

Synthesis of IL-13 by human B lymphocytes: Regulation and role in IgE production

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Background: Our laboratory has demonstrated previously that human tonsillar B lymphocytes express IL-13 mRNA

Objective: We sought to investigate IL-13 production by human B cells and the association between B cell-derived IL-13 and IgE secretion.

Methods: Human B lymphocytes were isolated from tonsils and purified by means of rosetting with sheep RBCs or positive or negative selection with magnetic beads. They were stimulated with anti-CD40 antibodies with or without recombinant IL-4. Total mRNA was extracted, and IL-13 mRNA was measured by means of standard RT-PCR or by means of real-time PCR with commercially available primers. B cells were cultured with or without IL-13 neutralizing antibodies, and C ϵ transcripts and supernatant IgE levels were measured.

Results: IL-13 mRNA was detected in human B lymphocytes stimulated with anti-CD40 antibodies and IL-4 or IL-2 but not in unstimulated B cells. Real-time PCR demonstrated a 10- to 15-fold increase in IL-13 mRNA, maximizing at 36 hours. IL-13 protein was detected from B lymphocytes on day 3 and accumulated through day 7. The synthesis of IL-13 required both CD40 and IL-4 stimulation. The presence of IL-13 was confirmed by means of intracellular staining of cultured B lymphocytes and antigen-stimulated nasal biopsy specimens from atopic individuals. Addition of IL-13 neutralizing antibodies to purified B-cell cultures inhibited IgE production by up to 80% and diminished IgE (C ϵ) transcripts by 50%.

Conclusion: Human B lymphocytes express IL-13 mRNA after ligation of CD40 and the addition of cytokines. Human B lymphocytes produce significant IL-13, and neutralization of IL-13 impairs IgE synthesis. IL-13 might be an important autocrine growth factor for IgE-producing B lymphocytes. (*J Allergy Clin Immunol* 2004;114:657-63.)

Key words: B lymphocytes, cytokines, IL-13, allergy, IgE

IgE plays a crucial role in the pathogenesis of atopic diseases, and diminishing IgE synthesis or inhibiting its action are key goals for therapies of asthma and related allergic conditions.¹ The synthesis of IgE by B lympho-

Abbreviation used

α CD40: Antibodies to CD40

cytes is under stringent controls, requiring antigens, cytokines, and cell-cell contact between B and T lymphocytes. CD40-CD40 ligand contact plus obligatory T_H2 cytokine signals are necessary for switching the genetic program of a B cell from IgM to IgE.²

After activation by antigen and T cells, B cells clonally expand, growing in colonies as they mature into either plasma cells or memory B cells.³ Factors that maintain maturation of B-lymphocyte colonies to memory or plasma cells are incompletely studied. Cytokines from cells in the lymph node microenvironment likely play a role in the latter stages of B-cell maturation, but this has not been well defined. B lymphocytes might produce cytokines or growth factors, such as IL-6 or IL-10, yet there are few data pertaining to autocrine cytokine production in the development of IgE-secreting B cells. The T_H2 cytokines IL-4 and IL-13 are the primary candidates for this function. Both induce phosphorylation of Janus kinases and signal transducer and activator of transcription 6. These signals are required for IgE (ϵ) gene transcripts, including the genetic switch region (I ϵ) and the constant region, C ϵ .⁴ We have shown previously that cultured B lymphocytes from tonsils are capable of expressing both IL-4 and IL-13 mRNA after stimulation with the potent lipid mediator platelet-activating factor.⁵ In the current studies we have detected IL-13, but not IL-4, in anti-CD40/IL-4-stimulated purified B lymphocytes. We characterized production of IL-13 by human B lymphocytes and investigated the role of B cell-derived IL-13 in the production of IgE. Our data suggest that IL-13 is necessary to maintain development of IgE-secreting cells, and B cells might be an autocrine or paracrine source of IL-13 in this process.

METHODS

B-lymphocyte isolation

Human tonsillar B cells were isolated as described previously.⁶ Hypertrophied tonsils were obtained after routine tonsillectomy and finely minced. Mononuclear cells were purified by means of Ficoll-Hypaque density centrifugation (Pharmacia, Uppsala, Sweden). B cells were separated from T cells by means of E-rosetting with neuraminidase-treated (Sigma, St Louis, Mo) sheep RBCs (obtained from Faculté de Médecine Vétérinaire, Université de Montréal,

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St-Hyacinthe, Quebec, Canada). Alternatively, B lymphocytes were purified by means of positive selection on magnetic microbead columns with MACS CD19 microbeads or negative selection with the B cell Isolation Kit (Miltenyi Biotec, Auburn, Calif), according to the manufacturer's instructions. E-rosette-isolated fractions contained more than 98% CD19⁺ cells, as determined by means of flow cytometry. MACS-purified B cells were 99% CD19⁺, with less than 1% detectable CD3⁺ T cells.

RNA extraction and cDNA synthesis

Total cellular RNA was extracted from purified B cells with TRIZOL (Invitrogen Life Technologies, Mississauga, Ontario, Canada), according to the manufacturer's instructions. RNA pellets were dissolved in nuclease-free water. cDNA strands were generated in a 20- μ L reaction mixture by using 1 μ g of total RNA as a template, 0.5 μ g of oligo(dT)12-18, 0.25 mmol/L deoxyribonucleoside triphosphate mixture, and 200 U of M-MLV reverse-transcriptase enzyme (Invitrogen) in the presence of a ribonuclease inhibitor.

Conventional PCR and real-time PCR

Conventional PCR was performed with a mixture consisting of 1.5 mmol/L MgCl₂, 1 \times PCR buffer, 0.25 mmol/L deoxyribonucleoside triphosphate mixture, 2.5 U of Taq polymerase, 0.4 μ mol/L of each of the sense and antisense primers, and 1 μ L of the synthesized cDNA strand. Specific primers for IL-13 were synthesized by Invitrogen according to published sequences⁷: sense, 5'-CTC CTC AAT CCT CTC CTG TT-3'; antisense, 5'-GTT GAA CCG TCC CTC GCG AAA-3'. The samples were amplified in a thermal cycler for 35 cycles, consisting of 1 minute of denaturation at 95°C, 1 minute of annealing at 60°C, and 1 minute of extension at 72°C. PCR products were visualized by means of ethidium bromide staining after 1.5% agarose gel electrophoresis by using phosphoimaging. Quantification of the mRNA message coding for β_2 -microglobulin and IL-13 was performed by using Lightcycler (Roche Diagnostics, Laval, Quebec, Canada). IL-13 primers were purchased from Search LC (Heidelberg, Germany), as previously described.⁸ PCR reactions were performed in a volume of 20 μ L containing 1 μ L of cDNA, 0.3 μ mol/L of each primer, and 10 μ L of QuantiTect SYBR Green PCR (Qiagen, Mississauga, Ontario, Canada). The denaturation and amplification conditions for both β_2 -microglobulin and IL-13 were 95°C for 15 minutes, followed by 45 cycles of PCR. Each cycle included denaturation at 95°C for 20 seconds, annealing for 20 seconds at 57°C, and extension for 20 seconds at 72°C. The IL-13 mRNA copy ratio was obtained by calculating the gene copy number of β_2 -microglobulin using the formula provided with the Lightcycler software (Roche) and dividing into the IL-13 copy number, derived from the standard curve included with the IL-13 mRNA kit.

Immunocytochemical staining and microscopy of B-cell clones

Purified B lymphocytes were cultured in complete medium consisting of RPMI-1640 (Invitrogen), 10% FCS (HyClone, Logan, Utah), 5 mg/mL L-glutamine, 50 U/mL penicillin, and 50 μ g/mL streptomycin (Invitrogen) with or without antibodies for CD40 (α CD40; 1 μ g/mL) and IL-4 (400 U/mL; R&D Systems, Minneapolis, Minn). They were plated at 5×10^5 cells/mL onto glass chamber slides (ISC BioExpress, Kaysville, Utah). After 5 to 7 days, the cells were fixed on the slides with 4% paraformaldehyde for 20 minutes at room temperature. The slides were centrifuged at 1000 rpm for 5 minutes at 4°C, washed in PBS, air-dried for 30 minutes, and stored at -80°C. Slides were rehydrated in PBS and blocked with

protein blocking solution (DAKO Corp, Carpinteria, Calif) for 30 minutes. All procedures were subsequently performed at room temperature. For IL-13/CD19 double staining, slides were permeabilized with CytoPerm B (Caltag Laboratories, An-Der-Grub, Austria) and incubated with monoclonal anti-human IL-13 antibody (R&D Systems) for 1 hour. Slides were then washed and incubated with FITC-conjugated goat anti-mouse IgG (BD Pharmingen, San Diego, Calif) for 1 hour. Monoclonal anti-human CD19 antibody (BD Pharmingen) was labeled with Alexa Fluor 568 by using Zenon-One Labeling Kits (Molecular Probes, Eugene, Ore) and used within 30 minutes of preparation. After 3 washes, slides were incubated with labeled CD19 antibody for 1 hour. Negative control staining was performed with secondary antibodies alone. The stained slides were examined with a Zeiss Laser Scanning Confocal system mounted on an Axiovert 135 Zeiss inverted microscope (Carl Zeiss Inc, Oberkochen, Germany), equipped with ImageproPlus software (MediaCybernetics, Silver Spring, Md). After confocal examination, cover slips were washed off and slides were stained with hematoxylin and eosin and examined with an Olympus light microscope.

IL-13 measurement

Measurement of IL-13 in supernatants of tonsillar B-cell cultures was determined by means of specific ELISA (Pharmingen or Diaclone, Boston, Mass) as directed, with a lower limit of detection of 3.1 pg/mL.

In situ hybridization for IgE transcripts

B cells were cultured with α CD40 and IL-4 in 24-well plates for 5 days, with IL-13 neutralizing antibodies added as indicated. The cells were harvested and washed and then applied to polylysine-coated slides by using cytospin, and *in situ* hybridization with ³⁵S-labeled complementary RNA probes coding for C ϵ RNA was performed, as previously described.² After cell permeabilization and pre-hybridization, cytospin preparations were incubated overnight with a hybridization mixture containing the C ϵ probe (0.75×10^6 cpm/slide). Cytospin preparations were dipped in Amersham LM-2 emulsion (Amersham International, Buckinghamshire, United Kingdom), exposed to film for 9 days, developed in Kodak D-19 developer, fixed, and counterstained in Hematoxylin GII (Fisher Scientific, Montreal, Quebec, Canada) for 60 seconds. The samples were examined with a graduated microscope; silver grains were read as C ϵ^+ signals.

Measurement of IgE production by means of ELISA

Ninety-six-well plates (Costar, Corning Corp, Acton, Mass) were coated overnight at 4°C with 5 μ g/mL rat anti-human IgE (Biosource, Camarillo, Calif) in 0.05 mol/L carbonate-bicarbonate buffer, pH 9.6. After washing 3 times with PBS/0.1% Tween 20, the plates were blocked with PBS plus 0.5% gelatin (Sigma) for 2 hours at 37°C and then incubated for 2 hours at 37°C with cell culture supernatants or serial dilutions of human IgE standards. After washing, biotinylated goat anti-human IgE (Biosource) diluted 1:15,000 was added and incubated for 2 hours at 37°C. The plates were washed, and then streptavidin-horseradish peroxidase conjugate (Biosource) diluted 1:10,000 in blocking buffer was added. After incubation for 1 hour at 37°C, tetramethylbenzidine (Zymed Labs Inc, South San Francisco, Calif) was added, and the plate was incubated for 10 minutes at room temperature. The reaction was stopped with phosphoric acid (Sigma), and absorbance was measured with an ELISA reader at 450 nm. The limit of IgE detection was 50 pg/mL.

Immunofluorescent detection of IL-13 in B cells from nasal tissues

Nasal biopsy specimens were taken from atopic and nonatopic children as part of a project on nasal response to allergens.⁹ Frozen tissue sections from explanted nasal mucosa from ragweed-sensitive or nonatopic children were stimulated with ragweed (50 µg/mL) for 24 hours, as previously described.^{9,10} Twenty-four hours after tissue culture, tissue was fixed in 4% paraformaldehyde, washed in 15% sucrose-PBS and blocked in optimal cutting temperature (OCT) embedding medium (Tissue-Tek; Somagen Diagnostics, Edmonton, Alberta, Canada) by snap-freezing in isopentane cooled in liquid nitrogen. The tissue was sectioned at 5-mm thickness onto poly-L-lysine (0.1%)–coated glass slides (Microm HM500; Microm International GmbH, Walldorf, Germany) and baked overnight at 37°C. Slides were incubated with anti-CD19 antibody (R&D Systems) for 30 minutes at 37°C, followed by anti-mouse IgG secondary antibody conjugated to FITC (R&D Systems) for 30 minutes at room temperature, to identify B cells. After washing, slides were incubated with anti-human IL-13 conjugated to R. Phycoerythrin (undiluted; R&D Systems) for 30 minutes. All slides were evaluated at 660× magnification with a Zeiss LSM microscope.

RESULTS

IL-13 mRNA is induced in B lymphocytes

Because of the role IL-13 plays in IgE production, we examined whether, under conditions that would produce IgE, B lymphocytes could produce IL-13. Isolated tonsillar B cells stimulated with anti-CD40 antibodies (1 µg/mL) and recombinant IL-4 (400 U/mL) or IL-2 (10 ng/mL) consistently expressed mRNA for IL-13 (Fig 1, A). Anti-CD40, IL-4, or IL-2 alone was insufficient to induce IL-13 mRNA (data not shown). By means of real-time PCR, anti-CD40/IL-4 stimulation augmented IL-13 mRNA by greater than 10-fold over baseline levels by 24 hours (from 80 ± 50 copies per microliter to 945 ± 227 copies per microliter). This was approximately 50% of IL-13 mRNA induced in T cells stimulated with PHA (approximately 2000 copies per microliter). Importantly, IL-4 mRNA was not detected in anti-CD40/IL-4–stimulated B cells, whereas it was consistently detected in T cells (data not shown). IL-13 transcripts increased over time, maximizing between 24 and 36 hours (Fig 1, B). No significant differences were found between mRNA from B cells purified by means of positive selection with CD19⁺ magnetic beads or negative selection by means of T cell–monocyte–dendritic cell depletion.

IL-13 is produced in colonies of cultured B cells

Once stimulated, highly purified B lymphocytes grow in highly organized colonies (Fig 2, A). We examined these colonies for the presence of IL-13 by means of immunofluorescent staining of permeabilized B cells. After 5 to 7 days of culture, anti-CD40– and IL-4–activated B cells stain strongly for intracellular IL-13 (Fig 2, C) compared with unstimulated B cells (Fig 2, E). Staining for IL-4, performed on similarly prepared anti-CD40/IL-4–stimulated B lymphocytes, demonstrated no intracellular IL-4 in B cells (see Fig E1 in the Journal's

Online Repository at www.mosby.com/jaci). Only cells in organized formations stained positively for IL-13; few single cells in the culture chambers were positive for IL-13. By using double staining for intracellular IL-13 with an FITC-conjugated antibody and surface CD19 with a red fluorescent conjugate (Alexa 568), we demonstrated colocalization of IL-13 to a large number of B cells growing in colony formation (Fig 2, D).

IL-13 is detected in the supernatants of B cells committed to produce IgE

Highly purified B lymphocytes cultured with anti-CD40 antibodies and IL-4 (400 U/mL) produced significant and reproducible amounts of IL-13 detected after 7 days of culture (Fig 3). IL-13 was first detected in cell culture supernatants after 72 hours in culture and accumulated until it reached a plateau on day 7 (Fig 3). IL-13 secretion was dependent on activation of B cells because baseline levels were consistently undetectable, whereas stimulated B cells produced an average of 19.6 pg/mL (range, 8–37 pg/mL; n = 7). B cells required the combination of IL-4 and anti-CD40 to produce IL-13 protein because neither alone lead to significant detectable IL-13. These results were consistent whether B lymphocytes were purified by means of sheep RBC rosetting or positive (anti-CD19 magnetic beads) or negative selection (B-cell enrichment cocktail). No IL-13 was detected with the T_H1 cytokine IFN-γ in the presence or absence of anti-CD40 stimulation. Similar quantities of T cells or cultured follicular dendritic cells stimulated with αCD40 plus IL-4 did not produce detectable IL-13 protein.

Anti-IL-13 antibodies inhibit IgE production by B lymphocytes

To determine the role of B-cell derived IL-13 in the production of IgE, anti-CD40/IL-4–stimulated B lymphocytes were cultured in the presence or absence of increasing concentrations of neutralizing IL-13 antibodies. After 14 days, supernatants were harvested and IgE was measured by means of specific ELISA. In the presence of IL-13 neutralizing antibodies (3 µg/mL), IgE production was diminished by approximately 85% (Fig 4, A). This suppression was concentration dependent, although doses of greater than 12 µg/mL caused some agonistic effect. Addition of isotype control or irrelevant anti-CD3 antibodies did not inhibit the production of IgE (data not shown).

We wished to investigate whether there was an IL-13–dependent mechanism in the induction of Cε transcripts by αCD40/IL-4. B cells were stimulated with αCD40/IL-4 and cultured in the presence or absence of optimal concentrations of neutralizing anti-IL-13 antibodies (1–3 µg/mL). Baseline of IgE transcripts detected was less than 2%; this was increased to 10% on day 5 by means of stimulation with αCD40/IL-4 (Fig 4, B). Neutralizing IL-13 antibodies significantly diminished the number of Cε⁺ cells in a dose-dependent fashion.

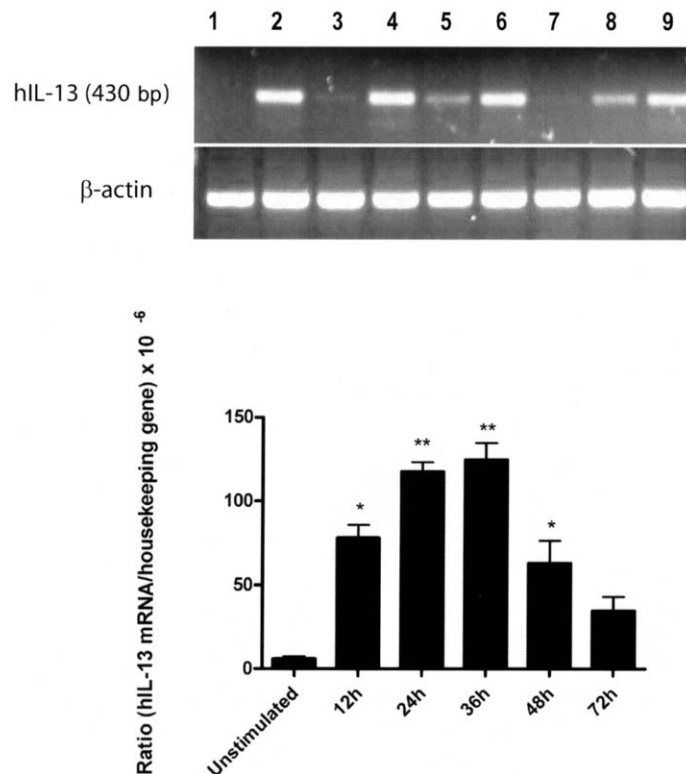


FIG 1. A, Analysis of human IL-13 mRNA expression by means of conventional RT-PCR in human tonsillar B and T cells. *Upper panel,* RT-PCR of human IL-13: *lane 1,* unstimulated T cells; *lane 2,* T cells plus PHA (24 hours); *lane 3,* unstimulated B cells (24 hours); *lane 4,* B cells plus α CD40/IL-4 (24 hours); *lane 5,* B cells plus α CD40/IL-2 (24 hours); *lane 6,* T cells plus PHA (48 hours); *lane 7,* unstimulated B cells (48 hours); *lane 8,* B cells plus α CD40/IL-4 (48 hours); *lane 9,* B cells plus α CD40/IL-2 (48 hours). *Lower panel,* RNA as above probed for β -actin. Results are representative of 3 identical experiments. **B,** Kinetics of human IL-13 mRNA induction in B lymphocytes by means of real-time PCR expressed as a ratio between IL-13 mRNA copies and β -microglobulin copies, calculated as in the Methods section. * $P < .05$, ** $P < .01$ by using the Student *t* test ($n = 3$).

Human B lymphocytes in tissues produce IL-13

We have demonstrated the presence of IgE-secreting B cells in appropriately stimulated nasal explant tissue¹¹ and hypothesized that IgE⁺ B cells in tissues might contain IL-13. To colocalize intracellular IL-13 within B-lymphocyte populations in nasal tissues, we double-stained B lymphocytes with CD19-FITC and intracellular IL-13-phycoerythrin from nasal biopsy specimens of atopic children ($n = 2$). Fig 5, A, shows the clusters of B cells within the nasal biopsy tissue, as demonstrated by CD19 staining. Within these clusters, several cells demonstrate double staining for IL-13 (yellow; Fig 5, C). Thus stimulated nasal tissues from atopic donors contain IL-13-secreting B cells, which might play a role in maintaining IgE production. B cells are rarely detected in nasal biopsy specimens of nonatopic children (data not shown).

DISCUSSION

In this article we have explored the synthesis of IL-13 by human B lymphocytes. Our data show for the first time that during maturation to IgE-secreting cells, human B

lymphocytes express IL-13 mRNA and produce IL-13 protein. This was confirmed by the colocalization of IL-13 and B cells in cultured cells and in tissues from atopic individuals. The neutralization of B cell-derived IL-13 abrogated IgE synthesis. Taken together, these data suggest that autocrine production of IL-13 might be crucial for the synthesis of IgE by B lymphocytes.

Autocrine cytokine secretion appears to be important for B-cell development. Stimulated B cells produce large amounts of IL-6 and GM-CSF,¹² whereas IL-12 and IFN- γ have been detected under certain conditions.¹³⁻¹⁵ B cell-derived IL-10 might also be an important regulatory molecule, particularly in autoimmune diseases.^{12,16-18} B cell-derived cytokines might influence surrounding cells in their environment by, for example, driving T cells to a T_H2 phenotype or aiding in the differentiation of other cells.¹⁹ Harris et al¹⁹ differentiated B cells from transgenic mice to secrete cytokines that resemble those of T_H1 and T_H2 lymphocytes, and other investigators have observed that B cells or their cytokines can influence T-cell development.²⁰⁻²² B cell-derived cytokines might also act as growth factors for B-cell clones. Cerruti et al²³ documented that neutralization of endogenously produced IL-10 and TGF- β in cultured CL-01 B cells diminished

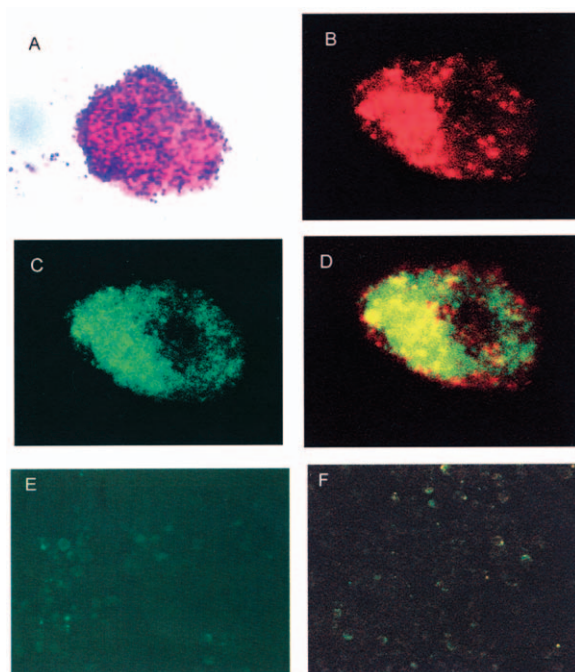


FIG 2. B lymphocytes cultured for 5 days with α CD40 and IL-4 in glass slide chambers. **A**, Colonies stained with hematoxylin and eosin: light microscopy (200 \times) showing follicle-like organization of cultured B cells. **B**, Fluorescent microscopy of B cells stained for surface CD19. **C**, Staining for intracellular IL-13. **D**, Analysis of double-stained cells. All cells staining yellow are both CD19⁺ and IL-13⁺. **E**, Unstimulated B lymphocytes stained identically as in **C** (300 \times). **F**, α CD40/IL-4-stimulated B-cell colony stained only with FITC-conjugated second antibody (300 \times). Results are representative of 4 identical experiments.

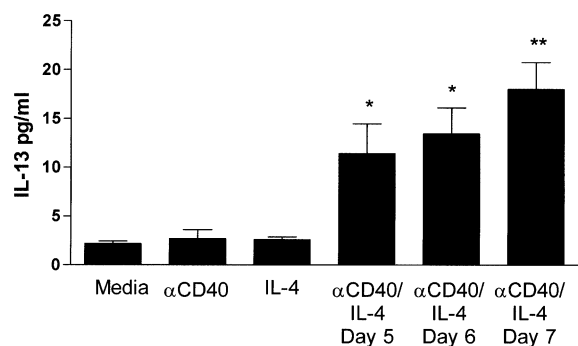


FIG 3. Detection of IL-13 in purified B-cell cultures in supernatant. Purified tonsillar B cells were cultured with or without anti-CD40 antibodies (1 μ g/mL) and IL-4 (400 U/mL) for up to 7 days. Supernatants were withdrawn, and IL-13 levels were measured with a commercial ELISA. * P < .05, ** P < .005, compared with media alone (n = 7).

production of IgG and IgA. Similarly, studies with isolated human B cells have shown that neutralization of IL-10 inhibits IgG, IgA, and IgM production,¹⁸ and inhibition of IL-6 impairs plasma cell development.²⁴

The expression of IL-13 mRNA and protein in stimulated but not resting tonsillar B lymphocytes suggests a role for IL-13 in the development of IgE-

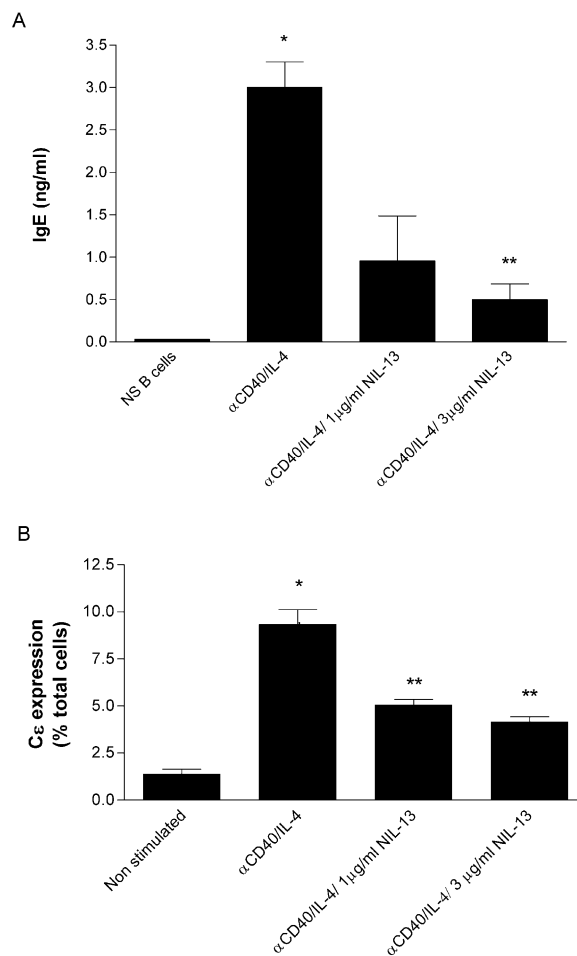


FIG 4. **A**, IgE production by B cells incubated with IL-4 and anti-CD40 with and without anti-IL-13 neutralizing antibodies (n = 3). **B**, Histogram showing enumeration of silver grains indicating C ϵ ⁺ cells after 5 days of culture, demonstrating a 60% decrease in C ϵ transcripts after IL-13 neutralization. For both, * P < .05 comparing α CD40/IL-4 and control and ** P < .005 comparing α CD40/IL-4⁺ anti-IL-13 with α CD40/IL-4.

secreting B cells. IL-13, but not IL-4, is present in the colonies that are formed as B cells differentiate after initial activation, as well as in B cells in the nasal mucosa of atopic individuals. IL-13 accumulates in B-cell culture supernatants after stimulation, and the amount detected at 7 days is similar to that produced by eosinophils or mast cells^{25,26} but less than that produced by cultured basophils or T cells.^{27,28} This difference in detection might be due to the rapid binding of IL-13 to its receptor on B cells, whereas human T cells in culture do not express IL-13 receptors.²⁹ Unfortunately, our attempts to completely block IL-13 uptake with anti-IL-13 receptor antibodies have not been successful to date, possibly because of incomplete penetration of the antibodies into the colonies. During B-cell development, IL-13 might influence early events, such as induction of IgE switching and IgE transcripts (C ϵ), or later maturation of IgE-secreting plasma cells. We found that neutralization of endogenous IL-13 by antibodies curtailed IgE synthesis by cultured B

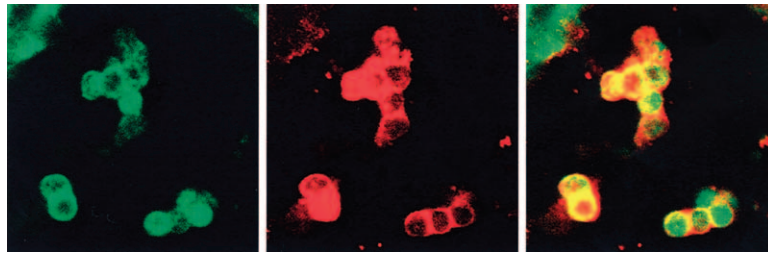


FIG 5. Direct visualization of intracellular IL-13 in human B Lymphocytes in tissues. Nasal biopsy tissue from atopic children was stimulated by an appropriate antigen for 24 hours. Permeabilized samples were stained for B cells (CD19-FITC) and IL-13–phycoerythrin and visualized by means of confocal microscopy. Results are representative of 2 experiments.

cells by 80% and IgE (C ϵ) transcripts by approximately 50%. The discordance between the presence of IgE transcripts and protein secretion suggests that IL-13 might play a more important role as a differentiation factor for the long-term survival of IgE-secreting B cells rather than as a crucial cytokine for isotype switching. A similar role has been proposed for IL-6, which has been shown to assist in the development of IgE-producing plasma cells.²⁴ In our experiments the significant production of IL-6 was not affected by IL-13 neutralizing antibodies (unpublished observation), yet IgE levels were markedly curtailed.

The IL-13 gene is located in a cluster that includes other T_H2 cytokine genes. Signals that induce IL-13 synthesis include those that contribute to T_H2 cell differentiation, such as GATA3³⁰ and signal transducer and activator of transcription 6.³¹ Ligation of CD28 leads to release of IL-13 from human eosinophils, whereas crosslinking of Fc ϵ RI induces IL-13 production by basophils and, in some cases, mast cells.^{26,32,33} Our results indicate that for B cells to release IL-13, both a cytokine signal and CD40 crosslinking need to be present. CD40 signaling through nuclear factor κ B and the p38 mitogen-activated protein kinase pathway are known to complement IL-4 and IL-2 signaling in B lymphocytes.³⁴ Further dissection of the role of these kinases in the regulation of IL-13 mRNA expression is currently underway in our laboratory, but preliminary data suggest a requirement for nuclear factor κ B translocation.

Previous studies have identified IL-13 mRNA in B-lymphocyte cell lines and in malignant clones.²⁸ These are the first studies to detect IL-13 protein in nontransformed human B cells. A possible explanation for this is that clonal organization or cell-cell interaction (Fig 2) is a prerequisite for optimal IL-13 synthesis by B cells. Although supernatant levels of IL-13 were consistent, we were not able to detect IL-13 intracellularly efficiently by means of flow cytometry or cytospin preparation. Direct staining of enlarging B-cell colonies optimized the detection of intracellular IL-13.

The finding of B cells staining for IL-13 in allergen-stimulated nasal mucosa is intriguing. These biopsy specimens were from atopic individuals, who were selected because these individuals had been shown previously to have B cells that were positive for C ϵ and I ϵ and were

able to secrete IgE *in situ*.¹¹ IgE-producing B lymphocytes are rarely seen in the nasal mucosa of nonallergic individuals.¹¹ The detection of locally produced IgE and the ability of B-cell clones to persist in extralymphoid mucosal tissues in the absence of external circulation³⁵ implies that internal factors are needed to sustain B-lymphocyte clones in tissues. IL-13 might be a crucial factor in sustaining IgE-committed B-cell clones. These clones might also influence adjacent nasal mucosa, not only through the production of IgE but also possibly through release of cytokines, such as IL-13.

We have clearly shown the presence of IL-13, and not IL-4, in the clonal expansion of committed IgE-secreting B cells. Further work must define the maturity and developmental pathways of IL-13⁺ B cells and to identify the role of IL-13⁺ B cells in the maintenance of plasma and memory B cells in allergic diseases. Better definition of the IL-13⁺ B-cell population in nasal mucosa and potentially in the inflammatory infiltrate of atopic asthmatic patients might demonstrate a role for B cells in allergic inflammation beyond that of IgE production alone.

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