

Role of interleukin-4 and interleukin-13 in synthesis of IgE and expression of CD23 by human B cells

Punnonen J, Aversa G, Cocks BG, de Vries JE. Role of interleukin-4 and interleukin-13 in synthesis of IgE and expression of CD23 by human B cells. Allergy 1994; 49: 576–586. © Munksgaard 1994.

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Accepted for publication 28 March 1994

The effector functions of antibodies, such as complement fixation, Fc-receptor binding, and traversal of basal membranes, are determined by the constant regions of the antibodies. IgE is the least abundant immunoglobulin (Ig) isotype in human serum, but it is the most important isotype mediating allergic reactions (102). IgE probably evolved because of its protective effect against parasitic infections (45, 105). It is only IgE that can bind to high-affinity IgE receptors (Fc_εRI) expressed on mast cells and basophils. The cross-linking of these receptors by interaction of the allergen and Fc_εRI-bound IgE leads to degranulation of the mast cells and rapid release of mediator molecules, such as histamine, prostaglandins, and leukotrienes, causing allergic symptoms in the target organs. In the absence of high-affinity IgE receptors, no anaphylactic reactions can occur (30).

B-cell proliferation and differentiation into Ig-secreting cells is initiated by specific recognition of antigen by cell-surface Ig. This generally occurs in the lymph nodes, leading to formation of germinal centers, where follicular dendritic cells serve as efficient antigen-presenting cells (15). The antigen recognition induces rapid B-cell proliferation, giving rise to dark zones of germinal centers. Differentiating B cells then move into the light zones, where their variable (V) region genes also undergo somatic mutations to increase the affinity of the secreted anti-

bodies (1, 90). The first isotypes to be produced are IgM and IgD; to produce Ig of other isotypes, B cells have to undergo class switching (18, 73). The genes for the constant regions of heavy chains (CH) are clustered at the 3' end of the Ig heavy-chain locus on human chromosome 14 and on mouse chromosome 12. The human heavy-chain locus comprises nine functional genes and two pseudogenes ordered 5' μ - δ - γ 3- γ 1- ψ ϵ - α 1- ψ γ - γ 2- γ 4- ϵ - α 2 3'. Heavy-chain class switching occurs by DNA recombination, which is mediated by repetitive segments known as switch regions (S) that are located a few kilobases 5' to each heavy chain locus (18, 32, 52, 111). This recombination, which occurs through S-S joining, brings a specific variable region adjacent to a new heavy-chain gene. B-cell differentiation and isotype switching are heavily regulated by CD4⁺ T helper cells, which provide costimulatory signals in both soluble and membrane-bound forms (15, 27, 78, 96). Isotype switching is directed by cytokines, possibly by regulation of the accessibility of CH genes and their S regions to a common recombinase system (32, 111).

Cytokines are required for both B-cell proliferation and differentiation, and they also direct Ig isotype switching. Murine B cells switch to IgG2a production in response to interferon- γ (IFN- γ) (97), and both murine and human B cells switch to IgE

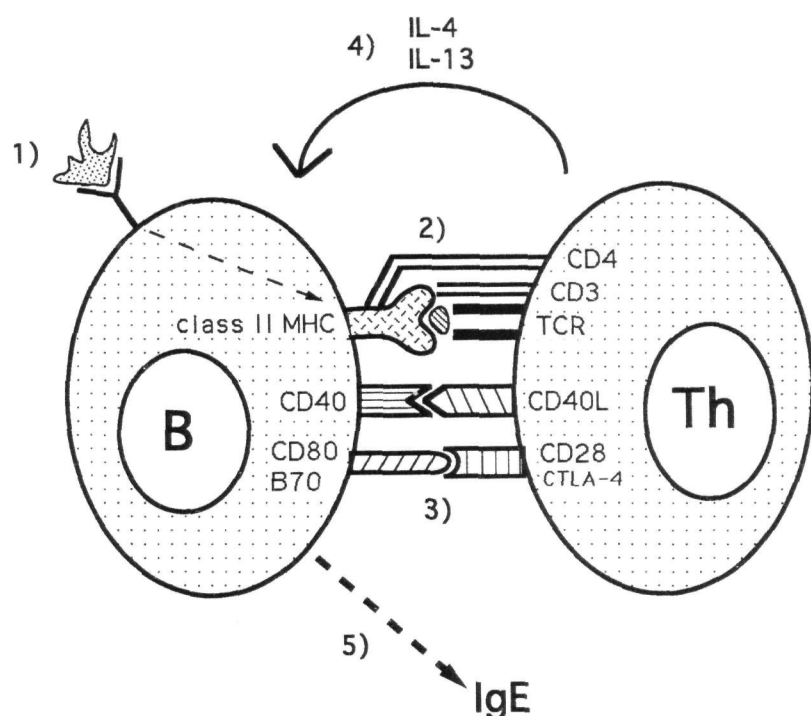


Fig. 1. Diagram of differentiation of resting B cell into IgE-secreting cell. 1) Antigen/allergen is recognized by sIgM, and is internalized and processed within cell. 2) Antigenic peptide is presented to T helper cell in context of MHC class II molecules. Peptide-MHC class II complex is recognized by complex which comprises T-cell receptor (TCR), and CD3 and CD4 molecules. 3) Recognition of antigenic peptide leads to T-cell activation and expression of ligand for CD40 (CD40L). CD40/CD40L interaction results in B-cell activation and upregulation of CD80 (B7) and B70. Interaction of CD80/B70 with their ligands CD28 and CTLA-4 further enhances T-cell activation. 4) Activated T helper cells produce cytokines, such as IL-4 and IL-13. 5) IL-4 and IL-13 induce B-cell proliferation, Ig isotype switching, and differentiation into IgE-secreting cells.

synthesis in response to interleukin-4 (IL-4) (20, 25, 39, 79, 97, 108). IL-4 also induces IgG1 and IgG4 production by murine and human B cells, respectively (39, 64, 97). In addition, transforming growth factor (TGF)- β directs IgA switching both in murine and human B cells (19, 23, 107). Recently, IL-13 was shown to be another cytokine which induces IgG4 and IgE switching in human B cells (17, 82, 116). In addition to cytokines, contact-mediated signals derived from activated CD4⁺ T cells are required for B-cell proliferation, differentiation, and Ig isotype switching (Fig. 1).

Signaling through CD40 is critical in Ig isotype switching

CD40 is a cell-surface glycoprotein, which belongs to the tumor-necrosis factor (TNF)/nerve growth factor receptor family (15). This receptor family includes, in addition to CD40, two TNF receptors (types I and II); the low-affinity nerve growth factor receptor; and CD27, CD30, and the Fas antigen. CD40 is expressed on immature and mature B cells, thymic epithelial cells, and bone-marrow-derived

dendritic cells, and follicular dendritic cells (15). Studies indicating that monoclonal antibodies (mAbs) specific for CD40 can trigger B-cell activation and proliferation suggest an important function for this molecule in the regulation of B-cell activation (14). Subsequently, anti-CD40 mAbs were shown to replace activated T cells in induction of IgE synthesis by IL-4 (40, 49, 92, 115). Furthermore, long-term B-cell lines were established with IL-4 and anti-CD40 mAbs cross-linked to Fc γ RII expressed on murine L cells (9).

The ability of anti-CD40 mAbs to replace T cells in induction of IgE synthesis suggested the presence of the ligand for CD40 (CD40L) on activated T cells. Recently, the ligands for both murine and human CD40 have been cloned from cDNA libraries constructed from activated T cells (3, 17, 47, 100). These cDNAs encode a 33-kDa type II glycoprotein with significant homology to TNF- α and TNF- β . In contrast to activated CD4⁺ T cells, resting T helper cells, which cannot assist B-cell differentiation, do not express CD40L. Transfectants expressing CD40L induced B-cell proliferation and differentiation into IgE-secreting cells in the presence of IL-4, indicating that all the signals required for IgE switching can be delivered by CD40L and IL-4 (3, 17, 47, 100). However, the signaling pathway through CD40 does not seem to be identical to that induced by activated CD4⁺ T cells (40), suggesting that additional membrane-bound molecules are involved in induction of B-cell proliferation, isotype switching, and differentiation.

The gene encoding CD40L was mapped on the X-chromosomal location q26.3–q27.1, the same region in which a gene defect in patients with X-linked hyper-IgM syndrome was localized (4, 29, 38). Patients with hyper-IgM syndrome, who have minimal levels of IgG, IgA, and IgE in their sera, have mutations in their CD40L gene, resulting in defective expression of CD40L and impaired isotype switching *in vivo* (4, 29, 38). In addition, T cells from a subset of patients suffering from common variable immunodeficiency were found to express suboptimal levels of CD40L (33), further supporting an essential role for CD40/CD40L interactions in productive T-cell and B-cell collaboration.

Expression of CD40L is not restricted to T cells. Human mast cells have been shown to express CD40L, which was functional in that mast cells could induce IgE synthesis by human B cells in the presence of IL-4 (43). Mast cells have been shown to produce IL-4 and IL-5 after activation (11, 43), suggesting that mast cells may play a role in the regulation of IgE synthesis *in vivo*. These results also suggest that Ig isotype switching may occur outside the germinal centers in the periphery. It remains to be determined whether mast cells are capable of

inducing IgE synthesis *in vivo*. This would be somewhat surprising, because no antigen-specific receptor molecules on mast cells have been identified, and we do not know how the specificity of the mast-cell-induced Ig synthesis could be controlled.

Other molecules involved in T-cell and B-cell interactions

The results indicating that anti-CD40 mAbs and activated CD4⁺ T cells have synergistic effects on IgE synthesis by B cells cultured in the presence of optimal concentrations of IL-4 suggest that membrane-bound molecules other than CD40L may also be involved in the events leading to B-cell activation, proliferation, and differentiation (40). Indeed, the 26-kDa membrane form of TNF- α (mTNF- α), expressed on CD4⁺ T cells after activation, is another molecule associated with productive T-cell and B-cell interactions. mAbs specific for the mTNF- α strongly inhibit IgE synthesis induced by activated CD4⁺ T-cell clones or their membranes (7). Similarly, mAbs specific for the type A TNF receptor (p55) inhibit polyclonal B-cell activation induced by activated CD4⁺ T cells and IL-4 (7). In contrast to anti-CD40 mAbs, anti-TNF- α or anti-TNF-receptor mAbs do not have any stimulatory effects on B cells. T cells infected with human immunodeficiency virus (HIV) were also shown to express high levels of mTNF- α , which may play a role in the polyclonal B-cell activation observed in patients infected with HIV (66). Thus, although CD40/CD40L interaction appears to be essential for B-cell differentiation and Ig isotype switching, other molecules, including mTNF- α , can modulate productive B-cell and T-cell interactions.

Activation of T helper cells is crucial for optimal expression of CD40L as well as for cytokine production. Recognition of the class II MHC-peptide complex by the TCR/CD3 alone does not lead to T-cell proliferation and cytokine synthesis. A second costimulatory signal that is mediated by CD28 is required (Fig. 1). The membrane antigen CD80 (B7), expressed on activated B cells, monocytes, and dendritic cells, has been shown to be the ligand for CD28 (36, 63). Anti-CD80 mAbs inhibit mixed lymphocyte reactions, and B-cell lines transfected with CD80 have enhanced capacity to stimulate primary T-cell responses (56, 62), showing the importance of CD28/CD80 interactions in T-cell activation. The CD28-mediated costimulatory signal also enhances cytokine production by CD4⁺ T helper cells through stabilization of cytokine mRNA (61, 62).

Recently, another ligand for CD28 has been cloned and sequenced (8, 37). This new protein, termed B70 or B7-2, which has limited homology to CD80, is also a member of the immunoglobulin superfamily.

It is expressed on resting monocytes and dendritic cells, and on activated T, NK, and B cells (8, 37). Cells transfected with B70/B7-2 induce T-cell activation, and mAbs specific for B70/B7-2 inhibit virtually completely the primary mixed lymphocyte response (MLR), whereas anti-CD80 mAbs only partially inhibit the alloantigen-derived response. These data suggest that B70/B7-2, constitutively expressed on monocytes, is the primary ligand for CD28 in induction of MLR (8).

The role of IL-4 in IgE switching

It is well established *in vitro* that IL-4 induces IgG4/IgE and IgG1/IgE switching in human and murine B cells, respectively (20, 25, 39, 64, 79, 97, 108). Several other cytokines are known to modulate IL-4-induced IgE synthesis. IFN- α (79), IFN- γ (79), TGF- β (41), IL-8 (53), and IL-12 (54) have inhibitory effects, whereas IL-2 (70), IL-5 (80), IL-6 (109), and TNF- α (41) enhance IL-4-induced IgE synthesis. IL-10 also blocks IgE synthesis by peripheral blood mononuclear cells (PBMC) by inhibiting the accessory cell function of monocytes (87), but it also directly stimulates B cells cultured in the presence of IL-4 and anti-CD40 mAbs cross-linked to CD32 (Fc γ R2) on murine L cells (93).

Studies using the murine model suggest an obligatory role for IL-4 in induction of IgE synthesis. IL-4-deficient mice, generated by targeted gene disruption, have normal levels of IgM, IgG2a, IgG3, and IgA in their sera (58). Although the levels of IgG1 are lower than in wild-type animals, IgG1 is produced in significant amounts, indicating that an IL-4-independent induction of IgG1 switching occurs in these animals. Importantly, these mice do not produce any IgE after nematode infection, suggesting that IL-4 is required for IgE switching and IgE synthesis *in vivo* (58). Moreover, administration of anti-IL-4 mAbs blocks virtually completely IgE synthesis in mice, supporting an obligatory role for IL-4 in the regulation of IgE synthesis in mice (34). In addition to deficient IgE synthesis, IL-4-deficient mice show a two- to threefold lower eosinophilia than wild-type mice after a primary nematode infection (55). These mice also have strongly impaired development of Th2-type T cells, and the production of not only IL-4, but also of other Th2-type cytokines such as IL-5 and IL-10, is highly impaired (55). Therefore, it is difficult to determine whether it is the deficiency of IL-4 as such that causes the defective eosinophilia and IgE synthesis, or whether they are due to the secondary lack of other Th2-type cytokines. The latter appears to be true at least in the case of defective eosinophilia, since injection of recombinant IL-5 completely restores the level of eosinophilia in IL-4-deficient mice (55).

In the human system, only indirect evidence about the role of IL-4 in the regulation of IgE synthesis *in vivo* is available. It has been suggested that the B cells of atopic patients differ from those of healthy persons in their requirements for induction of IgE synthesis (104). However, IL-4 has been shown to induce similar levels of IgE synthesis *in vitro* by B cells derived from healthy persons and atopic patients (13, 88). On the other hand, T cells derived from atopic patients, as well as allergen-specific T-cell clones, produce high levels of IL-4, which is likely to contribute to the enhanced IgE synthesis observed in these patients (26, 76, 113). In addition, anti-IL-4 mAbs strongly, but not completely, inhibit the IgE synthesis induced by allergen-specific T-cell clones without significantly affecting IgM, IgG, or IgA synthesis (24, 25, 27). IL-4 has been known as the only cytokine inducing germ-line or productive ϵ -transcription or IgE synthesis by human B cells. However, a non-IL-4-producing T-cell clone induced germ-line ϵ -transcription in purified B cells, indicating that an IL-4-independent pathway of induction of germ-line ϵ -transcription exists (41, 44). Recently, IL-13 has been shown to be another cytokine that, in addition to IL-4, can induce IgG4 and IgE synthesis by human B cells.

IL-13 induces IgE switching in human B cells

The human cDNA homologue of a mouse cDNA sequence encoding P600, a protein produced by murine Th2 clones after activation, was recently cloned and expressed (68, 69). Human P600 protein was biologically active and was shown to induce monocyte and B-cell growth and differentiation (68). Therefore, this novel cytokine was designated IL-13. The human cDNA sequence is 66% homologous to that of the mouse and encodes a protein that is 58% homologous to mouse IL-13 (68, 69). Although IL-13 and IL-4 share only $\approx 30\%$ homology, all the residues contributing to the hydrophobic structural core of IL-4 are conserved or have conservative hydrophobic replacements in IL-13 (117). Human IL-13 is a nonglycosylated protein of 132 amino acids with a mol. wt. of 12000. The gene encoding IL-13 is located in chromosome 5q31 in the same region as the genes encoding IL-3, IL-4, IL-5, and GM-CSF (116). It is produced by activated CD4⁺ and CD8⁺ T cells, but the expression seems to be more abundant in CD4⁺ T cells (68). Although it is a Th2 cytokine in the mouse, it appears to be produced by Th0-, Th1-, and Th2-like human T-cell clones (116).

IL-13 induces IgE synthesis by unfractionated PBMC as well as by highly purified B cells cultured in the presence of activated CD4⁺ T-cell clones or their membranes (82). In addition, IL-13 induces

B-cell proliferation and differentiation into IgE-secreting cells in the presence of the ligand for CD40 (17). IL-13 also induces significant levels of IgM, total IgG, and IgG4 production. Neutralizing anti-IL-4 mAbs failed to inhibit IL-13-induced IgE synthesis, indicating that the IgE synthesis observed was not mediated through induction of IL-4 production by T cells (82). Culturing of highly purified sIgD⁺ B cells in the presence of IL-13 and activated, non-IL-4-producing CD4⁺ T cells also resulted in induction of IgE synthesis, confirming that IL-13-induced IgE synthesis was due to Ig isotype switching, and not to an outgrowth of a few IgE-committed B cells. No additive or synergistic effects were observed when both IL-4 and IL-13 were used at optimal concentrations (82). These data suggest that IL-13 produced by allergen-specific CD4⁺ T cells may play an important role in induction of IgE switching and in the maintenance of IgE synthesis by B cells of atopic patients.

Like IL-4, IL-13 induces proliferation of human B cells activated by anti-IgM or anti-CD40 mAbs or by the ligand for CD40 (17, 68). The mean level of IgE produced in response to IL-13 is generally two- to fourfold lower than that induced by IL-4 (82). B-cell proliferation induced by IL-13 in the presence of anti-IgM mAbs is also significantly lower than that induced by IL-4 (68). However, IL-13 and IL-4 are equally effective in inducing proliferation of B cells activated through their CD40 molecule in the presence of either anti-CD40 mAbs or the ligand for CD40 (17, 68).

IL-13 has IL-4-like effects also on human monocytes. Human monocytes cultured in the presence of IL-13 develop long processes and aggregate homotypically, and IL-13 appears to prolong the survival of monocytes (68). In addition, IL-13 induces phenotypic changes and affects cytokine synthesis by monocytes in a manner similar to that of IL-4 (28). Importantly, IL-13 inhibits production of IFN- α and IL-12 by monocytes (28). These cytokines induce development of Th1-like cells, suggesting that IL-13 may promote the development of Th2-like cells, which produce high levels of IL-4 and IL-13 (71, 116). Therefore, IL-13 may increase IgE synthesis not only directly by inducing IgE switching, but also indirectly by enhancing the development of Th2 cells.

Despite their similar effects on human B cells, the functions of IL-4 and IL-13 are not identical. IL-13, unlike IL-4, does not have growth-promoting effects on activated T cells or T-cell clones (74, 98). Furthermore, IL-13 has not been shown to induce IgE synthesis by murine B cells stimulated with lipopolysaccharide, a system in which IL-4 efficiently induces IgE switching. These data are consistent with the fact that no IgE is detected in IL-4-deficient mice after nematode infection, and suggest that mu-

rine B cells, in contrast to human B cells, do not express functional IL-13R.

Induction of CD23 expression by IL-4 and IL-13

CD23 is the low-affinity receptor for IgE; it is also termed Fc_εRII (21). It shows no homology to the high-affinity IgE receptor (Fc_εRI) expressed on mast cells and basophils. Two forms of CD23, Fc_εRIIa and Fc_εRIIb, which differ only in their N-terminal cytoplasmic portions, are generated through the use of different transcription sites and alternative RNA splicing (112). Fc_εRIIa is expressed exclusively by B cells, but Fc_εRIIb is also expressed on monocytes and Langerhans' cells after activation by IL-4 (21, 112). CD23 is a labile protein, in that a soluble fragment is released from the carboxy terminal extracellular portion of the molecule, giving rise to soluble CD23 (sCD23).

Like IL-4, IL-13 strongly upregulates CD23 expression on purified human B cells (82). Incubation of purified B cells with IL-13 resulted in induction of high-level CD23 expression on a proportion of B cells. Unlike IL-13, IL-4 induces CD23 expression on virtually all mature B cells, supporting the conclusion that the effects of IL-4 and IL-13 are not identical. IL-13 also upregulated expression of class II MHC molecules, sIgM, and CD72, the ligand for CD5 (106). These effects of IL-13 were similar to those observed with IL-4. CD23 expression was already detectable after a culture period of 24 h, but maximal responses were observed after 72 h of culture. IL-13 did not affect the expression of CD19, CD20, CD25, CD40, class I MHC antigen, CD80 (B7), ICAM-1, LFA-1, and LFA-3 (82).

Although the exact role of CD23 in the regulation of IgE synthesis remains to be determined, a strong correlation between CD23 expression and induction of IgE synthesis has been observed (80, 95). The finding that IL-13 induces significant expression of CD23 within 24 h indicates that CD23 expression precedes IL-13-induced ϵ -switching, confirming the correlation between induction of CD23 expression and subsequent IgE synthesis. (82). The studies on the role of sCD23 in the regulation of IgE synthesis have resulted in somewhat contradictory results. Supernatants of CD23⁺ B-cell lines were shown to enhance IgE synthesis, and mAbs specific for CD23 inhibited B-cell proliferation and differentiation (10, 65, 95). However, other studies suggest that recombinant sCD23 does not have any B-cell growth-promoting activity *in vitro* (103). Recently, it was shown that administration of mAbs specific for CD23 to mice strongly inhibits antigen-specific IgE synthesis, suggesting a role for the CD23 molecule in the regulation of IgE production *in vivo* (35). In

addition, CD21 was shown to be another ligand for IgE (5). Since CD21 is expressed on B cells, some T cells, and follicular dendritic cells, CD23 may play a role also in direct cell-cell interactions, resulting in B-cell activation, proliferation, and differentiation into IgE-secreting cells.

Induction of germ-line ϵ -transcription by IL-4 and IL-13

Induction of isotype switching is preceded by expression of the corresponding germ-line C_H gene, which is likely to be essential in the initiation of class switching (44, 50, 60, 91, 101). Human germ-line ϵ -mRNA comprises, in addition to the C_ε exons, an I_ε (initiate) exon located 3.5 kb upstream from C_ε and 5' from S_ε (44). IL-4 induces germ-line ϵ -gene transcription in normal human B cells as well as in EBV-transformed B-cell lines and Burkitt's lymphoma cells (42, 44, 48, 50). Germ-line ϵ -mRNA is detectable already after 2 days of culture in IL-4, whereas productive ϵ -mRNA is detectable only after 7 days of incubation (44). It has been suggested that germ-line ϵ -mRNA synthesis is critical to the recombination events that result in switching to IgE production. The level of germ-line ϵ -transcription correlates to the level of subsequent IgE synthesis. In addition, TGF- β and TNF- α , which inhibit and enhance germ-line ϵ -transcription, also inhibit and enhance IgE production, respectively (41). The importance of germ-line transcription for isotype switching has also been shown by deletion of I_{γ1} or I_{γ2b} regions by gene-targeting which resulted in a shutdown of class switching to $\gamma1$ and $\gamma2b$, respectively (51, 114).

Consistent with its capacity to induce IgE synthesis, IL-13 has been shown to induce germ-line ϵ -transcription in human B cells (82, Cocks et al., paper submitted). IL-13-induced germ-line ϵ -transcription is strongly upregulated by anti-CD40 mAbs or the ligand for CD40 transfected to COS-7 cells, but the CD40-mediated signal alone does not induce germ-line ϵ -transcription. The level of IL-13-induced germ-line ϵ -mRNA is less than that induced by IL-4 in the absence of other stimuli, but no difference can be observed in IL-4- and IL-13-induced responses when B cells are cultured in the presence of anti-CD40 mAbs (82, Cocks et al., paper submitted). No synergy could be observed when IL-4 and IL-13 were added at optimal concentrations. The mechanisms regulating germ-line ϵ -transcription in normal B cells by IL-4 and IL-13 appear to be similar, because anti-CD40 mAbs, IFN- γ and TGF- β have similar effects on germ-line ϵ -transcription irrespective of whether B cells are cultured in the presence of IL-4 or IL-13 (41, Cocks et al., paper submitted).

Interestingly, IFN- γ and IFN- α strongly inhibited IL-4- and IL-13-induced germ-line ϵ -transcription in B-cell lines transfected with a 600 bp germ-line ϵ -promoter-reporter gene construct (Cocks et al., paper submitted). The inhibitory effects of IFN- γ and IFN- α were most readily detected in short culture periods of 24 h. It has earlier been shown that neither IFN- γ nor IFN- α affect germ-line ϵ -transcription in normal human B cells cultured in the presence of IL-4 for 5–12 days (41). In close proximity to the germ-line ϵ -promoter, there is a 12-bp sequence with striking similarity to IFN regulatory elements, suggesting that the same nuclear factors mediating induction of transcription by IFN- α or IFN- γ interact with the germ-line ϵ -promoter, resulting in inhibition of germ-line ϵ -mRNA synthesis. The IFN-mediated inhibition of germ-line ϵ -transcription may, at least in part, explain the IFN-mediated inhibitory effect on IgE synthesis.

IL-4 and IL-13 affect B cells early in ontogeny

Ig levels of fetal origin in cord blood are very low, and virtually no IgE can be detected (2, 77). The defective humoral immunity in neonates has been partly attributed to immaturity of their B-cell function (2, 12). IL-2 induces normal proliferation and IgM synthesis by cord-blood B cells activated by *Staphylococcus aureus* Cowan I, but no other isotypes were observed in these cultures (81). However, cord-blood B cells can be induced to switch to ϵ in the presence of exogenous IL-4 (77, 99). Cord-blood T cells were found to be deficient in IL-4 production, a finding which may explain the absence of IgE in cord blood (31, 77).

Studies on the effects of IL-4 on early B-cell precursors have resulted in partially contradictory results. B-cell precursors express IL-4R (59), but IL-4 appears to have different effects on different subpopulations of B-cell precursors (89).

IL-4 has been shown to induce growth and differentiation of human pre-B cells (46, 67), whereas other studies have found either no effect (110) or inhibition (75, 89) of B-cell precursor proliferation.

Immature B cells derived from fetal spleen, liver, bone marrow (BM) (84), or thymus (85) undergo Ig-isotype switching and differentiation into Ig-secreting cells in the presence of IL-4 and anti-CD40 mAbs or activated CD4⁺ T cells. In addition, more recently, it has been shown that highly purified CD19⁺, surface(s) μ ⁻, cytoplasmic(c) μ ⁺ pre-B cells derived from human fetal BM differentiate into Ig-secreting cells when cocultured with activated, cloned CD4⁺ T cells and IL-4 (83). These results indicate that induction of IgE synthesis by human B cells does not require previous contact with antigen or allergen. In addition, these data indicate that the ϵ -switching mechanism is operational in early B cells, suggesting that commitment to IgE synthesis may occur at a very early stage of B-cell ontogeny.

Like IL-4, IL-13 induces proliferation and IgE switching by human immature B cells derived from fetal BM (86). Similar mechanisms appear to regulate IL-4- and IL-13-induced IgE synthesis by immature B cells, because IL-6 enhanced, and IFN- α , IFN- γ , TGF- β , and IL-12 inhibited, both IL-4- and IL-13-induced IgE synthesis in the presence of cloned CD4⁺ T helper cells (86). The levels of Ig produced in response to IL-13 were consistently 5–15-fold lower than those induced by IL-4, irrespective of whether anti-CD40 mAbs or CD4⁺ T cells were used to costimulate the immature B cells. However, IL-13-induced proliferation of immature B cells was only slightly lower than that induced by IL-4 (86). The reason for the lower capacity of IL-13 to induce Ig synthesis remains to be determined. Answers to these questions await characterization of the IL-13R and information about its expression on different B-cell subsets.

In contrast to IL-4, IL-13 seems to activate B cells

Table 1. Effects of IL-4 and IL-13 on human B cells at different developmental stages

	Proliferation ¹		Differentiation ¹		IgE synthesis ¹		CD23 expression		Germ-line ϵ -transcription	
	IL-4	IL-13	IL-4	IL-13	IL-4	IL-13	IL-4	IL-13	IL-4	IL-13
Mature B cells (refs. 17, 25, 39, 44, 79, 82)	+++	+++	+++	++	+++	++	+++	++	+++	++
Immature B cells (refs. 77, 84, 86, 99)	+++	++	+++	++	+++	++	++	+	+++	++
Pre-B cells (refs. 46, 89, 75, 83, 86)	±	±	++	±	++	±	++	-	++	-

¹ B-cell proliferation, differentiation, and IgE synthesis by IL-4 or IL-13 were studied in presence of costimulatory signals derived from activated CD4⁺ T helper cells. Germ-line ϵ -mRNA synthesis and CD23 expression were studied in response to IL-4 and IL-13 alone.

directly only after they have passed the pre-B-cell stage in B-cell ontogeny. We did not observe any phenotypic changes on human pre-B cells cultured in the presence of IL-13 (86). However, IL-4 induced high levels of CD23 expression on 7–9% of cultured μ^- pre-B cells, and consistently enhanced the expression of CD40 and HLA-DR on these cells. Under similar culture conditions, IL-13 and IL-4 induce CD23 expression on 30–50% or 80–90%, respectively, of mature, adult B cells (22, 82), indicating that IL-13 and IL-4 are less effective in inducing activation of immature B cells and pre-B cells than of mature B cells. Nevertheless, IL-4 directly induces pre-B-cell activation in the absence of other T-cell-derived signals. Our recent data indicate that IL-4 and IL-13 also induce germ-line ϵ -mRNA synthesis in immature human B cells freshly isolated from fetal BM (Punnonen et al., paper in preparation). Moreover, IL-4, but not IL-13, induces germ-line ϵ -transcription in highly purified pre-B cells.

Although rare in fetal BM, T cells are abundant in normal adult BM (16). Atopic patients with elevated IgE levels have high frequencies of Th2-like cells producing high levels of IL-4 (26, 76, 113), suggesting that commitment to IgE production in a subset of B cells in these patients may occur in BM already at early stages of B-cell development. The effects of IL-4 and IL-13 on human B cells of different developmental stages are summarized in Table 1.

An IL-4 mutant protein is antagonist for both IL-4 and IL-13

IL-4 and IL-13 are similar in structure, and they also share many biologic functions, suggesting that the receptors for IL-4 and IL-13 may also be structurally related. This was supported by the finding that IL-4 and IL-13 do not have any synergistic or additive effects (82). Recent data indicate that the receptors for IL-4 and IL-13 are separate, but they share a common component that is involved in signal transduction (116, 117). IL-4R and IL-13R are not identical, because IL-13 does not bind to the 130-kDa IL-4-binding protein, and it does not have growth-promoting effects on activated human T cells expressing high levels of IL-4R (117). In addition, activated human T cells, which proliferate after activation by IL-4, do not bind IL-13. It has also been found that an IL-4 mutant protein, in which the tyrosine residue at position 124 has been replaced by aspartic acid (hIL-4.Y124D) (57, 117), binds to IL-4 receptors with high affinity, but induces only minimal receptor activation. The hIL-4.Y124D mutant protein competitively inhibits receptor binding not only of IL-4, but also of IL-13 (6, 117), further supporting the conclusion that IL-4R and IL-13R share

a subunit. A candidate for the common component of IL-4R and IL-13R is the IL-2R γ -chain, which has recently been shown to be part of IL-4R and IL-7R (72, 94). However, it remains to be determined whether the IL-2R γ -chain is also a component of the IL-13R.

The IL-4 mutant protein inhibits the biologic activities of both IL-4 and IL-13. hIL-4.Y124D almost completely blocks IL-4- and IL-13-induced B-cell proliferation and IgE synthesis when used in 100- or 20-fold excess in the presence of optimal concentrations of IL-4 and IL-13, respectively (6). hIL-4.Y124D efficiently blocks both IL-4- and IL-13-induced IgG4 and IgE synthesis by PBMC or by highly purified sIgD⁺ B cells cultured in the presence of anti-CD40 mAbs. hIL-4.Y124D also inhibits IL-4-induced CD23 expression on B cells (57). The mutant protein has a weak IL-4-like agonistic effect. When used at high concentrations, hIL-4.Y124D induces an IgE synthesis which is approximately 2% of that induced by IL-4 (6). Despite its weak agonistic effect, hIL-4.Y124D may prove to be of therapeutic potential in treating IgE-mediated allergies. In fact, hIL-4.Y124D may be a more effective inhibitor of IgE synthesis than specific IL-4 antagonists, such as anti-IL-4 mAbs or soluble IL-4R, because this mutant protein inhibits both IL-4- and IL-13-induced IgE responses.

Conclusion

Significant progress has been made in recent years in characterizing the molecular events resulting in B-cell proliferation, differentiation, and isotype switching. T-cell and B-cell collaboration, an essential part of which is the CD40/CD40L interaction, plays a crucial role in the regulation of ϵ -switching and IgE synthesis. In addition, two cytokines, IL-4 and IL-13, are now known to induce IgE switching in human B cells. The molecular characterization of the IL-4 protein led to the finding of an IL-4 mutant protein which acts as an antagonist for both IL-4 and IL-13. Although this mutant protein efficiently blocks IgE synthesis *in vitro*, more data are required on its effects *in vivo* and on the relative contribution of IL-4 and IL-13 to the induction of IgE switching and the maintenance of ongoing IgE synthesis *in vivo*. In addition, more knowledge is required about the reasons for the enhanced IgE synthesis in atopic patients. It is crucial to characterize the mechanisms that determine the resulting cytokine synthesis profile after antigen or allergen recognition by T helper cells. Understanding of these mechanisms may result in effective and specific means to control IgE synthesis in atopic patients.

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