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Immunology

Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells

(B-cell differentiation/isotype switching/allergy)

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ABSTRACT **Recently the cDNA encoding interleukin 13 (IL-13), a T-cell-derived cytokine, was cloned and expressed. The present study demonstrates that IL-13 induces IgG4 and IgE synthesis by human B cells. IL-13-induced IgG4 and IgE synthesis by unfractionated peripheral blood mononuclear cells and highly purified B cells cultured in the presence of activated CD4$^+$ T cells or their membranes. IL-13-induced IgG4 and IgE synthesis is IL-4-independent, since it was not affected by neutralizing anti-IL-4 monoclonal antibody. Highly purified, surface IgD$^+$ B cells could also be induced to produce IgG4 and IgE by IL-13, indicating that the production of these isotypes reflected IgG4 and IgE switching and not a selective outgrowth of committed B cells. IL-4 and IL-13 added together at optimal concentrations had no additive or synergistic effect, suggesting that common signaling pathways may be involved. This notion is supported by the observation that IL-13, like IL-4, induced CD23 expression on B cells and enhanced CD72, surface IgM, and class II major histocompatibility complex antigen expression. In addition, like IL-4, IL-13 induced germ-line IgE heavy-chain gene transcription in highly purified B cells. Collectively, our data indicate that IL-13 is another T-cell-derived cytokine that, in addition to IL-4, efficiently directs naive human B cells to switch to IgG4 and IgE production.**

B cells undergo immunoglobulin isotype switching and differentiation into immunoglobulin-secreting cells in response to cell surface IgM (sIgM)-mediated signals in the presence of costimulatory factors provided by CD4$^+$ T cells (1, 2). Antigen-specific T-cell/B-cell interactions require binding of the T-cell receptor to peptide–class II major histocompatibility complex (MHC) on B cells, which results in T-cell activation and cytokine synthesis. Once the T cells are activated they can activate B cells in an antigen-independent fashion.

Cytokines are essential for B-cell proliferation and differentiation; they not only determine immunoglobulin secretion quantitatively but also direct immunoglobulin isotype switching (3, 4). Interleukin 4 (IL-4) was shown to induce IgG4 and IgE switching (5–8), whereas transforming growth factor β (TGF-β) directs IgA switching (9, 10). In addition to cytokines, contact-mediated signals delivered by CD4$^+$ T cells are required for B-cell proliferation and immunoglobulin production. Recently the ligand for CD40, which is expressed on activated CD4$^+$ T cells, was shown to be one such membrane-associated molecule that acts as a costimulatory signal for IL-4-dependent IgE production by both murine and human B cells (11, 45). Moreover, several cytokines, including IL-2 (12), IL-5 (13), IL-6 (14), IL-8 (15), IL-10 (2), IL-12 (16), interferon α (IFN-α) (5), IFN-γ (5), tumor necrosis factor α (17) and TGF-β (17), modulate IL-4-induced IgG4 and IgE synthesis.

IL-4 has been thought to be the only cytokine capable of inducing IgE synthesis. Out of 16 cytokines tested, IL-4 was the only one that induced germ-line or productive IgE heavy-chain (ε) transcripts or IgE synthesis (17–19). In addition, anti-IL-4 antibodies preferentially inhibit IgE synthesis induced by IL-4-producing T-cell clones without significantly affecting IgM, IgG, or IgA synthesis (12, 20, 21). Also in murine models, anti-IL-4 antibodies strongly inhibit IgE synthesis *in vivo* without affecting the other immunoglobulin isotypes (22). Most importantly, IL-4-deficient mice fail to produce IgE following nematode infection (23). However, a non-IL-4-producing T-cell clone can induce germ-line ε transcription in purified B cells, indicating that an IL-4-independent pathway of induction of germ-line ε transcription is operational (17, 18).

The human cDNA homologue of a mouse cDNA sequence encoding P600, a protein produced by murine T_{h2} helper T-cell clones following activation (24), was recently cloned and expressed (46). The human cDNA sequence is 66% identical to that of the mouse and encodes a protein that is 58% identical to the mouse P600 (46). P600 and IL-4 are related with a homology of ≈30%, but no homology with other cytokines was detected (46). Human P600 protein was biologically active and was shown to induce monocyte and B-cell differentiation (46). Therefore, this cytokine was designated IL-13. Human IL-13 is a nonglycosylated protein of 132 amino acids with a molecular mass of 10 kDa. Here we show that IL-13 induces CD23 expression, germ-line ε mRNA synthesis, and IgG4 and IgE switching in human B cells. The activity of IL-13 is shown to be independent of IL-4.

MATERIALS AND METHODS

Reagents. Recombinant human IL-13 was purified as described (46). Recombinant IL-4, IFN-α, and IFN-γ were provided by Schering–Plough. Fluorescein isothiocyanate (FITC)-conjugated anti-CD72 monoclonal antibody (mAb) and neutralizing anti-TGF-β mAb were purchased from R & D Systems (Minneapolis). FITC- or phycoerythrin (PE)-conjugated mAbs specific for CD3, CD4, CD8, CD14, CD16, CD19, CD20, CD23, CD25, CD56, and HLA-DR, as well as control antibodies with irrelevant specificities, were obtained from Becton Dickinson. FITC- or PE-conjugated mAbs specific for lymphocyte function-associated antigen 1 (LFA-1) (L130), LFA-3, the cell adhesion molecule ICAM-1 (LB2),

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Abbreviations: IL, interleukin; IFN, interferon; PBMC, peripheral blood mononuclear cell; mAb, monoclonal antibody; sIg, surface Ig; MHC, major histocompatibility complex; TGF, transforming growth factor; LFA, lymphocyte function-associated antigen; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

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B7 (L307), and class I MHC antigen were kindly provided by J. Phillips (DNAX). FITC-conjugated anti-IgD and anti-IgM mAbs were obtained from Nordic (Tilburg, The Netherlands). The purified anti-CD40 mAb 89 (IgG1) (25) was a gift of J. Banchereau (Schering–Plough France, Dardilly, France). The neutralizing anti-IL-4 mAb 25D2.11 was kindly provided by J. Abrams (DNAX).

Cell Preparations. Blood samples and spleens were obtained from healthy volunteers or from patients undergoing splenectomy due to trauma, respectively. Mononuclear cells were isolated by centrifugation over Histopaque-1077 (Sigma).

Purified B cells were obtained by negative sorting using a fluorescence-activated cell sorter (FACStar Plus, Becton Dickinson) or magnetic beads (Dynal, Oslo). Briefly, splenic mononuclear cells were washed twice and PE-conjugated mAbs against CD3, CD4, CD8, CD14, CD16, and CD56 were added at saturating concentrations and incubated at 4°C for 30 min. The cells were washed twice with phosphate-buffered saline. Cells with the light-scattering characteristics of lymphocytes were gated, and PE^- cells were sorted. Alternatively, cells stained with mAbs against CD3, CD4, CD8, CD14, CD16, and CD56 were incubated for 30 min at 4°C with magnetic beads coated with anti-mouse immunoglobulin mAbs. The cells bearing murine immunoglobulin were removed by using a magnetic field. The remaining cells were washed, counted, and used in further experiments. For isolation of $sIgD^+$ B cells, positive sorting by FACStar Plus was used. Splenic mononuclear cells were stained with PE-conjugated mAbs against CD3, CD4, CD8, CD14, CD16, and CD56 and FITC-conjugated anti-IgD mAb, and $FITC^+$, PE^- cells were sorted. On reanalysis purities of the sorted cell populations were >98%, and those of cells isolated by using magnetic beads were >95%.

The $CD4^+$ T-cell clone B21 and the $CD4^+$ non-IL-4-producing T-cell clone SP-A3 were cultured as described (26). The cells were obtained 4–6 days after they had been activated by the feeder cell mixture and phytohemagglutinin. In addition, IL-2 (100 units/ml) was added to maintain the activation state of the T-cell clones.

Preparation of T-Cell Membranes. The membranes of a $CD4^+$ T-cell clone were obtained as described (27). Briefly, the $CD4^+$ T-cell clone B21 was harvested 12 days after activation with feeder cell mixture and phytohemagglutinin, and the cells were washed and restimulated with concanavalin A (10 μg/ml) for 7–8 hr at 37°C. During the last 30 min of the concanavalin A stimulation, methyl α-D-mannopyranoside (Sigma) was added at 100 μg/ml. From these cells, membranes were prepared by the methods of Brian (28) and Maeda *et al.* (29), and they were stored under liquid nitrogen (10^8 T-cell equivalents per ml = 0.2 mg of protein per ml of membrane preparation) until used.

Culture Conditions. Purified B cells were cultured at 5000 per well in quadruplicate, in round-bottomed 96-well plates (Linbro) at 37°C in a humidified atmosphere containing 5% CO_2. Each well contained 0.2 ml of Yssel's medium (30) supplemented with 10% fetal bovine serum. Unfractionated peripheral blood mononuclear cells (PBMCs) were cultured at 10^5 per well in 12 replicates. In coculture experiments, the $CD4^+$ T-cell clone SP-A3 was cultured at 5000 cells per well (T/B-cell ratio, 1:1). After a culture period of 12 days, immunoglobulin levels in the culture supernatants were measured by ELISA.

Measurement of Immunoglobulin Production. IgM, total IgG, IgA, IgE secretion (5) and IgG4 secretion (31) were determined by ELISA as described. The sensitivities of IgM, total IgG, and IgA ELISAs were 0.5–1 ng/ml, and the sensitivities of IgG4 and IgE ELISAs were 0.2 ng/ml.

Phenotypic Analysis of Cultured Cells. Purified B cells were cultured as described above and were harvested and washed twice. FITC- and PE-conjugated mAbs were added at saturating concentrations and incubated at 4°C for 30 min. FITC- and PE-conjugated mAbs with irrelevant specificities were used as negative controls. The cells were washed twice with phosphate-buffered saline, and cells with the light-scattering characteristics of lymphocytes were analyzed with a FACScan flow cytometer (Becton Dickinson).

RNA Isolation and Northern Analysis. Total RNA was isolated by using RNAzol B (CNNA; Biotech, Friendswood, TX) according to the manufacturer's instructions. RNA was electrophoresed through 0.85% agarose and transferred (32) to BA-S nitrocellulose (Schleicher & Schuell, Keone, NH). ^{32}P cDNA probes were made by random priming (33) using as templates (*i*) an *Eco*RI–*Hin*dIII fragment of pBSIgE1-4 (18) for germ-line ε and (*ii*) DNA complementary to the *Bgl* I–*Sma* I fragment of pHFγA-1 (34) for actin.

RESULTS

IL-13 Induces CD23 Expression on Purified B Cells. The effect of IL-13 on the expression of a variety of B-cell surface antigens was investigated by flow cytometry. Incubation of purified B cells with IL-13 (200 units/ml) resulted in strong induction of CD23 expression on a proportion of the B cells (Fig. 1). IL-13 also upregulated class II MHC antigen, sIgM, and CD72 expression on B cells. These effects of IL-13 were similar to those observed with IL-4 (refs. 35 and 36; unpub-

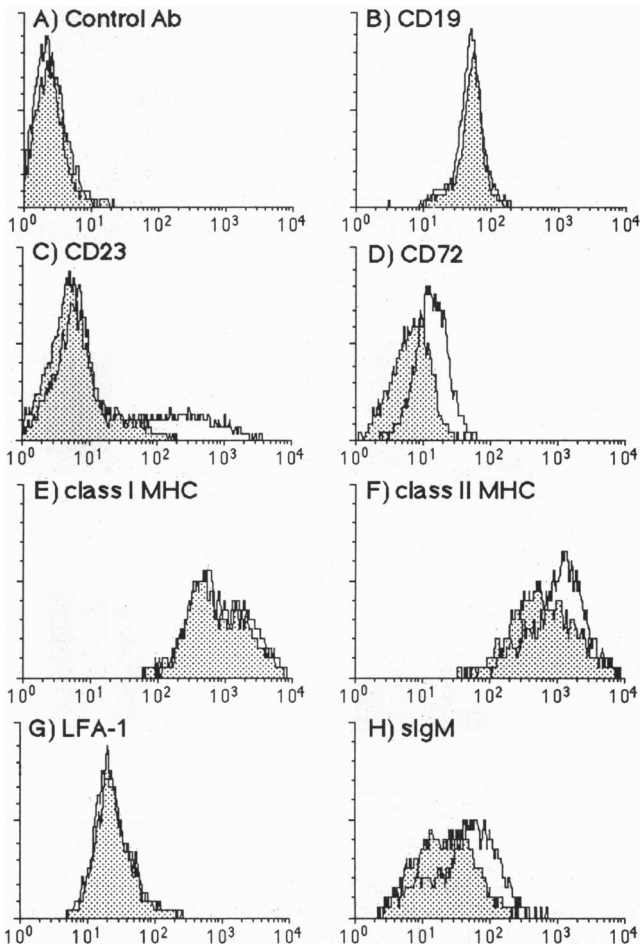

FIG. 1. Effect of IL-13 on B-cell phenotype. Purified B cells were cultured with or without IL-13 (200 units/ml) for 72 hr, and the cells were harvested, stained with mAbs indicated, and analyzed by FACScan flow cytometer. Open and stippled histograms indicate analysis of cells cultured with and without IL-13, respectively. Control antibody (Ab) used was FITC-conjugated mouse IgG2a.

lished observations). CD23 expression was already detectable after a culture period of 24 hr, but maximal responses were observed after 72 hr of culture. The expression of CD19, CD20, CD25, CD40, class I MHC antigen, B7, ICAM-1, LFA-1, and LFA-3 were not significantly modified by IL-13 (Fig. 1 and data not shown).

IL-13 Induces IgE Synthesis by PBMCs. Because CD23 expression on B cells has been associated with IgE synthesis (13, 37–39), we studied whether IL-13 could induce IgE synthesis by human PBMCs. IL-13 induced IgE synthesis by unfractionated PBMCs in a dose-dependent manner in the absence of exogenous IL-4 (Fig. 2 *B* and *C*). In addition, strong IgG4 production in response to IL-13 was observed (Fig. 2*A*). Interestingly, neutralizing anti-IL-4 mAbs failed to inhibit IL-13-induced IgE synthesis (Fig. 2*C*), whereas IL-4-induced IgE production was virtually completely blocked (Fig. 2*D*), indicating that IL-13-induced IgE synthesis was not mediated through induction of IL-4 production by PBMCs. Similarly to IL-4, maximal induction of IgE synthesis by IL-13 was usually obtained at concentrations of 50 units/ml. The mean level of IgE produced in response to IL-13 was somewhat lower (63 ng/ml, $n = 6$) than that induced by IL-4 (169 ng/ml, $n = 6$). No additive or synergistic effects were observed when both IL-4 and IL-13 were used at saturating concentrations (Fig. 2*D*).

IL-13 Induces IgG4 and IgE Switching in B Cells. We next studied the ability of IL-13 to induce IgG4 and IgE synthesis by purified B cells. IL-13 induced IgG4 and IgE synthesis by highly purified B cells cultured in the presence of membranes of an activated CD4$^+$ T-cell clone (Fig. 3*A*). Also in this culture system the levels of IL-13-induced IgG4 and IgE production were generally lower than those induced by IL-4. The difference was in the same range as that observed in the cultures of unfractionated PBMCs (Fig. 2 *C* and *D*) (data not shown). IL-13 also induced significant levels of IgM and total IgG production, but no IgA synthesis was observed (data not shown). In this aspect IL-13 is similar to IL-4, which generally inhibits IgA synthesis (40). These results show that IL-13 induces IgG4 and IgE synthesis by human B cells in the absence of IL-4 and indicate that IL-13 acts directly on B cells to induce IgG4 and IgE synthesis. Furthermore, these data strongly suggest that IL-13 induces immunoglobulin isotype switching to IgG4 and IgE in an IL-4-independent manner.

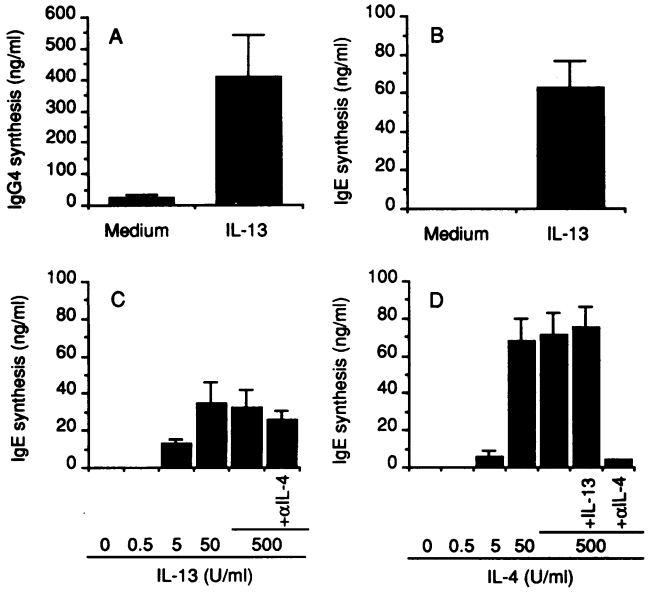

FIG. 2. IL-13 induces IgG4 and IgE synthesis by PBMCs. Unfractionated PBMCs were cultured with IL-13 (500 units (U)/ml), and IgG4 (*A*) and IgE (*B*) levels in the culture supernatants were measured after a culture period of 12 days. *C* and *D* show the effects of neutralizing anti-IL-4 mAb (αIL-4, 10 μg/ml) on IL-13- and IL-4-induced IgE synthesis, respectively. *D* also shows IgE synthesis induced by combination of IL-4 and IL-13 (500 U/ml). The data represent mean ± SEM of IgE levels of 12 replicates in three experiments (*A* and *B*) or one representative experiment out of three (*C* and *D*).

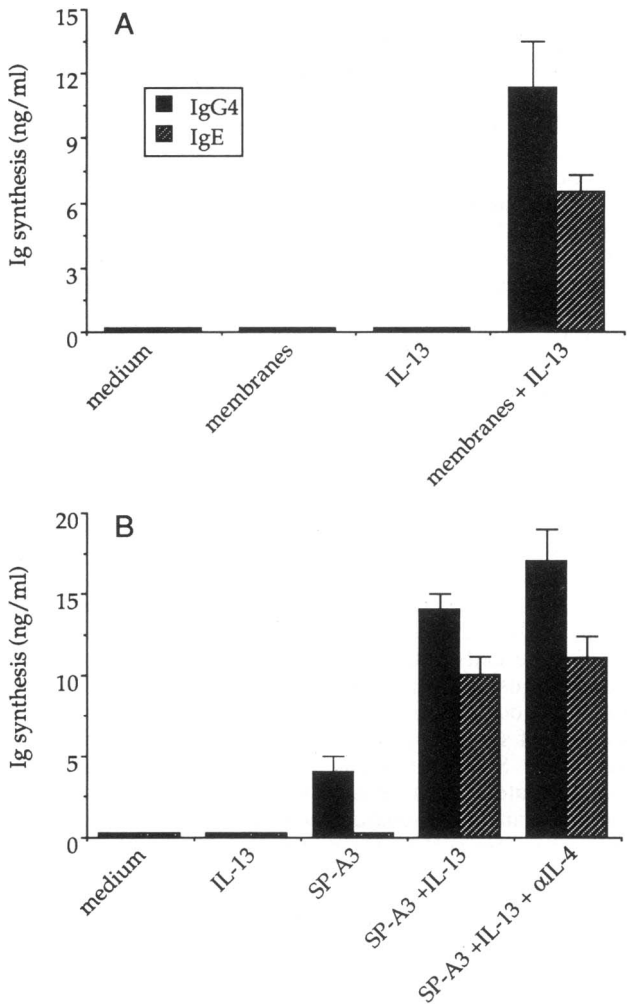

FIG. 3. IL-13-induced IgG4 and IgE synthesis reflects immunoglobulin isotype switching. Highly purified, sorted B cells (*A*) or sorted sIgD$^+$ B cells (*B*) were cultured in the presence of IL-13 (400 units/ml) and membranes of CD4$^+$ T cells (*A*) or with the activated, non-IL-4-producing T-cell clone SP-A3 (5000 cells per well) in the presence or absence of neutralizing anti-IL-4 mAb (αIL-4, 10 μg/ml) (*B*). IgG4 and IgE levels in the culture supernatants were measured by ELISA after a culture period of 12 days. The data represent mean ± SEM of quadruplicate cultures.

To confirm that IgE synthesis observed in above experiments was due to immunoglobulin isotype switching and not to an outgrowth of a few IgE-committed B cells, we studied the effects of IL-13 on naive sIgD$^+$ B cells. Culturing of highly purified sIgD$^+$ B cells with the activated, non-IL-4-producing T-cell clone SP-A3 in the presence of IL-13 resulted in induction of IgE synthesis (Fig. 3*B*). In addition, IL-13 enhanced IgG4 synthesis induced by this non-IL-4-producing T-cell clone alone. As was demonstrated above for PBMCs, IL-13-induced IgG4 and IgE synthesis could not be inhibited by anti-IL-4 mAbs.

Induction of Germ-Line ε Transcription by IL-13. So far, IL-4 has been the only cytokine known to induce germ-line ε transcription in B cells (17–19). Since switching to ε by IL-4 is preceded by the induction of germ-line ε RNA synthesis (17–19), we hypothesized that IL-13 might induce germ-line

ε transcription as well. Indeed, when highly purified B cells were cultured in the presence of IL-13 and anti-CD40 mAbs, germ-line ε mRNA synthesis, at levels comparable to that in the presence of IL-4 and anti-CD40 mAbs, was detected after a culture period of 5 days (Fig. 4). Since anti-CD40 mAbs alone do not induce germ-line ε transcription in B cells (17), these results indicate that IL-13 is another T-cell-derived cytokine that, like IL-4, can induce germ-line ε transcripts in B cells. In addition, these results confirm the correlation between germ-line ε transcription and subsequent switching to IgE synthesis.

DISCUSSION

The human cDNA encoding a T-cell-derived cytokine designated IL-13 was recently cloned and expressed (46). IL-13 is a nonglycosylated protein of 10 kDa. The first biological characterizations have indicated that IL-13 has monocyte and B-cell differentiation-inducing activities (46). The present study shows that IL-13 induces CD23 expression and IgG4 and IgE production by naive human B cells.

IL-4 has been considered the only cytokine to induce IgE switching in human or murine B cells. This was based on studies showing that anti-IL-4 mAbs preferentially block IgE synthesis both *in vitro* (12, 20, 21) and *in vivo* (22) and on the observation that no circulatory IgE could be detected in mice in which the IL-4 gene had been disrupted (23). However, our data show that IL-13-induced IgE synthesis is independent of IL-4, since IL-13 induced IgG4 and IgE synthesis in cultures of highly purified B cells in the absence of exogenous IL-4. In addition, anti-IL-4 mAbs, which efficiently blocked IL-4-induced IgE synthesis, failed to affect IL-13-induced IgE production. Moreover, IL-13-induced IgG4 and IgE synthesis, like that induced by IL-4, reflects immunoglobulin isotype switching and is not due to a selective outgrowth of a few B cells committed to IgG4 or IgE synthesis, since IL-13 also induced IgG4 and IgE synthesis by naive, sorted $sIgD^+$ B cells.

Switching to IgE by IL-13 was preceded by induction of germ-line ε mRNA synthesis, but costimulatory signals provided by activated T cells were required for induction of IgE production. This is consistent with the studies showing that IL-4-induced switching to ε in both murine and human B cells is preceded by the induction of germ-line ε RNA synthesis and that costimulatory signals provided by activated $CD4^+$ T-cell clones or by anti-CD40 mAbs are required for the induction of productive ε mRNA transcripts and IgE synthesis by IL-4 (17–19). Although their exact role remains to be determined, it has been suggested that germ-line ε transcripts play an important role in the ε-switch process (17–19). Despite the fact that IL-4 has been considered to be the only cytokine to induce germ-line ε transcription in B cells, an IL-4-independent pathway of induction of germ-line ε transcription is operational, since a non-IL-4-producing T-cell clone was also capable of inducing strong germ-line ε RNA synthesis (17). Although it remains to be determined, it is tempting to speculate that IL-13 produced by the non-IL-4-producing T-cell clones is responsible for the IL-4-independent induction of germ-line ε mRNA in B cells. The present findings may also explain why induction of IgE synthesis by IL-4-producing T-cell clones was never completely inhibited by anti-IL-4 mAbs (12, 20, 21).

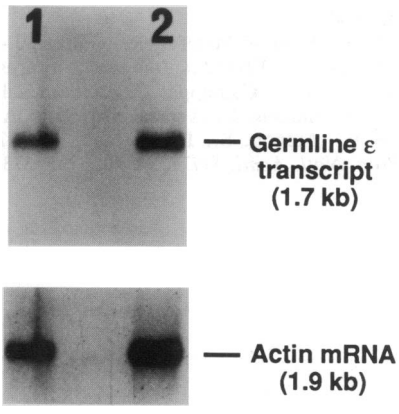

FIG. 4. IL-13- and IL-4-induced expression of germ-line ε transcripts in purified B cells. Sorted B cells (3×10^5) were incubated with either IL-4 (lane 1) or IL-13 (lane 2) at 400 units/ml with anti-TGF-β mAb at 10 μg/ml and anti-CD40 mAb 89 at 2 μg/ml. The anti-TGF-β mAb was added to prevent potential inhibition of germ-line ε transcript expression by TGF-β generated in the cultures (17). After 5 days total RNA was extracted and subjected to Northern analysis. Levels of germ-line ε (*Upper*) and actin (*Lower*) transcripts were detected on the same Northern blot by probing successively with appropriate ^{32}P-labeled cDNAs.

No additive or synergistic effects on IgE synthesis were observed when IL-4 and IL-13 were added together at optimal concentrations, suggesting that IL-4 and IL-13 may use common signaling pathways for induction of IgG4 and IgE switching. Indeed, recent studies have shown that receptors for IL-13 and IL-4 share a common subunit that functions in signal transduction (S. Zurawski, F. Vega, Jr., B. Huyghe, W. Dang, and G.Z., unpublished data). However, IL-13 does not bind to cells bearing the 130-kDa IL-4 receptor, indicating that IL-13 does not act through this IL-4-binding protein (S. Zurawski, F. Vega, Jr., B. Huyghe, W. Dang, and G.Z., unpublished data). The commonality between IL-13 and IL-4 was further supported by the observation that IL-13, like IL-4 (36), induced CD23 expression on purified B cells. Like IL-4 (ref.35; unpublished data), IL-13 also upregulated expression of class II MHC antigen, sIgM, and CD72, which is the ligand for CD5 (41). 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 (13, 37–39), and soluble forms of CD23 have been found to enhance IgE synthesis (42). Since IL-13 induced significant expression of CD23 within 24 hr, our data also indicate that CD23 expression precedes IL-13-induced ε switching, confirming the correlation between induction of CD23 expression and subsequent IgE synthesis.

Despite the similarities between IL-4 and IL-13 in their effects on B cells, the functions of IL-4 and IL-13 are not identical. The levels of IgG4 and IgE produced in response to IL-13 were generally lower than those induced by IL-4. Moreover, preliminary results have indicated that IL-13, in contrast to IL-4 (43, 44), does not act on T cells or T-cell clones. IL-13 has no T-cell growth-promoting activity and does not induce CD8α expression on $CD4^+$ T-cell clones (R.d.W.M. and H. Yssel, unpublished data), which may be due to lack of functional IL-13 receptors on T cells. The activation state of T cells is essential for their ability to deliver costimulatory signals required for B-cell proliferation and differentiation (8). Therefore, the lack of a T-cell activation-inducing effect of IL-13 may partially explain why maximal IgG4 and IgE synthesis by PBMCs in response to IL-13 was lower than that induced by IL-4.

The relative contribution of IL-13 in the regulation of IgE synthesis *in vivo* remains to be determined. Our data seem to be incompatible with the finding that IL-4-deficient mice have no detectable circulatory IgE following nematode infections (23). However, it is not clear whether IL-13 also induces IgE synthesis by murine B cells. Our preliminary data show that IL-13 is produced for much longer periods than IL-4 following T-cell activation (R.d.W.M. and J.E.d.V., unpublished data), suggesting an important role for IL-13 in the regulation of enhanced IgE synthesis in allergic individuals.

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