors have been identified, the suppressors of cytokine signaling SOCS, the protein inhibitors of activated STATS (PIAS) and the tyrosine phosphatases SHP1 and CD45 [111]. For example, the tyrosine phosphatase CD45 down-regulates both IL-4-dependent IgE switching and AID expression by dephosphorylating JAK1 and JAK3 (Fig. 3). This phosphatase thereby inhibits signals coming from the IL-4 receptor [112,113]. Although, both CD45 and PIAS are of importance for IgE CSR I will here primarily focus on the repressors of the SOCS family. Several member of this family are directly involved in regulating responses from TH1 or TH2 cytokine receptors.

The SOCS family consists of eight members, CIS and SOCS 1–7, which all have in common a central SH2 domain and a C-terminal SOCS box. SOCS family members are induced upon cytokine signaling and by a range of other stimuli like bacterial lipopolysaccharides (LPSs) and chemokines [111]. They are involved in down-regulating further signaling from the cytokine receptors by blocking the JAK–STAT pathway. The SOCS SH2 domains have been suggested to bind to phosphorylated tyrosine residues on signaling intermediates, e.g. on JAKs or the cytokine receptor. CIS is thought to act by blocking STAT recruitment to the receptor [111]. Several of the SOCS are of interest from an allergy view point, some are potent regulators of TH1 and some are of TH2 responses. For example, CIS over expression has been shown to favor TH2 responses and may thereby be involved in the development of asthma and atopic dermatitis [111]. This member of the SOCS family is induced by cytokines that activate STAT5, such as erythropoietin (EPO), growth hormone, prolactin, IL-2 and IL-3, and CIS is thought to act as a negative regulator of STAT5. However, the most important SOCS

regarding an allergic setting seem to be SOCS1, -3 and -5. SOCS3 is predominantly expressed in TH2 type cells and SOCS1 and SOCS5 primarily in TH1 cells [111].

SOCS1 is a potent inhibitor of STAT1, and thereby a potent inhibitor of IFN- γ receptor signaling. Deficiency of this SOCS family member results in a phenotype characterized by fatty degeneration of the liver, lymphopenia, macrophage infiltration of major organs and activation of peripheral T cells [111]. Affected mice normally die within three weeks after birth [111]. SOCS1 may also act on the signaling by other inflammatory cytokines, including IL-2, -4, -6, -12 and -15, and its absence may therefore be associated with a large number of primarily TH1 type inflammatory disorders [111]. However, increased expression of SOCS1 may instead lead to suppression of TH1 responses and an accelerated TH2 differentiation. SOCS1 may in this situation act as an inducer of allergic inflammation [114].

SOCS3 is transiently induced by a number of inflammatory and anti-inflammatory cytokines, including IFN- γ , IL-3, IL-6 and IL-10, and inhibits STAT3 signaling [115,116]. In addition, SOCS3 inhibits IL-12-mediated STAT4 activation. This latter activity may actually be the most important role of SOCS3 in regulating TH cell differentiation [116]. Mice that constitutively express or lack the SOCS3 gene show defects in fetal liver erythropoiesis or placental function, resulting in embryonic lethality [117,118]. SOCS3 transgenic mice show increased TH2 responses, which indicate that this factor is directly involved in regulating the TH1–TH2 balance. Interestingly, Seki et al. also observed a strong correlation between the level of SOCS3 expression, serum IgE levels in allergic patients and the severity of the asthma and atopic dermatitis, which further substantiates this conclusion [116].

SOCS5 is preferentially expressed in TH1 cells where it inhibits IL-4 and probably IL-13-dependent STAT6 activation, which results in a reduced TH2 differentiation [119].

8. Cell surface receptors on B cells and their role in IgE regulation

Several cell surface receptors, other than the cytokine receptors and CD40, have been shown to affect IgE regulation (Fig. 3). One of them the cell surface phosphatase CD45 has already been discussed in relative detail. Other cell surface molecules of interest are the B cell receptor (BCR), the low affinity receptor for IgE (CD23), and CTLA4.

Cross-linking of the BCR by monoclonal antibodies *in vitro* has been shown to inhibit a CD40+ IL-4-driven CSR to IgG1 and IgE, which indicates that the antigen receptor may play an important role in the selection of Ig isotypes during T cell-dependent immune responses [120]. This indicates that the isotype pattern during an immune response may be determined by the strength in the Ag–Ab interaction.

Cytotoxic T lymphocyte antigen 4 (CTLA4) is an inhibitory costimulatory molecule that is primarily expressed by T cells. However, CTLA4 is also induced by either CD40 or LPS stimulation on IL-4-treated B cells. When CTLA4 binds to its receptor (B7:1 or B7:2 (CD80 and CD86)) it becomes

activated, which leads to an inhibition of NF κ B and STAT6, and a block in the induction of IgG1 and IgE CSR (Fig. 3) [121].

The low-affinity IgE receptor (Fc ϵ R2), which also goes by the name CD23, acts as a receptor for antigen uptake and thereby antigen presentation. Cross-linking of this receptor on the surface of B cells has been shown to suppress the production of IgE in both human and animal studies (Fig. 3) [122,123]. Transgenic mice over-expressing CD23 show a decrease in IgE levels and conversely, mice deficient in CD23 show increased IgE levels [124]. Soluble CD23, generated by proteolytic cleavage has also been shown to enhance the production of IgE [125]. A probable mechanism is that soluble CD23 binds to IgE and thereby limits its access to cell-bound CD23.

9. IgE, stress and complement components

It has recently been shown that both stress (increased cortisol levels) and complement components directly can affect IgE synthesis.

Interestingly the synthetic corticosteroid hydrocortisone has been found to induce IgE production in IL-4-stimulated human B cells *in vitro* [126]. This IgE production is CD40L-dependent as B cells from CD40L-deficient patients fail to produce IgE upon hydrocortisone stimulation. Moreover, soluble CD40 can inhibit hydrocortisone-induced IgE production by normal B cells [127]. Hydrocortisone also upregulates the expression of the CD40L on both B and T cells. Notably, the hydrocortisone levels conferring optimal CD40L expression are in the μ M range, the same range that has been observed for cortisol in humans under stress [128]. Stress can thereby initiate IgE synthesis, and increase CD40L expression, relatively rapid. The increase in CD40L can in turn activate CD40-expressing cells, including macrophages, dendritic cells and eosinophils and thereby contributes to the elimination of pathogens before antigen-specific T cells being increased in numbers [48].

The complement regulatory protein C4 binding protein (C4BP) was recently found to bind CD40 and to stimulate B cells in a way that mimics activation by CD40L. C4BP is produced in the liver, where it is upregulated by glucocorticoids and inflammatory cytokines such as IFN- γ , IL-1, IL-6 and TNF- α [129,130]. In the presence of IL-4, C4BP induces ϵ GL transcription and the expression of AID. This in turn leads to CSR and the production and secretion of IgE. C4BP may also be of importance for B cell survival and activation in germinal centers. Co-localization of C4BP with B cells has namely been observed in germinal centers of human tonsils. This complement regulatory protein may here contribute to sustained CD40 signaling after the initial activation by CD40L on T cells [48].

10. Site of production – local or systemic

The site of IgE production may also have interesting implications for potential treatment strategies. If the majority of IgE

is produced in spleen and lymph nodes a systemic administration of for example anti-IgE should be the most favorable way of treatment. However, if IgE primarily is produced in local tissue we should instead aim at more local administration. It is well-known that the majority of IgA is produced in mucosal tissues and that CSR to IgA also occurs at these sites. A potent activator of IgA CSR is here a high local TGF- β concentration.

Recently several articles describing local IgE production have also appeared in the literature. IgE producing plasma cells have been detected in local tissues such as nose and skin [131]. A marked increase in IgE positive cells in the nasal mucosa in rhinitis patients has also been observed [132]. In addition, IgE GL-transcripts have been detected in local tissues (sinus mucosa) by *in situ* hybridization indicating that CSR occurs in peripheral organs [133]. IgE producing B cells have been found to be more than 1000 times more frequent in the nose than in peripheral blood [134]. One interesting possibility in the light of these findings is that local allergic reactions may be confined to specific tissue sites, and that treatment strategies should aim at targeting these specific sites.

11. Factors influencing the half-life of IgG and IgE

The plasma half-life of IgG and IgE are known to differ markedly, with 21 and 2.5 days in humans, respectively. Interestingly, a recent knock out for the FcRN [135] has been shown to reduce the plasma half-life of IgG1 in mice from 9 days to 1.4 days. Thereby, IgG1 in these mice has approximately the same half-life as all other Ig isotypes in mice 1–2 days [135]. This finding clearly indicates that the main reason for the difference in half-life between IgG and IgE is this stabilizing interaction between IgG and FcRN. Thus, the turnover of IgE may be much faster than that of IgG and a substantially higher amount of IgE may be produced than what is reflected in its plasma levels.

For these calculations we have, however, to take into consideration that the majority of the produced IgE is probably bound to high-affinity Fc ϵ RI on the surface of basophils and mast cells. This in marked contrast to IgG that instead is primarily found in free circulating form. The IgE–Fc ϵ RI interaction has a potent stabilizing effect on IgE half-life. The half-life of receptor bound IgE when has been estimated to approximately 14 days, which indicate that the majority of IgE may have a half-life similar to IgG, despite its much lower plasma half-life. No studies of the turnover of all IgE, plasma and cell bound, have, however, been performed (to my knowledge), which would clarify this issue.

12. Susceptibility genes for allergy and asthma

Most of the available data on the regulation of IgE expression have been obtained from experiments using traditional cell and molecular biology techniques like, cell assays, protein purification, cDNA cloning, and during more recent years transgene and knock out techniques. However, during the past 10–15 years, genome-based techniques have started to enter the arena. Single nucleotide polymorphisms (SNIPs)

have been used in attempts to identify novel atopy and asthma susceptibility genes. Complex diseases like asthma are, however, difficult to study with this technique, because a potentially large number of genes are involved. After many years of intense work, these efforts have now resulted in a number of interesting candidate genes. Four of these are in more advanced stages, namely PHF11, DPP10, ADAM33 and GPRA. Although not all of them may be directly involved in regulating IgE levels, they may still be of interest in the context of this review. One of them, the plant homeo domain zinc finger gene 11 (PHF11) on human chromosome 13q14 is a potential transcriptional regulator. PHF11 was identified on consistent linkage to atopy (high IgE concentrations) and severe asthma [136]. This gene is highly expressed in stomach, tonsils and B cells, and encodes NY-REN-34, a protein originally identified from patients with renal cell carcinoma. This protein contains two plant homeo domain (PHD) zinc finger motifs and is probably involved in chromatin-mediated transcriptional regulation. No function of the gene product has, however, yet been presented. Two other genes, SETDB2 and RCBTB1, are also located in the same region and may be involved in the observed phenotype [136].

Another gene that was identified as a candidate gene associated with asthma is the dipeptidyl peptidase homologue (DPP10) on chromosome 2q14 [137]. The encoded protein belongs to the conserved family of dipeptidyl peptidases that can cleave off terminal dipeptides from chemokines and cytokines, and might thus regulate their activities. However, a mutation in one of the three amino acids needed to form the catalytic triad probably renders this member of a serine protease family inactive. Namely, the crucial serine has been replaced with a glycine. As an alternative function, DPP10 has been suggested to associate with type A K⁺ channel subunits in neurons, based on its high homology to DPP6, which holds such a function [138]. However, it is not possible to tell the potential role of this protein in regulating IgE and atopy before the functional characteristics of this protein have been clarified.

A third gene identified through positional cloning is disintegrin and metalloproteinase-33 gene (ADAM33) on chromosome 20p13. This gene has also been linked to asthma phenotype, combined with either bronchial hyperreactivity or high total or specific IgE titers [139]. Due to its expression in bronchial fibroblasts and smooth muscle cells, this protein has been proposed to play a role in airway remodeling. There is, however, still controversy concerning the linkage of this gene to asthma-related phenotypes. This linkage has namely not been observed in several other studies [140].

A fourth gene identified through positional cloning is GPRA (G-protein coupled receptor for asthma susceptibility), which is situated on chromosome 7p14–p15. This gene is linked to high IgE levels in the Finnish Kainuu population [141]. The same locus was later found in a screening on asthma phenotype in both a French Canadian and a second Finnish sample, and in several other genome scans [140]. GPRA encodes an orphan G-coupled receptor with yet unknown function. Two isoforms have been described that are differentially expressed in bronchial epithelial cells and

smooth muscle cells of asthmatics and healthy controls [142]. A potential ligand peptide that may derive from an uncharacterized precursor has also been identified. This peptide, which is phylogenetically conserved across mammals, induces both calcium mobilization and adenylate cyclase accumulation by GPRA, indicating that it is a functional receptor involved in cellular signaling [143]. However, as for the other candidate genes no direct role in regulating IgE levels or asthma severity has yet been presented.

Taken together, although these four genes are very interesting candidates for novel unrecognized regulatory pathways no or only little functional data are so far available. A lot of additional work is therefore probably still needed to characterize these candidate genes for their role in asthma pathogenesis and IgE regulation. Before their biological function is known it is also difficult to evaluate their potential as future targets for therapy.

13. Potential targets of intervention

Based on our present knowledge of IgE and IgE regulation there are a relatively large number of potential target molecules to consider for therapeutic intervention (Fig. 4). Among the most interesting ones are the various cytokines, from the classical TH2 cytokines IL-4 and IL-13 to the more recently identified cytokines that positively regulate the TH2 environment, such as TSLP, IL-25, IL-18, IL-33, IL-15 and IL-3 (Fig. 4). Several of the TH1-inducing cytokines are also interesting candidates as they increase cell-mediated immunity and thereby often down-regulate TH2 responses. However, the administration and *in vivo* distribution of TH1 cytokines

may here pose a major problem. Injection of even minute amounts of IL-12, one of the major TH1-inducing cytokines, has for example shown to induce very severe adverse effects. With a dose of 500 ng/kg of recombinant (r) IL-12, during a phase II clinical trial these effects were so severe that two persons died [144]. Administration of 0.5 or 1 µg of rIL-12 in mice for 6 consecutive days also resulted in 100% mortality [144]. It therefore appears extremely difficult to balance the appropriate dosing and *in vivo* distribution such that it grants the wanted positive effects without causing many severe side effects. Similar, although not so extreme, results have also been seen after administration of IL-2. The aim with the injection of this cytokine has primarily been to improve T cell responses in cancer or HIV patients. The problem is here that relatively large amounts of IL-2 have to be injected to render the desired response, which also increases the risk of causing severe side effects [145,146].

Although there are numerous examples of difficulties in using pure recombinant cytokines for therapy there are also success stories. One such example is recombinant EPO, which clearly has revolutionized the treatment of anemic patients with kidney diseases [147,148]. The administration is here, however, more easy. EPO normally works at a distance, as an endocrine factor. It does not stay at the injection site, but does instead move with the blood to reach its target, the bone marrow. This is similar to its normal *in vivo* route, from the kidney to the bone marrow. Many of the TH1 cytokines are, however, not aimed to act at a distance and may therefore be more difficult to administrate.

The modulation of excessive amounts of a cytokine seems, however, to be more easy to control. There are several

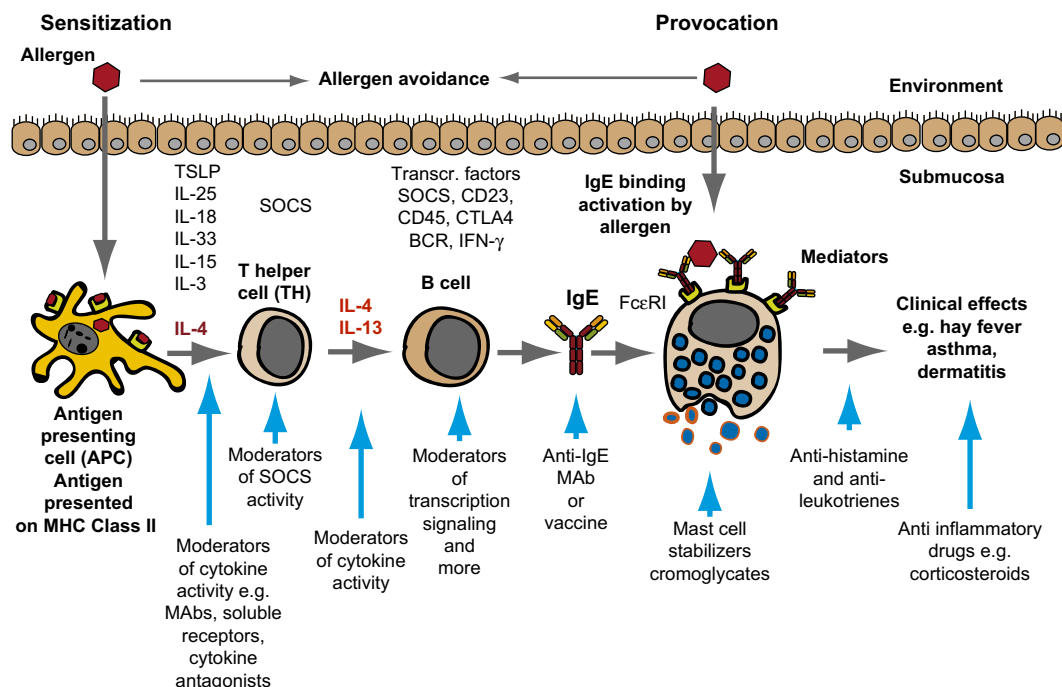


Fig. 4. Potential targets of intervention. A summary of different potential target molecules and at what level during the allergic sensitization or activation phase they act. Ways of targeting these molecules are also listed at the bottom (indicated by blue arrows).

alternative strategies, and at least three of them have already been studied in relative detail. The most frequently used strategies are monoclonal antibodies that block cytokines from binding to their receptors. These antibodies may also, depending on the epitope, target the cytokine to immune complexes that can be removed by phagocytosis. Another alternative is to use soluble receptors. These receptors can compete with natural cell surface bound receptor for binding to the cytokine and thereby limits its effects on target cells. A third alternative is to use antagonistic or blocking cytokines, e.g. by introducing mutations in the cytokine that allow receptor binding but hamper signaling. One interesting example of this latter alternative has been studied extensively, an IL-4 analog. A human IL-4 mutant was first identified which could bind, but did not activate, the IL-4 receptor [149]. However, to perform preclinical conceptual studies in an animal model, the corresponding mouse analog had first to be constructed. A mouse IL-4 variant (Q116D, Y119D) was therefore produced and tested for its therapeutic potential in allergic sensitization and in established disease in a murine asthma model. Inhibition of the IL-4/IL-13 system in the allergic sensitization phase resulted in a dose dependent reduction in ovalbumin-specific IgE and an inhibition of airway eosinophilia. However, if the IL-4/IL-13 system was inhibited at various time points after allergy was established, only little effect on all measured allergic parameters was observed in this short-term study [150]. However, it is possible that more long-term administration may also reduce already established sensitization.

Several additional therapies that inhibit or block cytokine action have already reached the clinic or are in advanced clinical studies. One very important example is the targeting of TNF- α in patients with rheumatoid arthritis and other inflammatory disorders. The treatment is here based on monoclonal antibodies or the soluble receptor [151]. Many patients have experienced a remarkable improvement with these new biodrugs and the side effects are relatively modest, with a moderate increase in sensitivity to mycobacterial infections [151,152].

In addition to the cytokine targets where the development in many cases has reached a very advanced clinical stage or are already on the market, there are a number of less well-explored targets to consider. SOCS1, -3 and -5 are certainly interesting candidates, due to their involvement in regulating the TH1–TH2 balance and because they are expressed in either TH1 or TH2 cells (Fig. 4). They are, however, intracellular proteins and are structurally related. This increases the complexity in finding and designing activators or inhibitors with sufficient specificity that reaches their targets inside the cell in sufficient quantities.

Inhibiting the binding of transcription factor to their target sites in switch promoters is may be even more difficult, but is certainly of interest (Fig. 4). Stabilized double-stranded oligonucleotides modified to enter cells through the plasma membrane may here have the potential to block the activation of the ϵ GL promoter.

In addition to the potential targets discussed in this review there is one important area where the development already has

reached a relatively advanced stage, the IgE molecule itself (Fig. 4). In a collaborative effort between Tanox, Genentech and Novartis, a monoclonal antibody has been developed which targets the receptor-binding region of human IgE. The mouse monoclonal originally isolated has been humanized, and the recombinant humanized anti-IgE antibody is now produced under GMP for injection into patients with severe asthma. All the clinical trials needed to take this drug to the market have been performed, and this monoclonal is now sold under the name Xolair. A relatively good add on effect on other treatments has been seen with this new monoclonal, and a 26–38% reduction in the rate of clinically significant asthma exacerbations. A review that summarizes the clinical studies on Omalizumab, the active component in Xolair, was recently published in Respiratory Medicine 2006 [153]. One major disadvantage with this type of treatment is, however, the high cost. Relatively large amounts of high quality recombinant protein have to be injected into the patient, up to 375 mg of recombinant antibody every two or four weeks [154]. The cost per patient and year has been estimated to 15 000–44 000\$ [155].

In order to address the problems with large amounts of recombinant protein required per patient, the large number of visits to the clinic, and the high cost of treatment we have looked into an alternative strategy to target IgE, vaccination. This strategy induces the patient's own immune system to produce anti-IgE antibodies. Preclinical studies in rat and dog have shown that it is possible to trigger the immune system to produce antibodies against a self-protein [10,14,156]. By covalent coupling of the receptor interacting domain of IgE, the CH3 domain, to a foreign (non-self) carrier molecule the immune system is triggered to produce an antibody response to both the carrier and the self-protein. Sufficiently high titers of anti-IgE antibodies are produced in the animal models to suppress allergen triggered skin reactions [10,156]. The vaccination is reversible, appears very safe and comparably very small amounts of recombinant protein are needed per injection. We estimate that 10 000–20 000 times lower amounts of recombinant protein is needed to treat a patient with the vaccine compared to injections of monoclonal antibodies [10]. Therapeutic vaccines targeting various self-molecules are, however, facing their limitations at the level of the immune response [157]. Presently, only very weak immuno-stimulatory substances, adjuvants, are allowed for human use, but the induction of therapeutic antibody responses against self-proteins apparently needs strong adjuvants. The self-tolerance is potent and needs to be overruled in order to obtain good therapeutic effects. The only clinical trials with therapeutic vaccines against self-proteins that have been performed have used the weak adjuvant Alum [158] (Her-2 studies at Pharmexa, www.pharmexa.com). However, these studies not even come close to the very positive results obtained with potent adjuvant in preclinical studies in animal models [10,156,159–162]. The optimization of adjuvants is therefore urgently needed to move therapeutic vaccines from animal models into successful treatment regimens in the clinic [157].

14. Conclusions

The genes encoding IgG and IgE have, from originally being two identical genes, diversified quite remarkably during the past 200–250 million years of mammalian evolution. The gene product of one of these two genes, IgE, has become an extremely potent inducer of inflammation through its interaction with high-affinity receptors on mast cells and basophils, while IgG has become the major plasma isotype involved in phagocytosis, ADCC and complement activation. The different IgG isotypes and IgE are also being regulated very differently, where size of the switch region, positive and negative transcriptional regulators and cytokines, both directly regulating class switch and the general TH1 and TH2 environment, are involved. The progress in our understanding of the regulatory pathways and the components involved has opened a number of new ways of interfering with the production of IgE, and thereby new treatment strategies against atopic allergies. Some of the potential target molecules are already very well characterized and we know their various effects on our immune system. This category comprises the classical TH2 cytokines IL-4, IL-13. A number of additional regulators of TH2 responses such as TSLP, IL-25, IL-18, IL-33 and IL-15 can now also be added to the list of highly interesting target molecules for potential intervention. Also the IgE molecule itself should be added here, as well as SOCS and CD23. In addition, several candidate genes have been identified as the result of genome wide screenings for atopy or asthma susceptibility. These genes have, however, still some way to go before they can be evaluated as potential targets for intervention. An increasing number of potential targets are being identified which at least in theory should lead to a more rapid progress in our attempts to develop new treatment strategies against atopic allergies. However, a selection from the many new targets will have to be made carefully and novel ways to modulate their *in vivo* effects have to be made such that good therapeutic effects can be achieved with minimal side effects.

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References

- [1] Lanning DK, Zhai SK, Knight KL. Analysis of the 3' Cmu region of the rabbit Ig heavy chain locus. *Gene* 2003;309(2):135–44.
- [2] Vernersson M, Aveskogh M, Munday B, Hellman L. Evidence for an early appearance of modern post-switch immunoglobulin isotypes in mammalian evolution (II): cloning of IgE, IgG1 and IgG2 from a monotreme, the duck-billed platypus, *Ornithorhynchus anatinus*. *Eur J Immunol* 2002;32(8):2145–55.
- [3] Vernersson M, Aveskogh M, Hellman L. Cloning of IgE from the echidna (*Tachyglossus aculeatus*) and a comparative analysis of epsilon chains from all three extant mammalian lineages. *Dev Comp Immunol* 2004;28(1):61–75.
- [4] van Rheede T, Bastiaans T, Boone DN, Hedges SB, de Jong WW, Madsen O. The platypus is in its place: nuclear genes and indels confirm the sister group relation of monotremes and therians. *Mol Biol Evol* 2006;23(3):587–97.
- [5] Woodburne MO, Rich TH, Springer MS. The evolution of tribospheny and the antiquity of mammalian clades. *Mol Phylogenet Evol* 2003;28(2):360–85.
- [6] Johansson SG, Bennich H. Immunological studies of an atypical (myeloma) immunoglobulin. *Immunology* 1967;13(4):381–94.
- [7] Bennich H, Ishizaka K, Ishizaka T, Johansson SG. A comparative antigenic study of gamma E-globulin and myeloma-IgND. *J Immunol* 1969;102(4):826–31.
- [8] Bennich H, Johansson SG. Structure and function of human immunoglobulin E. *Adv Immunol* 1971;13:1–55.
- [9] Ishizaka T, Ishizaka K. Biology of immunoglobulin E. Molecular basis of reaginic hypersensitivity. *Prog Allergy* 1975;19:60–121.
- [10] Vernersson M, Ledin A, Johansson J, Hellman L. Generation of therapeutic antibody responses against IgE through vaccination. *FASEB J* 2002;16(8):875–7.
- [11] Kumagai Y, Hirano T, Watanabe N, Okumura K, Ovary Z. Studies on IgE production in mice. I. Spontaneous suppression of IgE production in SJA/9 mice. *Immunogenetics* 1983;18(2):147–53.
- [12] Azuma M, Hirano T, Miyajima H, Watanabe N, Yagita H, Enomoto S, et al. Regulation of murine IgE production in SJA/9 and nude mice. Potentiation of IgE production by recombinant interleukin 4. *J Immunol* 1987;139(8):2538–44.
- [13] Watanabe N, Katakura K, Kobayashi A, Okumura K, Ovary Z. Protective immunity and eosinophilia in IgE-deficient SJA/9 mice infected with *Nippostrongylus brasiliensis* and *Trichinella spiralis*. *Proc Natl Acad Sci U S A* 1988;85(12):4460–2.
- [14] Ledin A, Bergvall K, Hillbertz NS, Hansson H, Andersson G, Hedhammar A, et al. Generation of therapeutic antibody responses against IgE in dogs, an animal species with exceptionally high plasma IgE levels. *Vaccine* 2006;24(1):66–74.
- [15] Wagner B, Radbruch A, Rohwer J, Leibold W. Monoclonal anti-equine IgE antibodies with specificity for different epitopes on the immunoglobulin heavy chain of native IgE. *Vet Immunol Immunopathol* 2003;92(1–2):45–60.
- [16] Iio A, Strober W, Broder S, Polmar SH, Waldmann TA. The metabolism of IgE in patients with immunodeficiency states and neoplastic conditions. *J Clin Invest* 1977;59:743–55.
- [17] Anton IM, Jones GE. WIP: a multifunctional protein involved in actin cytoskeleton regulation. *Eur J Cell Biol* 2006;85(3–4):295–304.
- [18] Buckley RH. The hyper-IgE syndrome. *Clin Rev Allergy Immunol* 2001;20(1):139–54.
- [19] Mills FC, Brooker JS, Camerini-Otero RD. Sequences of human immunoglobulin switch regions: implications for recombination and transcription. *Nucleic Acids Res* 1990;18(24):7305–16.
- [20] Selsing E. Ig class switching: targeting the recombinational mechanism. *Curr Opin Immunol* 2006;18(3):249–54.
- [21] Luby TM, Schrader CE, Stavnezer J, Selsing E. The mu switch region tandem repeats are important, but not required, for antibody class switch recombination. *J Exp Med* 2001;193(2):159–68.
- [22] Khamlichi AA, Glaudet F, Oruc Z, Denis V, Le Bert M, Cogne M. Immunoglobulin class-switch recombination in mice devoid of any S mu tandem repeat. *Blood* 2004;103(10):3828–36.
- [23] Gauchat JF, Leberman DA, Coffman RL, Gascan H, de Vries JE. Structure and expression of germline epsilon transcripts in human B cells induced by interleukin 4 to switch to IgE production. *J Exp Med* 1990;172(2):463–73.
- [24] Jung S, Rajewsky K, Radbruch A. Shutdown of class switch recombination by deletion of a switch region control element. *Science* 1993;259(5097):984–7.
- [25] Gerondakis S. Structure and expression of murine germ-line immunoglobulin epsilon heavy chain transcripts induced by interleukin 4. *Proc Natl Acad Sci U S A* 1990;87(4):1581–5.
- [26] Kaminski DA, Stavnezer J. Antibody class switching: uncoupling S region accessibility from transcription. *Trends Genet* 2004;20(8):337–40.
- [27] Bottaro A, Lansford R, Xu L, Zhang J, Rothman P, Alt FW. S region transcription per se promotes basal IgE class switch recombination

- but additional factors regulate the efficiency of the process. *EMBO J* 1994;13(3):665–74.
- [28] Hein K, Lorenz MG, Siebenkotten G, Petry K, Christine R, Radbruch A. Processing of switch transcripts is required for targeting of antibody class switch recombination. *J Exp Med* 1998;188(12):2369–74.
- [29] Honjo T, Muramatsu M, Fagarasan S. AID: how does it aid antibody diversity? *Immunity* 2004;20(6):659–68.
- [30] Storb U, Stavnezer J. Immunoglobulin genes: generating diversity with AID and UNG. *Curr Biol* 2002;12(21):R725–7.
- [31] Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* 2000;102(5):553–63.
- [32] Rolink A, Melchers F, Andersson J. The SCID but not the RAG-2 gene product is required for S mu-S epsilon heavy chain class switching. *Immunity* 1996;5(4):319–30.
- [33] Casellas R, Nussenzweig A, Wuerffel R, Pelanda R, Reichlin A, Suh H, et al. Ku80 is required for immunoglobulin isotype switching. *EMBO J* 1998;17(8):2404–11.
- [34] Manis JP, Gu Y, Lansford R, Sonoda E, Ferrini R, Davidson L, et al. Ku70 is required for late B cell development and immunoglobulin heavy chain class switching. *J Exp Med* 1998;187(12):2081–9.
- [35] Snapper CM, Finkelman FD, Stefany D, Conrad DH, Paul WE. IL-4 induces co-expression of intrinsic membrane IgG1 and IgE by murine B cells stimulated with lipopolysaccharide. *J Immunol* 1988;141(2):489–98.
- [36] Zarrin AA, Tian M, Wang J, Borjeson T, Alt FW. Influence of switch region length on immunoglobulin class switch recombination. *Proc Natl Acad Sci U S A* 2005;102(7):2466–70.
- [37] Stavnezer J, Amemiya CT. Evolution of isotype switching. *Semin Immunol* 2004;16(4):257–75.
- [38] Thienes CP, De Monte L, Monticelli S, Busslinger M, Gould HJ, Vercelli D. The transcription factor B cell-specific activator protein (BSAP) enhances both IL-4- and CD40-mediated activation of the human epsilon germline promoter. *J Immunol* 1997;158(12):5874–82.
- [39] Stutz AM, Woisetschlager M. Functional synergism of STAT6 with either NF-kappa B or PU.1 to mediate IL-4-induced activation of IgE germline gene transcription. *J Immunol* 1999;163(8):4383–91.
- [40] Delphin S, Stavnezer J. Characterization of an interleukin 4 (IL-4) responsive region in the immunoglobulin heavy chain germline epsilon promoter: regulation by NF-IL-4, a C/EBP family member and NF-kappa B/p50. *J Exp Med* 1995;181(1):181–92.
- [41] Sugai M, Gonda H, Kusunoki T, Kataaki T, Yokota Y, Shimizu A. Essential role of Id2 in negative regulation of IgE class switching. *Nat Immunol* 2003;4(1):25–30.
- [42] Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, et al. Essential role of Stat6 in IL-4 signalling. *Nature* 1996;380(6575):627–30.
- [43] Sha WC, Liou HC, Tuomanen EI, Baltimore D. Targeted disruption of the p50 subunit of NF-kappa B leads to multifocal defects in immune responses. *Cell* 1995;80(2):321–30.
- [44] Chen CL, Yull FE, Cardwell N, Singh N, Strayhorn WD, Nanney LB, et al. RAG2-/-, I kappa B-alpha-/- chimeras display a psoriasisiform skin disease. *J Invest Dermatol* 2000;115(6):1124–33.
- [45] Messner B, Stutz AM, Albrecht B, Peiritsch S, Woisetschlager M. Cooperation of binding sites for STAT6 and NF kappa B/rel in the IL-4-induced up-regulation of the human IgE germline promoter. *J Immunol* 1997;159(7):3330–7.
- [46] Iciek LA, Delphin SA, Stavnezer J. CD40 cross-linking induces Ig epsilon germline transcripts in B cells via activation of NF-kappaB: synergy with IL-4 induction. *J Immunol* 1997;158(10):4769–79.
- [47] Mao CS, Stavnezer J. Differential regulation of mouse germline Ig gamma 1 and epsilon promoters by IL-4 and CD40. *J Immunol* 2001;167(3):1522–34.
- [48] Geha RS, Jabara HH, Brodeur SR. The regulation of immunoglobulin E class-switch recombination. *Nat Rev Immunol* 2003;3(9):721–32.
- [49] Harris MB, Mosteck J, Rothman PB. Repression of an interleukin-4 responsive promoter requires cooperative BCL-6 function. *J Biol Chem* 2005;280(13):13114–21.
- [50] Harris MB, Chang CC, Berton MT, Danial NN, Zhang J, Kuehner D, et al. Transcriptional repression of Stat6-dependent interleukin-4-induced genes by BCL-6: specific regulation of epsilon transcription and immunoglobulin E switching. *Mol Cell Biol* 1999;19(10):7264–75.
- [51] Yokota Y, Mansouri A, Mori S, Sugawara S, Adachi S, Nishikawa S, et al. Development of peripheral lymphoid organs and natural killer cells depends on the helix-loop-helix inhibitor Id2. *Nature* 1999;397(6721):702–6.
- [52] de Vries JE, Punnonen J, Cocks BG, de Waal Malefyt R, Aversa G. Regulation of the human IgE response by IL4 and IL13. *Res Immunol* 1993;144(8):597–601.
- [53] McKenzie GJ, Fallon PG, Emson CL, Grecis RK, McKenzie AN. Simultaneous disruption of interleukin (IL)-4 and IL-13 defines individual roles in T helper cell type 2-mediated responses. *J Exp Med* 1999;189(10):1565–72.
- [54] Punnonen J, Yssel H, de Vries JE. The relative contribution of IL-4 and IL-13 to human IgE synthesis induced by activated CD4+ or CD8+ T cells. *J Allergy Clin Immunol* 1997;100(6 Pt 1):792–801.
- [55] Lorentz A, Bischoff SC. Regulation of human intestinal mast cells by stem cell factor and IL-4. *Immunol Rev* 2001;179:57–60.
- [56] Wynn TA. IL-13 effector functions. *Annu Rev Immunol* 2003;21:425–56.
- [57] Leonard WJ, O'Shea JJ. Jaks and STATs: biological implications. *Annu Rev Immunol* 1998;16:293–322.
- [58] Roy B, Cathcart MK. Induction of 15-lipoxygenase expression by IL-13 requires tyrosine phosphorylation of Jak2 and Tyk2 in human monocytes. *J Biol Chem* 1998;273(48):32023–9.
- [59] Van Kooten C, Banchereau J. CD40–CD40 ligand: a multifunctional receptor–ligand pair. *Adv Immunol* 1996;61:1–77.
- [60] Stavnezer J. Antibody class switching. *Adv Immunol* 1996;61:79–146.
- [61] Manis JP, Tian M, Alt FW. Mechanism and control of class-switch recombination. *Trends Immunol* 2002;23(1):31–9.
- [62] Zhang K, Clark EA, Saxon A. CD40 stimulation provides an IFN-gamma-independent and IL-4-dependent differentiation signal directly to human B cells for IgE production. *J Immunol* 1991;146(6):1836–42.
- [63] Chaudhuri J, Tian M, Khuong C, Chua K, Pinaud E, Alt FW. Transcription-targeted DNA deamination by the AID antibody diversification enzyme. *Nature* 2003;422(6933):726–30.
- [64] Mond JJ, Lees A, Snapper CM. T cell-independent antigens type 2. *Annu Rev Immunol* 1995;13:655–92.
- [65] Szomolanyi-Tsuda E, Welsh RM. T-cell-independent antiviral antibody responses. *Curr Opin Immunol* 1998;10(4):431–5.
- [66] Cyster JG. B cells on the front line. *Nat Immunol* 2000;1(1):9–10.
- [67] Fagarasan S, Honjo T. T-Independent immune response: new aspects of B cell biology. *Science* 2000;290(5489):89–92.
- [68] Cerutti A, Qiao X, He B. Plasmacytoid dendritic cells and the regulation of immunoglobulin heavy chain class switching. *Immunol Cell Biol* 2005;83(5):554–62.
- [69] Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, et al. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. *Nat Immunol* 2002;3(9):822–9.
- [70] Liu YJ. Thymic stromal lymphopoietin: master switch for allergic inflammation. *J Exp Med* 2006;203(2):269–73.
- [71] Huston DP, Liu YJ. Thymic stromal lymphopoietin: a potential therapeutic target for allergy and asthma. *Curr Allergy Asthma Rep* 2006;6(5):372–6.
- [72] Soumelis V, Reche PA, Kanzler H, Yuan W, Edward G, Homey B, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002;3(7):673–80.
- [73] Wang YH, Ito T, Wang YH, Homey B, Watanabe N, Martin R, et al. Maintenance and polarization of human TH2 central memory T cells by thymic stromal lymphopoietin-activated dendritic cells. *Immunity* 2006;24(6):827–38.
- [74] Yoo J, Omori M, Gyarmati D, Zhou B, Aye T, Brewer A, et al. Spontaneous atopic dermatitis in mice expressing an inducible thymic stromal

- lymphopoietin transgene specifically in the skin. *J Exp Med* 2005; 202(4):541–9.
- [75] Ito T, Wang YH, Duramad O, Hori T, Delespesse GJ, Watanabe N, et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* 2005;202(9):1213–23.
- [76] Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity* 2001;15(6):985–95.
- [77] Hurst SD, Muchamuel T, Gorman DM, Gilbert JM, Clifford T, Kwan S, et al. New IL-17 family members promote Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol* 2002; 169(1):443–53.
- [78] Ikeda K, Nakajima H, Suzuki K, Kagami S, Hirose K, Suto A, et al. Mast cells produce interleukin-25 upon Fc epsilon RI-mediated activation. *Blood* 2003;101(9):3594–6.
- [79] Fallon PG, Ballantyne SJ, Mangan NE, Barlow JL, Dasvarma A, Hewett DR, et al. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J Exp Med* 2006;203(4):1105–16.
- [80] Okamura H, Tsutsi H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, et al. Cloning of a new cytokine that induces IFN-gamma production by T cells. *Nature* 1995;378(6552):88–91.
- [81] Tsutsui H, Yoshimoto T, Hayashi N, Mizutani H, Nakanishi K. Induction of allergic inflammation by interleukin-18 in experimental animal models. *Immunol Rev* 2004;202:115–38.
- [82] Leite-De-Moraes MC, Hameg A, Pacilio M, Koezuka Y, Taniguchi M, Van Kaer L, et al. IL-18 enhances IL-4 production by ligand-activated NKT lymphocytes: a pro-Th2 effect of IL-18 exerted through NKT cells. *J Immunol* 2001;166(2):945–51.
- [83] Yoshimoto T, Tsutsui H, Tominaga K, Hoshino K, Okamura H, Akira S, et al. IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. *Proc Natl Acad Sci U S A* 1999;96(24):13962–6.
- [84] Konishi H, Tsutsui H, Murakami T, Yumikura-Futatsugi S, Yamanaka K, Tanaka M, et al. IL-18 contributes to the spontaneous development of atopic dermatitis-like inflammatory skin lesion independently of IgE/stat6 under specific pathogen-free conditions. *Proc Natl Acad Sci U S A* 2002;99(17):11340–5.
- [85] Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity* 2005;23(5):479–90.
- [86] Brint EK, Xu D, Liu H, Dunne A, McKenzie AN, O'Neill LA, et al. ST2 is an inhibitor of interleukin 1 receptor and Toll-like receptor 4 signaling and maintains endotoxin tolerance. *Nat Immunol* 2004;5(4): 373–9.
- [87] Coyle AJ, Lloyd C, Tian J, Nguyen T, Eriksson C, Wang L, et al. Crucial role of the interleukin 1 receptor family member T1/ST2 in T helper cell type 2-mediated lung mucosal immune responses. *J Exp Med* 1999; 190(7):895–902.
- [88] Lohning M, Stroehmann A, Coyle AJ, Grogan JL, Lin S, Gutierrez-Ramos JC, et al. T1/ST2 is preferentially expressed on murine Th2 cells, independent of interleukin 4, interleukin 5, and interleukin 10, and important for Th2 effector function. *Proc Natl Acad Sci U S A* 1998;95(12):6930–5.
- [89] Lohning M, Grogan JL, Coyle AJ, Yazdanbakhsh M, Meisel C, Gutierrez-Ramos JC, et al. T1/ST2 expression is enhanced on CD4+ T cells from schistosome egg-induced granulomas: analysis of Th cell cytokine coexpression ex vivo. *J Immunol* 1999;162(7):3882–9.
- [90] Moritz DR, Rodewald HR, Gheyselinck J, Klemenz R. The IL-1 receptor-related T1 antigen is expressed on immature and mature mast cells and on fetal blood mast cell progenitors. *J Immunol* 1998;161(9):4866–74.
- [91] Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J Exp Med* 2000;191(6):1069–76.
- [92] Xu D, Chan WL, Leung BP, Huang F, Wheeler R, Piedrafita D, et al. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. *J Exp Med* 1998;187(5):787–94.
- [93] Wilkinson PC, Liew FY. Chemoattraction of human blood T lymphocytes by interleukin-15. *J Exp Med* 1995;181(3):1255–9.
- [94] Mori A, Suko M, Kaminuma O, Inoue S, Ohmura T, Nishizaki Y, et al. IL-15 promotes cytokine production of human T helper cells. *J Immunol* 1996;156(7):2400–5.
- [95] Hamelmann E, Oshiba A, Paluh J, Bradley K, Loader J, Potter TA, et al. Requirement for CD8+ T cells in the development of airway hyperresponsiveness in a murine model of airway sensitization. *J Exp Med* 1996;183(4):1719–29.
- [96] Ruckert R, Brandt K, Braun A, Hoymann HG, Herz U, Budagian V, et al. Blocking IL-15 prevents the induction of allergen-specific T cells and allergic inflammation in vivo. *J Immunol* 2005;174(9):5507–15.
- [97] Eglite S, Pluss K, Dahinden CA. Requirements for C5a receptor-mediated IL-4 and IL-13 production and leukotriene C4 generation in human basophils. *J Immunol* 2000;165(4):2183–9.
- [98] Li H, Sim TC, Alam R. IL-13 released by and localized in human basophils. *J Immunol* 1996;156(12):4833–8.
- [99] Gibbs BF, Haas H, Falcone FH, Albrecht C, Vollrath IB, Noll T, et al. Purified human peripheral blood basophils release interleukin-13 and preformed interleukin-4 following immunological activation. *Eur J Immunol* 1996;26(10):2493–8.
- [100] Devouassoux G, Foster B, Scott LM, Metcalfe DD, Prussin C. Frequency and characterization of antigen-specific IL-4- and IL-13-producing basophils and T cells in peripheral blood of healthy and asthmatic subjects. *J Allergy Clin Immunol* 1999;104(4 Pt 1):811–9.
- [101] Poorafshar M, Helmby H, Troye-Blomberg M, Hellman L. MMCP-8, the first lineage-specific differentiation marker for mouse basophils. Elevated numbers of potent IL-4-producing and MMCP-8-positive cells in spleens of malaria-infected mice. *Eur J Immunol* 2000;30(9):2660–8.
- [102] Gessner A, Mohrs K, Mohrs M. Mast cells, basophils, and eosinophils acquire constitutive IL-4 and IL-13 transcripts during lineage differentiation that are sufficient for rapid cytokine production. *J Immunol* 2005; 174(2):1063–72.
- [103] Gauchat JF, Henchoz S, Mazzei G, Aubry JP, Brunner T, Blasey H, et al. Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* 1993;365(6444):340–3.
- [104] Pene J, Rousset F, Briere F, Chretien I, Bonnefoy JY, Spits H, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons gamma and alpha and prostaglandin E2. *Proc Natl Acad Sci U S A* 1988;85(18):6880–4.
- [105] Chen YH, Bieneman AP, Creticos PS, Chichester KL, Schroeder JT. IFN-alpha inhibits IL-3 priming of human basophil cytokine secretion but not leukotriene C4 and histamine release. *J Allergy Clin Immunol* 2003;112(5):944–50.
- [106] Leonard WJ, Spolski R. Interleukin-21: a modulator of lymphoid proliferation, apoptosis and differentiation. *Nat Rev Immunol* 2005;5(9): 688–98.
- [107] Wurster AL, Rodgers VL, Satoskar AR, Whitters MJ, Young DA, Collins M, et al. Interleukin 21 is a T helper (Th) cell 2 cytokine that specifically inhibits the differentiation of naive Th cells into interferon gamma-producing Th1 cells. *J Exp Med* 2002;196(7):969–77.
- [108] Strengell M, Sareneva T, Foster D, Julkunen I, Matikainen S. IL-21 up-regulates the expression of genes associated with innate immunity and Th1 response. *J Immunol* 2002;169(7):3600–5.
- [109] Strengell M, Matikainen S, Siren J, Lehtonen A, Foster D, Julkunen I, et al. IL-21 in synergy with IL-15 or IL-18 enhances IFN-gamma production in human NK and T cells. *J Immunol* 2003;170(11):5464–9.
- [110] Ozaki K, Spolski R, Feng CG, Qi CF, Cheng J, Sher A, et al. A critical role for IL-21 in regulating immunoglobulin production. *Science* 2002; 298(5598):1630–4.
- [111] Elliott J, Johnston JA. SOCS: role in inflammation, allergy and homeostasis. *Trends Immunol* 2004;25(8):434–40.
- [112] 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* 2002;277(32): 28830–5.
- [113] 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* 2003;170(4):1887–93.
- [114] Diehl S, Anguita J, Hoffmeyer A, Zapton T, Ihle JN, Fikrig E, et al. Inhibition of Th1 differentiation by IL-6 is mediated by SOCS1. *Immunity* 2000;13(6):805–15.
- [115] Yasukawa H, Sasaki A, Yoshimura A. Negative regulation of cytokine signaling pathways. *Annu Rev Immunol* 2000;18:143–64.
- [116] Seki Y, Inoue H, Nagata N, Hayashi K, Fukuyama S, Matsumoto K, et al. SOCS-3 regulates onset and maintenance of T(H)2-mediated allergic responses. *Nat Med* 2003;9(8):1047–54.
- [117] Marine JC, McKay C, Wang D, Topham DJ, Parganas E, Nakajima H, et al. SOCS3 is essential in the regulation of fetal liver erythropoiesis. *Cell* 1999;98(5):617–27.
- [118] Roberts AW, Robb L, Rakar S, Hartley L, Cluse L, Nicola NA, et al. Placental defects and embryonic lethality in mice lacking suppressor of cytokine signaling 3. *Proc Natl Acad Sci U S A* 2001;98(16):9324–9.
- [119] Seki Y, Hayashi K, Matsumoto A, Seki N, Tsukada J, Ransom J, et al. Expression of the suppressor of cytokine signaling-5 (SOCS5) negatively regulates IL-4-dependent STAT6 activation and Th2 differentiation. *Proc Natl Acad Sci U S A* 2002;99(20):13003–8.
- [120] Rush JS, Hasbold J, Hodgkin PD. 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* 2002;168(6):2676–82.
- [121] 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* 2000;165(10):5530–6.
- [122] Sherr E, Macy E, Kimata H, Gilly M, Saxon A. Binding the low affinity Fc epsilon R on B cells suppresses ongoing human IgE synthesis. *J Immunol* 1989;142(2):481–9.
- [123] Yu P, Kosco-Vilbois M, Richards M, Kohler G, Lamers MC. Negative feedback regulation of IgE synthesis by murine CD23. *Nature* 1994;369(6483):753–6.
- [124] Payet M, Conrad DH. IgE regulation in CD23 knockout and transgenic mice. *Allergy* 1999;54(11):1125–9.
- [125] Saxon A, Ke Z, Bahati L, Stevens RH. 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* 1990;86(3 Pt 1):333–44.
- [126] Jabara HH, Ahern DJ, Vercelli D, Geha RS. Hydrocortisone and IL-4 induce IgE isotype switching in human B cells. *J Immunol* 1991;147(5):1557–60.
- [127] Jabara HH, Brodeur SR, Geha RS. Glucocorticoids upregulate CD40 ligand expression and induce CD40L-dependent immunoglobulin isotype switching. *J Clin Invest* 2001;107(3):371–8.
- [128] Beato M, Herrlich P, Schutz G. Steroid hormone receptors: many actors in search of a plot. *Cell* 1995;83(6):851–7.
- [129] Moffat GJ, Tack BF. Regulation of C4b-binding protein gene expression by the acute-phase mediators tumor necrosis factor-alpha, interleukin-6, and interleukin-1. *Biochemistry* 1992;31(49):12376–84.
- [130] Moffat GJ, Vik DP, Noack D, Tack BF. Complete structure of the murine C4b-binding protein gene and regulation of its expression by dexamethasone. *J Biol Chem* 1992;267(28):20400–6.
- [131] Fokkens WJ, Vinke JG, KleinJan A. Local IgE production in the nasal mucosa: a review. *Am J Rhinol* 2000;14(5):299–303.
- [132] KleinJan A, Vinke JG, Severijnen LW, Fokkens WJ. Local production and detection of (specific) IgE in nasal B-cells and plasma cells of allergic rhinitis patients. *Eur Respir J* 2000;15(3):491–7.
- [133] Ghaffar O, Durham SR, Al-Ghamdi K, Wright E, Small P, Frenkiel S, et al. Expression of IgE heavy chain transcripts in the sinus mucosa of atopic and nonatopic patients with chronic sinusitis. *Am J Respir Cell Mol Biol* 1998;18(5):706–11.
- [134] Takhar P, Smurthwaite L, Coker HA, Fear DJ, Banfield GK, Carr VA, et al. Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis. *J Immunol* 2005;174(8):5024–32.
- [135] Roopenian DC, Christianson GJ, Sproule TJ, Brown AC, Akilesh S, Jung N, et al. The MHC class I-like IgG receptor controls perinatal IgG transport, IgG homeostasis, and fate of IgG–Fc-coupled drugs. *J Immunol* 2003;170(7):3528–33.
- [136] Zhang Y, Leaves NI, Anderson GG, Ponting CP, Broxholme J, Holt R, et al. Positional cloning of a quantitative trait locus on chromosome 13q14 that influences immunoglobulin E levels and asthma. *Nat Genet* 2003;34(2):181–6.
- [137] Allen M, Heinzmann A, Noguchi E, Abecasis G, Broxholme J, Ponting CP, et al. Positional cloning of a novel gene influencing asthma from chromosome 2q14. *Nat Genet* 2003;35(3):258–63.
- [138] Nadal MS, Ozaita A, Amarillo Y, Vega-Saenz de Miera E, Ma Y, Mo W, et al. The CD26-related dipeptidyl aminopeptidase-like protein DPPX is a critical component of neuronal A-type K⁺ channels. *Neuron* 2003;37(3):449–61.
- [139] Van Eerdewegh P, Little RD, Dupuis J, Del Mastro RG, Falls K, Simon J, et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002;418(6896):426–30.
- [140] Kere J, Laitinen T. Positionally cloned susceptibility genes in allergy and asthma. *Curr Opin Immunol* 2004;16(6):689–94.
- [141] Laitinen T, Daly MJ, Rioux JD, Kauppi P, Laprise C, Petays T, et al. A susceptibility locus for asthma-related traits on chromosome 7 revealed by genome-wide scan in a founder population. *Nat Genet* 2001;28(1):87–91.
- [142] Laitinen T, Polvi A, Rydman P, Vendelin J, Pulkkinen V, Salmikangas P, et al. Characterization of a common susceptibility locus for asthma-related traits. *Science* 2004;304(5668):300–4.
- [143] Gupte J, Cutler G, Chen JL, Tian H. Elucidation of signaling properties of vasopressin receptor-related receptor 1 by using the chimeric receptor approach. *Proc Natl Acad Sci U S A* 2004;101(6):1508–13.
- [144] Leonard JP, Sherman ML, Fisher GL, Buchanan LJ, Larsen G, Atkins MB, et al. Effects of single-dose interleukin-12 exposure on interleukin-12-associated toxicity and interferon-gamma production. *Blood* 1997;90(7):2541–8.
- [145] Clark JI, Kuzel TM, Lestingi TM, Fisher SG, Sorokin P, Martone B, et al. A multi-institutional phase II trial of a novel inpatient schedule of continuous interleukin-2 with interferon alpha-2b in advanced renal cell carcinoma: major durable responses in a less highly selected patient population. *Ann Oncol* 2002;13(4):606–13.
- [146] Dillman RO, Wiemann MC, Tai DF, Depriest CB, Soori G, Stark JJ, et al. Phase II trial of subcutaneous interferon followed by intravenous hybrid bolus/continuous infusion interleukin-2 in the treatment of renal cell carcinoma: final results of Cancer Biotherapy Research Group 95-09. *Cancer Biother Radiopharm* 2006;21(2):130–7.
- [147] Vanrenterghem Y, Barany P, Mann JF, Kerr PG, Wilson J, Baker NF, et al. Randomized trial of darbepoetin alfa for treatment of renal anemia at a reduced dose frequency compared with rHuEPO in dialysis patients. *Kidney Int* 2002;62(6):2167–75.
- [148] Cody J, Daly C, Campbell M, Donaldson C, Khan I, Rabindranath K, et al. Recombinant human erythropoietin for chronic renal failure anaemia in pre-dialysis patients. *Cochrane Database Syst Rev* 2005;(3):CD003266.
- [149] Kruse N, Tony HP, Sebald W. Conversion of human interleukin-4 into a high affinity antagonist by a single amino acid replacement. *EMBO J* 1992;11(9):3237–44.
- [150] Hahn C, Teufel M, Herz U, Renz H, Erb KJ, Wohlleben G, et al. Inhibition of the IL-4/IL-13 receptor system prevents allergic sensitization without affecting established allergy in a mouse model for allergic asthma. *J Allergy Clin Immunol* 2003;111(6):1361–9.
- [151] Feldmann M, Brennan FM, Foxwell BM, Taylor PC, Williams RO, Maini RN. Anti-TNF therapy: where have we got to in 2005? *J Autoimmun* 2005;25(Suppl.):26–8.
- [152] Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwiertman WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 2001;345(15):1098–104.
- [153] Nowak D. Management of asthma with anti-immunoglobulin E: a review of clinical trials of omalizumab. *Respir Med* 2006.
- [154] Buhl R. Anti-IgE antibodies for the treatment of asthma. *Curr Opin Pulm Med* 2005;11(1):27–34.

- [155] Belliveau PP, Lahoz MR. Evaluation of omalizumab from a health plan perspective. *J Manag Care Pharm* 2005;11(9):735–45.
- [156] Hellman L. Profound reduction in allergen sensitivity following treatment with a novel allergy vaccine. *Eur J Immunol* 1994;24(2):415–20.
- [157] Johansson J, Ledin A, Vernerström M, Lovgren-Bengtsson K, Hellman L. Identification of adjuvants that enhance the therapeutic antibody response to host IgE. *Vaccine* 2004;22(21–22):2873–80.
- [158] Waterston AM, Gumbrell L, Bratt T, Waller S, Gustav-Aspland J, L'Herménier C, et al. Phase I study of TNF α autovaccine in patients with metastatic cancer. *Cancer Immunol Immunother* 2005;54(9):848–57.
- [159] Dalum I, Butler DM, Jensen MR, Hindersson P, Steinaa L, Waterston AM, et al. Therapeutic antibodies elicited by immunization against TNF- α . *Nat Biotechnol* 1999;17(7):666–9.
- [160] Hertz M, Mahalingam S, Dalum I, Klysner S, Mattes J, Neisig A, et al. Active vaccination against IL-5 bypasses immunological tolerance and ameliorates experimental asthma. *J Immunol* 2001;167(7):3792–9.
- [161] Renard V, Sonderbye L, Ebbeløj K, Rasmussen PB, Gregorius K, Gottschalk T, et al. HER-2 DNA and protein vaccines containing potent Th cell epitopes induce distinct protective and therapeutic antitumor responses in HER-2 transgenic mice. *J Immunol* 2003;171(3):1588–95.
- [162] Waterston AM, Salway F, Andreaskos E, Butler DM, Feldmann M, Coombes RC. TNF autovaccination induces self anti-TNF antibodies and inhibits metastasis in a murine melanoma model. *Br J Cancer* 2004;90(6):1279–84.