

Original article

Expression of IL-9 receptor α chain on human germinal center B cells modulates IgE secretion

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Background: IL-9 has been shown to affect the differentiation pathway of different cell types. However, its potential role in the maturation pathway of antigen-driven B-cell differentiation and its functional effects remain unknown.

Objective: To characterize IL-9 receptor α chain (IL-9R α) expression on human tonsillar B cells at different maturational stages, and to assess its effect on IgE production.

Methods: Freshly purified human tonsillar B cells were fractionated into 3 populations: low-density (LD), medium-density, and high-density cells. Expression levels of IL-9R α were determined by using immunohistochemistry and flow cytometry. IL-9R α^{high} -expressing cells were stimulated with IL-9 in the presence or absence of IL-4, and IgE release was measured by ELISA.

Results: IL-9R α was expressed on human LD tonsillar B cells, with an ability to transduce signals through activation of signal transducer and activator of transcription 3 and 5. Although IL-9 was unable to induce IgE secretion by itself, it potentiated IL-4-mediated IgE production from LD cells. Moreover, increased IgE was paralleled by an upregulation of IL-9R α and CD27, with the latter a memory B-cell marker implicated in increased IgE secretion.

Conclusion: These results highlight a crucial role for IL-9 in modulating T-cell-dependent B-cell differentiation and establish a new paradigm for understanding the synergistic role of T_H2 cytokines and their modulatory effect on B-cell maturation and IgE production.

Clinical implications: IL-9 appears to be involved in memory B-cell differentiation and T_H2-mediated allergic diseases such as asthma. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Germinal center B cells, B-cell maturation, IL-9, IgE

IL-9 exerts its action by binding to the IL-9 receptor (IL-9R), a heterodimer consisting of the IL-9R α and the common γ chain. IL-9 and the IL-9R α can affect differentiation of human T cells,¹ eosinophils,² and mast cells.³ However, despite reports suggesting a role for IL-9 in immunoglobulin production using peripheral blood lymphocytes,⁴ there are no reports describing the expression of IL-9R α on human B lymphocytes.

Human and mouse genetic studies implicate IL-9R α expression and IL-9 in the pathogenesis of asthma and allergic inflammation.^{5,6} Overexpression of IL-9 in mice systemically⁷ or via a lung-specific promoter⁸ results an asthma phenotype including mucus overproduction, sub-epithelial fibrosis, increased intraepithelial mast cells, lung eosinophilia, airway hyperresponsiveness, and increased responsiveness to antigen stimulation.^{8,9}

The effect of IL-9 on different inflammatory and structural cells has been studied in the context of asthma.¹⁰ Functional studies showed that stimulation of polymorphonuclear leukocytes from patients with asthma with IL-9 results in the release of IL-8.¹¹ Airway epithelial cells are activated by IL-9 to produce chemokines and proteases and to upregulate expression of ion channels and selective mucin genes.^{12,13} Airway smooth muscle cells are also a target for IL-9, which amplifies recruitment of eosinophils and neutrophils into the airways through upregulation of the IL-5R α receptor and secretion of IL-8.¹⁴

As part of our studies on the contribution of B lymphocytes and IgE to allergic airways disease, we sought to determine expression of IL-9R α on human tonsillar B cells. Here, we present novel evidence of the presence of the IL-9R α on primary human tonsillar B lymphocytes with predominantly higher expression on germinal center (GC) B cells and on B cells with a memory phenotype. The presence of IL-9R α on GC cells was specifically shown to be functional, because stimulation with IL-9 resulted in upregulation of phosphorylation of signal transducer and activator of transcription (STAT)-3 and STAT5 transcription factors and the ability of IL-9 to potentiate IgE production. Moreover, CD27 expression

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Abbreviations used

FACS:	Fluorescence-activated cell sorting
FITC:	Fluorescein isothiocyanate
FM:	Follicular mantle zone
GC:	Germinal center
HD:	High-density
IL-9R α :	IL-9 receptor α chain
LD:	Low-density
MD:	Medium-density
PE:	Phycoerythrin
STAT:	Signal transducer and activator of transcription
TBS:	TRIS-buffered saline

on IL-9R α ⁺ low-density (LD) B cells was observed, characterizing the phenotype of IgE producers in the context of CD40 stimulation of B cells as memory B cells. We provide clear evidence for IL-9's role in the late phase of B-cell differentiation in secondary lymphoid follicles, suggesting a role for IL-9 in the regulation of peripheral B-cell differentiation and immunoglobulin production in human beings.

METHODS

B-cell purification

Fresh B lymphocytes were isolated from human tonsils discarded after surgery. The tonsils were thoroughly minced; resuspended in wash medium consisting of RPMI 1640 (Invitrogen, Burlington, Ontario, Canada) supplemented with 2% FCS (Hyclone Laboratories Inc, Logan, Utah), 50 U/mL penicillin, 50 μ g/mL streptomycin, and amphotericin B (1/500 wt/vol) from Life Technologies (Burlington, Ontario, Canada); and layered onto a Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) gradient. Tonsil lymphocytes were separated by rosetting with neuraminidase-treated sheep red blood cells and Ficoll-Paque density centrifugation. Monocytes were removed from the E-rosette-negative fraction by adherence depletion; the remaining B cells were routinely >98% pure on flow cytometry by CD20 staining, with <1% CD14⁺ and <1% CD3⁺.

Percoll gradient centrifugation

Adherence-depleted B cells were layered onto Percoll gradients (Pharmacia Biotech) according to the methods of Liu et al¹⁵ and Suzuki et al.¹⁶ Cells recovered below the 60% layer were considered high-density (HD) cells; between the 50% and 60% layers were medium-density (MD); and LD cells (GC cells) were recovered from the 30% to 50% layers.^{15,17}

Immunocytochemistry

Cytospins were prepared and slides washed with TRIS-buffered saline (TBS; pH 7.6). After saturation for 10 minutes with Universal Blocking Solution (DAKOCytomation Inc, Mississauga, Ontario, Canada), the slides were incubated with mouse anti-IL-9R α (R&D Systems, Cedarlane Laboratories Ltd, Burlington, Ontario, Canada) or isotype-matched control (5 μ g/mL) overnight at 4°C. After washing, rabbit antimouse immunoglobulin (1:60) (DAKOCytomation Inc) was added for 30 minutes at room temperature followed by alkaline phosphatase antialkaline phosphatase (1:60) for 30 minutes at room temperature. The slides were developed by using Fast Red (Sigma, Toronto, Ontario, Canada) and counterstained with hematoxylin Gill II (Surgipath Canada, Winnipeg, Manitoba, Canada).

Immunohistochemistry

Freshly obtained tonsillar tissue was cut into small pieces (1 $\times$ 1 $\times$ 0.5 cm), and a cryoblock was made by immersing tissues covered with optimal cutting temperature embedding medium into isopentane cooled in liquid nitrogen until tissue sections were blocked. Cryoblocks were kept at -80°C until use. Cryostat sections of 5 μ m each were prepared, dried, and fixed in acetone:methanol. Fixed sections were blocked, washed with TBS, and incubated in a humid chamber overnight at 4°C with a rabbit antihuman IL-9R α antibody (1/100; Santa Cruz Biotechnology, Santa Cruz, Calif) and mouse antihuman CD20 (1/100; DAKOCytomation Inc).

After washing, sections were incubated with a second antibody (horse-antimouse IgG biotin, 1/100; Vector Labs Canada, Burlington, Ontario, Canada) for CD20 and swine antirabbit IgG biotin (DAKOCytomation Inc; 1/100) for IL-9R α . After another wash, sections were incubated with Streptavidin-alkaline phosphatase (1/200 in TBS; DAKOCytomation Inc) for CD20 and horseradish peroxidase for IL-9R α for 30 minutes (DAKOCytomation Inc). This was followed by incubation in the alkaline phosphatase substrate Fast Red for 20 minutes and a 5-minute incubation with 3,3'-diaminobenzidine (DAKOCytomation Inc) + H₂O₂, revealing the IL-9R α chain. Slides were counterstained with hematoxylin, dehydrated, and mounted.

Immunofluorescence

Slides were processed for immunofluorescent labeling as described for immunohistochemistry. Primary mAbs directed against CD19 (1:10; BD Biosciences, Mississauga, Ontario, Canada) were labeled with Alexa Fluor 568 (Molecular Probes, Portland, Ore) by using the Zenon Red kit (Molecular Probes, Portland, Ore). mAb against IL-9R α (1:50; R&D Systems) was followed by a fluorescein isothiocyanate (FITC)-conjugated goat antimouse IgG Ab (Pharmin-gen). Slides were mounted after washing in TBS with PermaFluor (Immunon, Thermo-Fisher Scientific, Waltham, Mass) and viewed with a confocal fluorescent microscope.

Flow-cytometric analysis

Three-color analysis was performed with unlabeled IL-9R α mAb and FITC-goat antimouse antibody, directly phycoerythrin (PE)-conjugated mouse antihuman IgD, and allophycocyanin-conjugated mouse antihuman CD38 (BD Biosciences, Mississauga, Ontario, Canada). Two-color analysis was performed with unlabeled IL-9R α mAb and FITC-goat antimouse antibody, and directly PE-conjugated mouse antihuman CD27 (BD Biosciences). PE-conjugated IgG₁ was used as an isotype control for IgD staining, APC-conjugated IgG₁ was used as an isotype control for CD38, and mouse IgG₁ followed by FITC-conjugated goat antimouse was used as a control for IL-9R α FITC staining. FACSCalibur (BD Biosciences) was used to analyze 5×10^5 cells/sample with Cell Quest software (BD Biosciences).

Detection of STAT phosphorylation

Phosphorylated STAT proteins were labeled with phosphospecific antibodies (rabbit polyclonal) that detected phosphorylated tyrosine residues pTyr 694 for activated STAT5 (Cell Signaling Technology, Danvers, Mass), pTyr 705 for STAT3, or pTyr 701 for STAT1. The antibodies were used at a 1/100 dilution. Cellular fixation and immunostaining were performed as described previously.¹⁸ Intracellular levels of phosphorylated STATs were measured by flow cytometry.

Culture and detection of IgE

Purified LD tonsillar B cells were cultured with or without anti-CD40 (1 μ g/mL) and IL-4 (400 U/mL) in the presence or absence of IL-9 (10 ng/mL) at 5×10^5 /mL at 1 mL/well for 14 days at 37°C, 5%

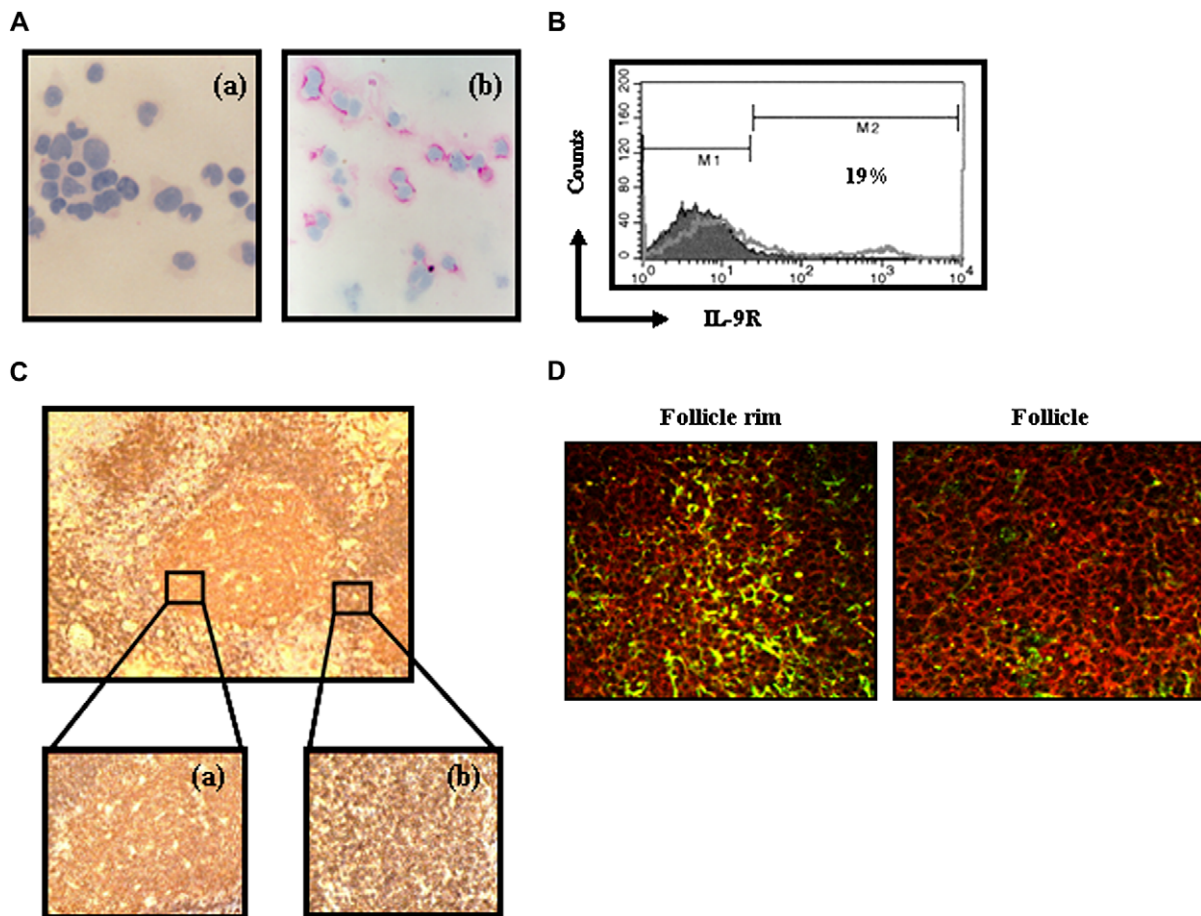


FIG 1. Detection of IL-9R α on tonsillar B cells. **A**, Immunocytochemistry for IL-9R α . *a*, Isotype control; *b*, B cells stained for human IL-9R α . **B**, FACS for IL-9R α expression. Representative of 3 individual experiments; mean expression, $21.33\% \pm 3.21\%$. **C**, Immunohistochemistry for IL-9R α on tonsillar sections. Alkaline-phosphatase antialkaline-phosphatase double-immunostaining for IL-9R α and CD20 expression of a secondary follicle (magnification $\times 20$). *a*, Inside the follicle ($\times 40$); *b*, edge of the follicle ($\times 40$). **D**, Immunohistochemistry for IL-9R α on tonsillar sections. Confocal microscopy. Follicle rim staining IL-9R α -positive cells (green) and CD19 (red); coexpression of IL-9R α and CD19 (yellow). Follicle staining shows scant IL-9R α expression on B cells. *M1*, Isotype control; *M2*, IL-9R α positive population.

CO₂. Culture media was replenished at day 7 of cell culture without added cytokines. The cultured supernatants were harvested, and IgE was measured according to the protocol of Hajoui et al.¹⁹ The limit of IgE detection was 50 pg/mL.

RESULTS

IL-9R α is expressed on tonsillar B cells

We assessed expression of IL-9R α on B cells using freshly isolated purified human tonsillar B cells. Human tonsils receive repeated antigen stimulation and display all stages of peripheral B-cell development; hence, they constituted an ideal tissue to determine IL-9R α expression at different stages of B-cell maturation. Immunocytochemistry was first performed on cytopspins of unfractionated B cells by using an affinity-purified mAb to IL-9R α . B cells stained positively for IL-9R α compared with the isotype control (Fig 1, *A, a, b*). Flow cytometry analysis of B cells stained with a mAb to IL-9R α confirmed this

observation, showing positive reactivity with $21.33\% \pm 3.21\%$ IL-9R α -positive cells (Fig 1, *B*).

Immunohistochemistry of IL-9R α expression on tonsillar B cells

These findings prompted us to investigate the localization of IL-9R α -positive B cells within lymphoid follicles. We therefore performed immunohistochemistry on sections of human tonsils. By using double staining with anti-IL-9R α and anti-CD20 (Fig 1, *C*), or anti-IL-9R α and anti-CD19 (immunofluorescence; Fig 1, *D*), we found that CD20⁺CD19⁺ B cells within the secondary lymphoid follicles and especially cells on the edge of these follicles displayed IL-9R α . Comparing the intensities of expression of IL-9R α inside the follicle versus the edge of the secondary lymphoid follicles, we observed that although IL-9R α -positive cells are found in both areas, cells on the edge of the follicles displayed a higher frequency of expression (Fig 1, *C and D*). Taken together, these results

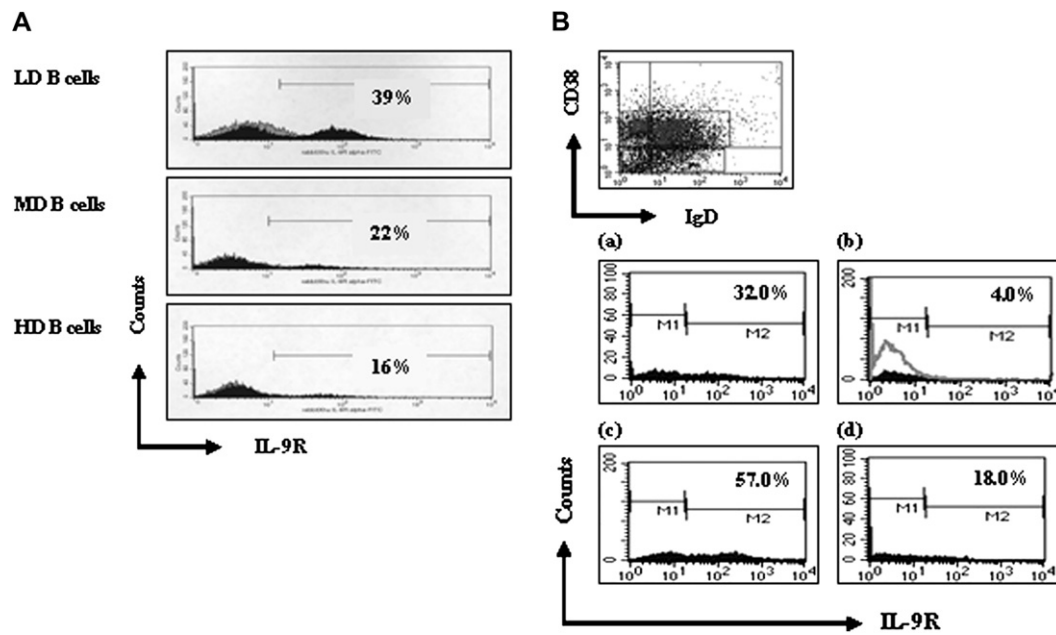


FIG 2. IL-9R α expression on tonsillar B-cell subsets. **A**, Expression of IL-9R α on tonsillar B-cell subsets. Percentages indicate IL-9R α high cells within LD, MD, and HD fractions. Representative of 3 experiments; mean, 36.1% $\pm$ 4.8% for LD cells, MD (17.5% $\pm$ 4.6%), and HD B cells (18.4% $\pm$ 2.2%). Isotype in gray; black represents IL-9R α ⁺ cells. **B**, FACS for IL-9R α /IgD and CD38 expression on LD B cells. Three-color FACS staining of LD cells with anti-IL-9R α , anti-CD38, and anti-IgD. Results are shown for 1 of 3 individual tonsillars. A representative dot plot is shown for IgD and CD38-positive cells, and histograms for the selected gates are shown: a, FM: IgD⁺CD38[−] mean, 29.4% $\pm$ SD 6.2%; b, GC: IgD[−]CD38⁺ 8.1% $\pm$ 4.8%; c, founder GC: IgD⁺CD38⁺ 54.3% $\pm$ 16.2%; d, memory: IgD[−]CD38[−] 19.7% $\pm$ 8.5%. IL-9R α -positive cells in the figure represent 1 of 3 experiments.

suggest that the IL-9R α is principally expressed on follicular mantle B cells.

The distribution of IL-9R α expression among B-cell subsets

We then fractionated human tonsillar B lymphocytes by using discontinuous Percoll density gradients. Using this method, we obtained 3 populations: LD (recovered in the 30% to 50% Percoll fraction), which represents mainly GC B cells; MD (50% to 60%), which are primarily centroblasts and centrocytes, and HD (>60%) or mature/resting B cells.¹⁷ Using flow cytometry, IL-9R α expression was higher in the LD or GC fraction of B cells (39%) compared with MD (22%) and HD fractions (16%; Fig 2, A).

Phenotype of GC cells expressing IL-9R α

To characterize further the phenotype of GC cells expressing the IL-9R α , 3-color flow cytometry was used to trace the IL-9R α expression on human tonsillar B cells through their progression in the follicular GCs from naive GC cells (follicular mantle zone [FM] B cells; IgD⁺CD38[−]), to founder GC cells (IgD⁺CD38⁺), to memory B cells and plasma cells (IgD[−]CD38[−] and IgD[−]CD38⁺, respectively).²⁰ On analysis of CD38⁺ and IgD⁺ cells for IL-9R α expression, we could clearly distinguish 2 distinct populations on the basis of IgD expression: IgD[−]IL-9R^{lo} and IgD⁺IL-9R^{hi} cells (Fig 2, B).

IL-9R α expression was highest within the IgD⁺CD38⁺ founder GC cells (54.3% $\pm$ 16.2%; Fig 2, B, c), and IgD⁺CD38[−] FM B cells (29.4% $\pm$ 6.2%; Fig 2, B, a). In contrast, GC cells that have lost IgD expression and are CD38⁺—that is, centroblasts and centrocytes—show no significant expression of IL-9R α (GC, IgD[−]CD38⁺ 8.1% $\pm$ 4.8%; Fig 2, B, b). In addition, a population of memory B cells (IgD[−]CD38[−]) was positive for IL-9R α (19.7% $\pm$ 8.5%; Fig 2, B, d), indicating a possible role for IL-9 in regulating secondary immune responses.

IL-9 induces tyrosine phosphorylation in LD cells

To ascertain the functionality of the IL-9R α , we assessed protein tyrosine phosphorylation on unfractionated tonsillar B cells as well as LD B cells, following activation with IL-9 for 15 minutes. Tyrosine phosphorylation was observed in IL-9 stimulated LD cells and not in unstimulated LD cells (data not shown). Unfractionated B cells did not have perceptible increases in tyrosine phosphorylation. This upregulation was diminished on addition of a neutralizing antibody to IL-9.

Using specific antibodies that detect phosphorylated tyrosine residues associated with activated STAT proteins, including STAT1, 3, and 5 (Tyr 694), we performed immunostaining of IL-9 stimulated LD cells and expression levels of phosphorylated STATs by flow cytometry. These 3 STAT proteins have all been associated with IL-

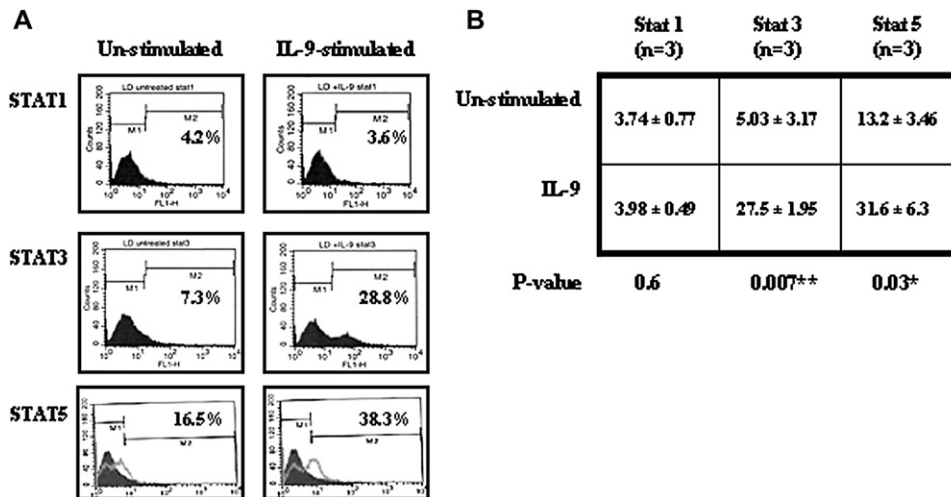


FIG 3. IL-9 induces STAT phosphorylation. **A**, Staining for phosphorylated tyrosine residues associated with activated STAT1, 3, and 5 was performed on unstimulated and IL-9-stimulated LD cells and analyzed by flow cytometry. Representative of 3 independent experiments. **B**, Mean of 3 experiments + SE. * $P \leq .05$ and ** $P \leq .01$ by paired Student *t* test.

9R α signaling in other cells, including IL-9R α transfectants.^{21,22} The results show an upregulation of STAT3 and 5 phosphorylation after IL-9 stimulation, specifically in LD cells, as shown by flow cytometry (Fig 3).

Synergistic effect of IL-9 on IL-4-induced IgE production

We have shown by immunohistochemistry and fluorescence-activated cell sorting (FACS) analysis that tonsillar LD cells have the highest expression of IL-9R α , with a preferential expression on IgD⁺ and memory B-cell populations in GCs. To determine the effect of IL-9 on IgE production by LD cells, we cultured LD cells in the presence or absence of anti-CD40 with or without IL-4, IL-9, or both cytokines. IL-9 alone in the presence of anti-CD40 does not induce IgE production compared with treatment with anti-CD40 and IL-4 (Fig 4). Addition of IL-9 to anti-CD40 and IL-4 consistently potentiated IgE production, albeit with donor to donor variability (Fig 4). We detected little IgE in culture before day 5; thus, IgE was measured on days 7 and 14 of culture. A synergistic effect of IL-9 is observed both on days 7 and 14 of culture. Although IL-9 is unable to induce IgE production by itself, it can potentiate IL-4's activity by upregulating IgE production.

IL-9R α and CD27 are upregulated on CD40-stimulated LD B cells

We sought to determine the phenotype of cells with increased IgE production. For this purpose, we stained for IL-9R α expression at days 1 and 10 of cell culture. Our results show that IL-9R α expression is upregulated through CD40 stimulation and maintained throughout the culture period (Fig 5, A). Cells were also stained for CD27 expression, which showed a pattern similar to the observed IL-9R α expression, whereby the observed

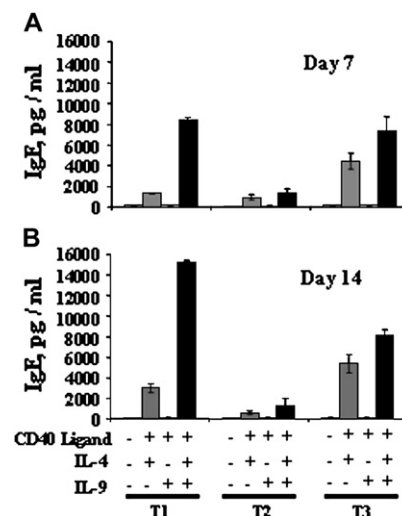


FIG 4. Synergistic effect of IL-9 on IL-4-induced IgE production. IgE measurement from supernatants of cultured LD B lymphocytes. IgE was assayed by ELISA. Values are given in pg/mL and represent the mean of 2 values $\pm$ SD. Results shown are for 3 independent tonsils (T1, T2, T3).

increase correlated specifically with CD40 and IL-4 stimulation (Fig 5, B and C). Interestingly, the increase in CD27 expression occurred predominantly on IL-9R α positive cells, suggesting a link with IgE production. Analysis of IL-9R α expression on peripheral blood lymphocytes from normal donors showed a similar pattern for CD27 expression (Fig 5, D).

DISCUSSION

T_H2 cytokines play a crucial role in the genesis of allergic disease. IL-9 is produced by T_H2 cells during immune

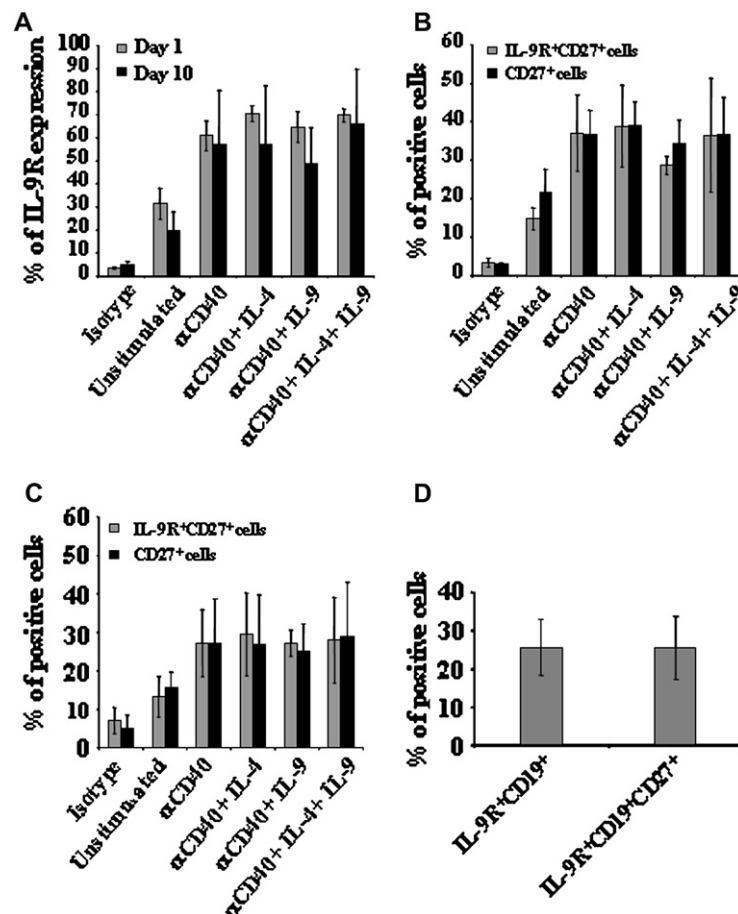


FIG 5. A, IL-9Rα expression during 10-day cultures. LD cells were stained for IL-9Rα after culture with anti-CD40/IL-4 and/or IL-9 for the indicated periods. B, IL-9Rα CD27 and CD27 expression on day 1 (n = 3). C, IL-9Rα CD27 and CD27 expression on day 10 (n = 3). D, PBMC staining for IL-9Rα. Staining for IL-9Rα on PBMCs purified from nonatopic donors. IL-9Rα⁺CD19⁺ 25.60% of total cells ± 7.34%; IL-9Rα⁺CD19⁺CD27⁺ 25.54% ± 8.32% (n = 3).

inflammation and is well recognized as a contributor to bronchial reactivity. There has been little work on the role of IL-9 in other important T_H2-driven processes such as IgE production. In spite of limited data identifying a possible influence of IL-9 on IgE production, it was unknown if IL-9 directly affected B lymphocytes or whether its action was mediated via other cells. In this study, we identify the presence and signaling pathways of IL-9Rα on human tonsillar B cells. We have specifically identified the LD Percoll-fractionated subset of cells, which consists of FM and GC B cells¹⁷ as the major tonsillar B-cell population expressing the IL-9Rα chain (Fig 2, A). We further characterized the LD fraction, identifying that a significant proportion of IL-9Rα-positive cells express IgD (Fig 2, B), with the majority on IgD⁺CD38⁺ (57%) cells and IgD⁺CD38⁻ (32%) cells.²⁰ Moreover, a population of IL-9Rα⁺ cells is found to coexpress CD27, the marker of memory B cells. This is of interest, because IL-9Rα⁺CD27⁺ cells in tonsils may be recirculating memory B cells re-entering the GC and undergoing a second round of selection as has been proposed by McHeyzer-Williams and McHeyzer-Williams,²³ and supported by a recent

report describing the presence of CD27⁺ B cells on the entire follicular periphery around the GC.²⁴

IL-9Rα expression corresponds to the entry of antigen-exposed FM B cells to the GC FM B cells (IgD⁺CD38⁻) are antigen-activated B cells that enter secondary follicles from periphery. Depending on their B-cell receptor affinities, a few are selected as founder GC cells (IgD⁺CD38⁺), which undergo somatic hypermutation of their IgV region genes and proliferate in the dark zone of the follicle as centroblasts. Centrocytes are selected on the basis of their affinity to antigen expressed by follicular dendritic cells or T cells; with low affinity, centrocytes die by apoptosis. Positively selected centrocytes present peptides from FDC-derived antigen to local T cells, and may class-switch their immunoglobulin genes from IgM to IgG or IgE and differentiate into plasma cells or CD27⁺ memory B cells, or undergo receptor editing to enhance antigen affinity. We demonstrate significant expression of IL-9Rα on maturing B-cell subsets, enhanced by T-cell-derived signals, CD40 ligation, and IL-4 (Fig 5). Both the MD and HD fractions, which contain more IgD⁻CD38⁺ and IgD⁻CD38⁻ cells, have significantly

lower expression of IL-9R α and decreased signaling through IL-9 compared with the LD fraction (Fig 2, A).

Signal transducer and activator of transcription proteins that have been linked to IL-9 signaling include STAT1, STAT3, and STAT5.^{21,22} Staining for tyrosine phosphorylated residues in STAT1 (Tyr 701) and STAT3 (Tyr 705) in total tonsillar B cells and LD cells demonstrate specific activation of STAT3 and 5, but not STAT1 in LD cells in the presence of IL-9 (Fig 3, A). This is particularly interesting because STAT3 is upstream of differentiation genes in IL-9 signaling events,²¹ whereas STAT5 phosphorylation has been linked to cellular growth and proliferation.²⁵ In mice, STAT3 has been shown to be required for T-dependent IgG plasma cell differentiation.²⁶ Taking into account the species difference, a similar role in human B cells seems likely.

We have demonstrated STAT3 and STAT5 phosphorylation by IL-9 in primary B cells. Phosphorylated STAT5 previously was shown only in studies overexpressing the receptor.^{22,27} STAT5 was recently reported to be phosphorylated in a subset of tonsillar B cells, linked to upregulation of B-cell lymphoma protein 6, a transcription factor involved in memory B-cell differentiation.²⁸ CD27⁺ B cells from the LD fraction may well coexpress CD38, as we and others have shown.²⁹ This subset behaves like other memory cells but is made up of potent immunoglobulin secreting cells. The fact that IL-9 can potentially act on this memory B-cell subset as well as early centrocytes (IgD⁺CD38⁺) suggests that IL-9 may indeed potentiate immunoglobulin secretion, which is borne out by our data on IgE synthesis. Our laboratory is currently addressing this possibility.

One of the best characterized cytokines produced by T cells in the context of a productive T-B cellular collaboration is IL-4, which is required for IgG₁ and IgE production.³⁰ IL-4 mediates IgE gene rearrangements and class switching via the transcription factor STAT6. Indeed, the actions of the IL-4 pathway can be replicated in part by IL-13, also released by activated T_H2 cells. The IL-13 receptor complex shares the IL-4R α chain with the IL-4 receptor complex, and IL-13 also signals predominantly via STAT6. Importantly, IL-13 receptor expression in B lymphocytes appears to be induced following initial T-cell signals, making it unlikely that in early B-cell activation, IL-4 and IL-13 act at the same time.²⁹ In contrast, IL-9 is able to act synergistically with IL-4. IL-9R α is present on both resting and activated B cells; IL-9 signaling, via STAT3 and STAT5 in our study, is distinct from IL-4 or IL-13. IL-9 thus can synergize with IL-4 to upregulate IgE production (Fig 4). This is not mediated by an increase in cellular proliferation, because the use of different modes of activation, suboptimal IL-4, and incremental doses of IL-9 did not affect B-cell proliferation on its own or in the presence of CD40 antibody and IL-4 (data not shown). CD40 and IL-4 stimulation also upregulated coexpression of IL-9R α and CD27 on LD B cells. This suggests a potential role for IL-9 in memory B-cell differentiation or in development of immunoglobulin-secreting cells characterized by coexpression of CD38 and CD27.

In humans, expression of the IL-9R α chain has been documented on different cell types involved in the pathogenesis of asthma. IL-9R α was detected in cultured human airway smooth muscle cells, primary cultured airway smooth muscle cells, and bronchial smooth muscle cells within biopsies from patients with asthma.³¹ In addition, a few reports have linked allergen challenge to an increase in IL-9R-positive cells in bronchoalveolar lavage fluid from patients with atopic asthma.³² IL-9 seems to be acting by synergizing with other inflammatory cytokines. IL-9 synergizes with TNF- α to induce IL-8 secretion and synergizes with IL-13 to increase eotaxin release from airway smooth muscle cells *in vitro*.¹⁴ Moreover, IL-9 synergizes with IL-5 in driving eosinophilic maturation of precursors.^{33,34}

Taken together, these data show that IL-9R α is expressed on human FM B cells and founder GC cells, specifically on IgD⁺ B cells, strongly suggesting a role for IL-9 in GC B-cell maturation and differentiation. Moreover, our data show a synergistic role for IL-9 in IgE production from human primary GC cells with IL-4 in the CD40/IL-4 culture system. This is particularly relevant to *in vivo* situations whereby IL-4 and IL-9 may affect B-cell differentiation, especially in the light of genetic evidence linking IL-9 genes to increased susceptibility to asthma.

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