

IgE⁺, Kit⁻, I-A/I-E⁻ myeloid cells are the initial source of IL-4 after antigen challenge in a mouse model of allergic pulmonary inflammation

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Background: IL-4 is generated within hours after antigen lung challenge and influences events that take place early in the induction of pulmonary inflammation. However, the cells responsible for this early IL-4 production in the lung are unknown.

Objectives: We sought to characterize the initial inflammatory events in the lung after antigen challenge and to identify cells responsible for producing IL-4 at early time points.

Methods: Mice were sensitized with ovalbumin or passive IgE and challenged intranasally. Histologic measures of inflammation were used, and lung tissue cytokine production was analyzed by means of RT-PCR. Cells producing IL-4 were characterized by means of in situ hybridization and flow cytometry.

Results: IL-4 mRNA was detectable 100 minutes after challenge in sensitized animals. Blockade of this early IL-4 down-regulated vascular cell adhesion molecule 1 mRNA expression and attenuated the early recruitment of eosinophils to the lung. CD4-depleted and mast cell-deficient mice both expressed early IL-4. Cellular analysis revealed the presence of IL-4 protein at 100 minutes exclusively in IgE⁺ myeloid cells that did not express CD3, Kit, or I-A/I-E. Moreover, IL-4 production induced by means of passive IgE sensitization and abrogated in FcR γ -chain-deficient mice supports the conclusion that this IL-4 production is dependent on IgE/ γ -chain interaction.

Conclusion: IL-4 production by an IgE/ γ -chain-dependent mechanism occurs rapidly after allergen challenge. At these early time points, IL-4 is produced by a myeloid cell with the characteristics of a mouse basophil (IgE⁺, Kit⁻, I-A/I-E⁻). These data thus suggest that strategies targeting basophils should be considered in the treatment of early lung inflammation. (*J Allergy Clin Immunol* 2002;110:117-24.)

Key words: Mast cells, basophils, asthma, IL-4, IgE, Fc receptors

IL-4 influences events that take place early in the induction of pulmonary inflammation. Thus, in a murine model of allergic asthma, in which asthmatic inflammation is provoked in sensitized mice by means of antigen

Abbreviations used

BAL: Bronchoalveolar lavage
DNP: Dinitrophenyl
ISH: In situ hybridization
OVA: Ovalbumin
SINC: Single intranasal challenge
VCAM-1: Vascular cell adhesion molecule 1

challenge, IL-4 promotes IgE synthesis, contributes to early recruitment of eosinophils,¹⁻⁴ and has been detected as early as 3 hours after challenge.⁵ The role of IL-4 in modulating this eosinophil influx is believed to be through upregulation of vascular cell adhesion molecule 1 (VCAM-1) on lung endothelial cells.⁶⁻⁹ Additionally, early IL-4 production is followed sequentially by production of IL-5 and other cytokines also known to be important in asthma pathogenesis.⁸ Despite these observations, little is known of the cells that produce IL-4 within the first few hours after antigen challenge. The purpose of our study was thus to identify the cells responsible for producing IL-4 in the context of early inflammatory events.

Mast cells, basophils, eosinophils, macrophages, natural killer cells, and B and T lymphocytes have been documented to produce IL-4.¹⁰⁻¹⁸ Because initial IL-4 expression within pulmonary tissue occurs soon after challenge in our sensitized mice, it is likely that this early IL-4 production is dependent on an early-response population that responds to IgE-dependent stimulation.^{10,19} We thus hypothesized that IL-4 was produced by pulmonary mast cells within the first hour after antigen challenge. As will be shown with a variety of approaches, the principal cells producing IL-4 were not found to be mast cells but consisted of a population of IgE⁺, Kit⁻, I-A/I-E⁻ myeloid cells. These characteristics define a basophil population.

METHODS

Sensitization and challenge

BALB/c (NCI, Frederick, Md), FcR γ -chain knockout ($-/-$; B6129P-Fcrlg^{tm1Rav}), and mast cell-deficient W/W^v or Sl/Sl^d mice with respective littermate control animals (Jackson Laboratory, Bar Harbor, Me) aged 6 to 10 weeks were used in these experiments. Sterile caging and food and water were supplied ad libitum. Experiments involving mice were performed in accordance with a protocol approved by the National Institute of Allergy and Infectious Diseases Animal Care and Use Committee.

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Mice were sensitized by means of intraperitoneal injection of 1 mg of chicken ovalbumin (OVA; grade V; Sigma Chemicals, St Louis, Mo) in 5 mg of aluminum hydroxide (alum, Sigma) or with PBS alone (sham). On day 10, mice were anesthetized with methoxyflurane and subjected to a single intranasal challenge (SINC) with either 1 mg of OVA in 100 μ L of PBS or PBS alone (sham). For *in vivo* neutralization of IL-4,¹ groups of mice received either a single intravenous injection of 700 μ g of anti-IL-4 mAb (clone 11B11, NCI) or PBS immediately before antigen challenge. *In vivo* depletion of T-cell subsets²⁰ was performed by means of intraperitoneal injection of 2 mg of anti-CD4 mAb (clone GK1.5, NCI) or anti-CD8 mAb (clone 2.43, NCI) 2 days before antigen challenge.

We also examined a passive cutaneous anaphylaxis model²¹ wherein BALB/c mice were injected through the tail vein with either 10 μ g of IgE anti-dinitrophenyl (anti-DNP; clone SPE-7, Sigma) or PBS alone (sham). After 24 hours, mice underwent SINC with 50 μ g of DNP-human serum albumin (Sigma) in 100 μ L of PBS or with PBS alone (sham).

IgE and OVA-IgE ELISA

At day 9 after intraperitoneal OVA injection, serum total IgE levels were measured by using a standard sandwich ELISA (Pharmingen, San Diego, Calif), according to the manufacturer's protocol. For measurement of OVA-specific IgE, 96-well plates were coated with OVA at 1 μ g/mL overnight and blocked with PBS in 3% BSA. Serum samples were applied to the plates, coated with 100 μ L of 2 μ g/mL biotinylated anti-mouse IgE, and detected with avidin-peroxidase (Sigma), followed by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) substrate solution (Sigma). Optical density was read at 410 nm. Individual serum samples were compared with a reference sample of pooled sera from OVA-alum-immunized mice and expressed as arbitrary units per milliliter of OVA-IgE relative to that reference.

Histologic studies

Bronchoalveolar lavage (BAL) was performed through a tracheal catheter with 3 successive washes of 0.5 mL of cold PBS. Cells (5×10^3) from each specimen were cytocentrifuged (Cytospin 3; Shandon, Pittsburgh, Pa) and stained with Kwik-Diff (Shandon). After counting 3 random fields per specimen under light microscopy (200 $\times$), the mean percentage of eosinophils per total cell population was reported. After BAL, lungs were perfused with 10% buffered formalin, embedded in paraffin, and cut into 6- μ m sections. For rating overall inflammation, Giemsa-stained sections were blinded by 3 different observers and scored on the basis of the presence of overall cellularity or perivascular, peribronchial, or pleural infiltrates.²²

In situ hybridization

In situ hybridization (ISH) procedures were performed at Molecular Histology, Inc (Gaithersburg, Md). Briefly, lung specimens were fixed in paraformaldehyde, embedded in plastic, and digested with proteinase K solution. Sense or antisense ³⁵S-methionine-labeled full-length IL-4 probes (clone 37561; American Type Culture Collection, Rockville, Md) were applied and incubated overnight. Nonspecifically bound probe was removed by means of RNase digestion. The slides were dipped in photographic emulsion, incubated, and developed.

RT-PCR for IL-4, IL-5, IFN- γ , and VCAM-1

Lungs and hearts were removed en bloc, and lungs were perfused with sterile PBS to remove any remaining peripheral blood. Lung parenchyma was dissected away from other tissues and homogenized in 5 mL of Tripure (Boehringer Mannheim Corp, Indianapolis, Ind) for 30 seconds on ice. RNA was extracted with chloroform and precipitated with isopropanol. RNA purity and

quantity were assessed by means of UV absorbance at 260 and 280 nm. The possibility of genomic DNA contamination was excluded by omitting reverse transcriptase in the reaction mixture for each RNA sample analyzed. RT-PCR reactions were carried out with the GeneAmp PCR System 9600 Thermal Cycler (Perkin-Elmer Corp, Foster City, Calif), according to the manufacturer's protocol. Total RNA (1 μ g) was reverse transcribed with oligo dT. Titration curves for each gene examined were performed to ensure optimal amplification. The resulting cDNA was amplified in parallel by 35 cycles of PCR with cytokine-specific primer pairs (Clontech, Palo Alto, Calif) for mouse IL-4, IL-5, IFN- γ , VCAM-1, and reduced glyceraldehyde-phosphate dehydrogenase.

Intracellular cytokine staining and flow cytometry

Intracellular cytokine staining was used to examine cytokine expression after *in vivo* activation with allergen.²³ One hundred minutes after challenge, lung parenchyma was isolated and dissociated into a single cell suspension with a Medimachine (Becton Dickinson Immunocytometry Systems, San Jose, Calif), according to the manufacturer's instructions. Cells were incubated at 37°C with DNase I (180,000 dornase U/mL; Calbiochem, San Diego, Calif) for 30 minutes to lyse cell clumps, followed by Brefeldin A incubation (10 μ g/mL, Sigma) for 2 hours. Cold 0.1 mol/L EDTA was added, and after washing with cold PBS, cells were fixed in 4% paraformaldehyde (Sigma) and then frozen at -80°C until use.

Cryopreserved fixed lung cells were stained for cell-surface markers and intracellular cytokines, as previously described.²³ Briefly, after permeabilization and blockade of nonspecific binding, cells were incubated at 4°C with either rat IgG1 (R3-34) as a negative isotype control or rat anti-mouse IL-4 (11B11, Pharmingen). Cells were simultaneously stained with lineage-specific mAbs (Pharmingen), including anti-IgE (R35-72), anti-CD117/Kit (2B8), anti-I-A^d/I-E^d (2G9), and anti-CD3e (145-2C11). Anti-CD16/32 (2.4G2) and anti-CD23 (B3B4) mAbs (Pharmingen) were added 10 minutes before addition of primary antibodies to ensure specific IgE receptor binding. After 30 minutes, samples were washed and analyzed with a 2-laser FACSCalibur (Becton Dickinson) and CELL-QUEST software.

Sorting of live lung cells

Groups of 10 mice were sensitized with either OVA or IgE anti-DNP and were killed 100 minutes after antigen challenge. Lungs were dispersed as above, treated with DNase, washed, and stained with appropriate concentrations of anti-IgE FITC and anti-CD3 phycoerythrin. Sorting experiments were performed on a modified FACS Star Plus. IgE⁺ and IgE⁻ gated events within the predetermined leukocyte population were sorted separately and collected. Samples were pooled from 10 mice, yielding 1 to 2 $\times 10^4$ IgE⁺ sorted events. Sorted cells were cytocentrifuged and stained with Wright-Giemsa.

Data analysis

Data are expressed as means $\pm$ SEM. Analysis of results used the paired sample Student *t* test. Differences were considered statistically significant at a *P* value of less than .05. For relative quantitation of mRNA, gels were digitally imaged and analyzed by means of densitometry (Kodak Digital Science, Eastman Kodak Co, Rochester, NY), and the ratio of cytokine/reduced glyceraldehyde-phosphate dehydrogenase intensity was calculated.

RESULTS

General characteristics of mouse model

Preliminary studies demonstrated that 1 mg of OVA was an optimal dose to induce systemic sensitization and

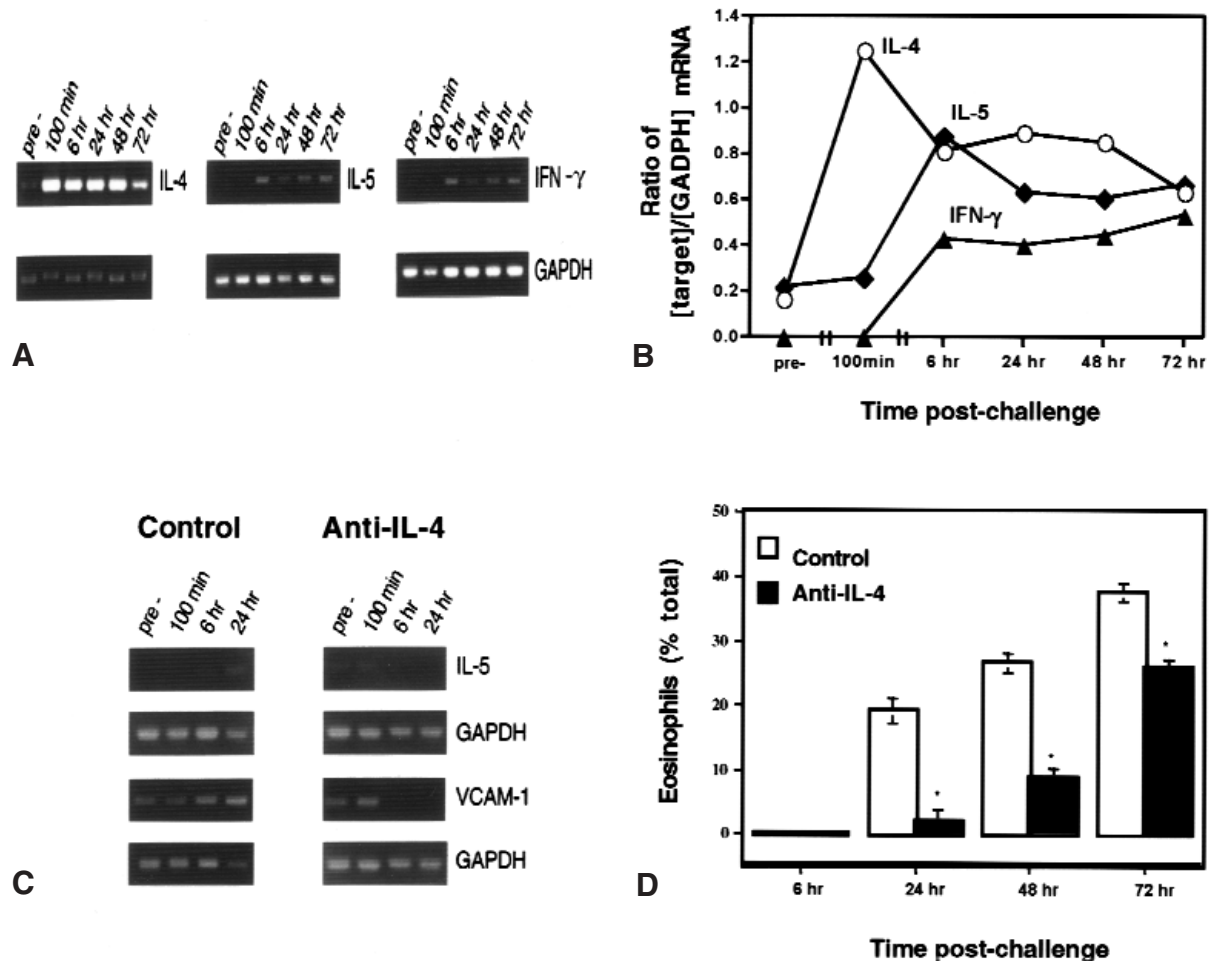


FIG 1. Kinetics of cytokine mRNA expression in mouse lung and effect of anti-IL-4 mAb on IL-5 and VCAM-1 mRNA expression and eosinophil influx. **A**, Semiquantitative RT-PCR for IL-4, IL-5, and IFN- γ on lung RNA of OVA-sensitized mice at time points after SINC. **B**, Graphic representation of above data with densitometric analysis of cytokine PCR normalized for reduced glyceraldehyde-phosphate dehydrogenase (GADPH) expression. **C**, OVA-sensitized mice were administered anti-IL-4 mAb or PBS (control) intravenously immediately before SINC. Semiquantitative RT-PCR for IL-5 and VCAM-1 was performed on lung RNA at time points after SINC. **D**, Percentage of total eosinophil count in BAL (mean $\pm$ SEM) was determined from anti-IL-4-treated and control mice at time points after SINC. * $P < .05$ compared with control group.

immediate inflammatory responses after a single active immunization protocol (data not shown). Peritoneal immunization with OVA and alum thus resulted in the production of OVA-specific IgE (mean $\pm$ SEM; 274.3 ± 87.4 vs 42.7 ± 13.7 U/mL in control animals, $P = .0307$) and an increase in total IgE levels (91.7 ± 16.9 vs 13.4 ± 5.9 ng/mL in control animals, $P = .0024$) in serum by day 9. After an OVA SINC at day 10, histologic analyses showed the presence of an inflammatory infiltrate composed primarily of eosinophils and mononuclear cells that was appreciable by 24 hours and peaked at 72 hours after challenge (data not shown).

Early IL-4 in inflammation

IL-4 mRNA (Fig 1, A) increased 6-fold by 100 minutes after challenge (Fig 1, B). IL-4 mRNA expression was repeatedly found to exhibit biphasic kinetics, with an initial peak at 100 minutes, followed by a decrease at 6

hours and a subsequent increase. IL-5 and IFN- γ mRNAs were observed to be upregulated by 6 hours after challenge, and these levels persisted through 72 hours. Thus, IL-4 upregulation occurred earlier than that of IL-5 and IFN- γ and, at this early time point, preceded evidence of a significant inflammatory infiltrate in the lung.

OVA-sensitized mice were treated with neutralizing doses of anti-IL-4 mAb (11B11) intravenously or with an equivalent volume of PBS (control group) immediately before antigen challenge to establish the importance of this early IL-4 in promotion of subsequent inflammatory events after SINC. As expected, IL-4 blockade resulted in downregulation of mRNAs for proinflammatory molecules, such as IL-5 and VCAM-1, by 6 hours (Fig 1, C). Additionally, there was a significant attenuation ($P < .05$) of BAL eosinophils at 24, 48, and 72 hours after SINC compared with that seen in untreated control animals (Fig 1, D). Thus, IL-4 produc-

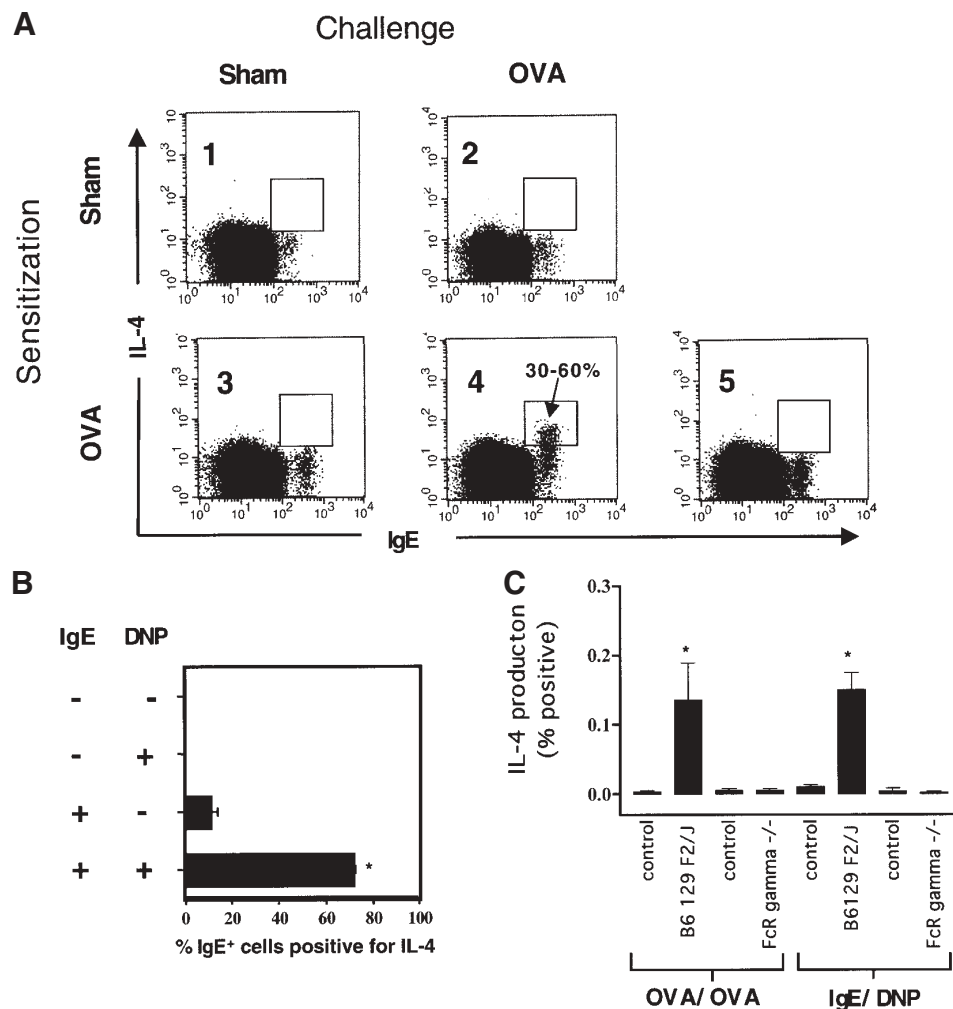


FIG 2. IgE/FcR γ -chain dependent IL-4 production by IgE⁺ cells. **A**, Lungs from BALB/c mice sensitized and challenged to OVA or sham treated at 100 minutes were stained with mAb to IgE and to either IL-4 (panels 1, 2, 3, and 4) or IgG isotype control (panel 5). Data are representative of 6 independent experiments. **B**, Lungs from BALB/c mice, passively sensitized with PBS (-) or IgE anti-DNP (+) and challenged with either PBS (-) or DNP (+) at 100 minutes, were stained with mAb to IgE and IL-4. Data represent mean $\pm$ SEM percentage of IgE⁺ cells expressing IL-4 (n = 6). * P < .001 compared with all control groups. **C**, FcR γ -chain (gamma) $-/-$ mice and their littermate control animals (B6129 F2/J) were sensitized and challenged with OVA or passively sensitized with IgE and challenged with DNP as described. Lung cells were then stained with mAb to IL-4 or IgG isotype (control). Data are presented as the percentage of lung cells (mean $\pm$ SEM, n = 6) producing IL-4 at 100 minutes. * P < .05 between knockout and control groups.

tion early in inflammation appears critical for early events after antigen challenge.¹⁻⁴

CD4⁺ lymphocytes have been shown to be a major source of IL-4.^{8,24} Therefore we investigated the effects of in vivo CD4 and CD8 depletion on the production of IL-4 mRNA through 72 hours. Administration of anti-CD4 mAb had minimal effect on IL-4 expression at 100 minutes but led to a loss of detectable IL-4 mRNA by 24 hours, which persisted through 72 hours. Administration of anti-CD8 mAb had no effect on IL-4 mRNA production (data not shown). Thus, the early peak of IL-4 mRNA production appeared to be largely independent of both CD4⁺ and CD8⁺ lymphocytes, whereas production at 24 hours and thereafter appeared to be dependent on the CD4⁺ lymphocyte population.

Cells producing IL-4 protein after SINC are IgE⁺ nonmast cells

Mast cells and basophils are known to express Fc ϵ RI, and both are capable of producing IL-4.^{10,19} To identify Fc ϵ RI-expressing cells,²⁵ we stained with anti-IgE and examined such IgE⁺ cells for IL-4 production. By using intracellular cytokine staining of dispersed whole lung at 100 minutes, IL-4 protein was consistently detected exclusively in the IgE⁺ population from OVA-sensitized and OVA-challenged (OVA/OVA) mice but not in sham-sensitized control mice (Fig 2, A). Moreover, 30% to 60% (mean, 40%) of IgE⁺ cells expressed IL-4 after SINC. Thus, the cells producing IL-4 by 100 minutes stain brightly for IgE.

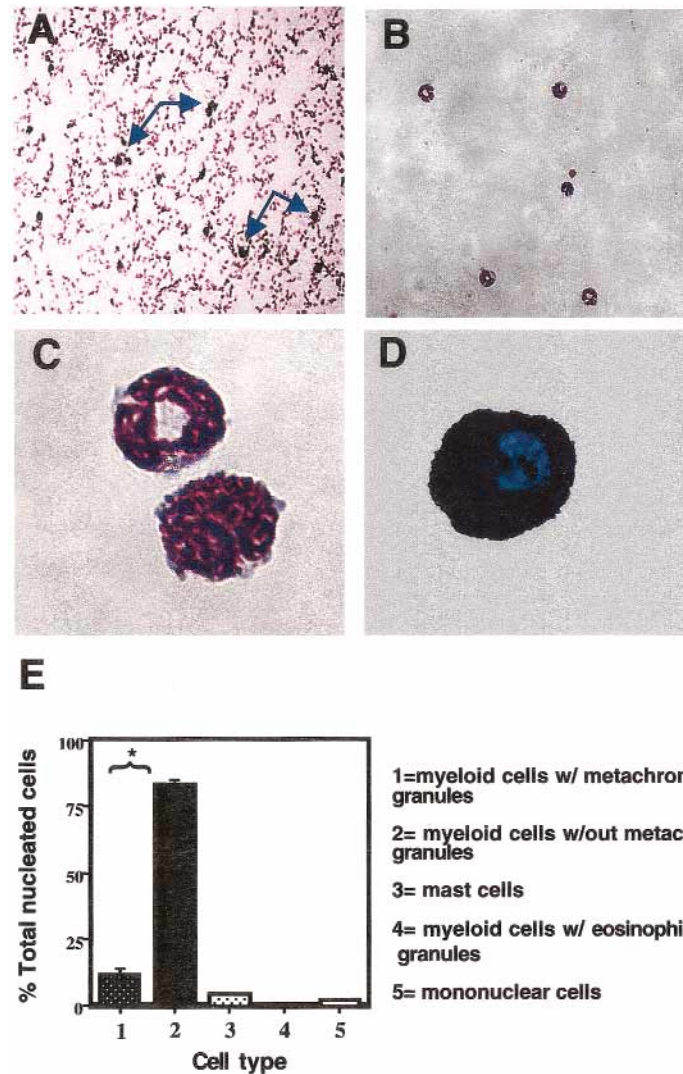


FIG 3. Distribution and morphologic appearance of IgE⁺ cells from whole lung. **A**, ISH for IL-4⁺ cells (blue arrows) from an OVA-immunized mouse lung 100 minutes after SINC (original magnification 400×). **B**, Lungs from OVA-sensitized and OVA-challenged mice were FACS sorted and stained with Wright-Giemsa. Photo shows IgE⁺ myeloid cells (original magnification 400×). Note the ring-like nuclei typical of myeloid cells in the mouse. **C**, Photo compares a post-sort nongranulated myeloid cell (upper cell) with a myeloid cell containing metachromatic granules (lower cell), the latter of which has characteristics consistent with a mature basophil (original magnification 1000×, oil). **D**, Photo of a post-sort lung mast cell with characteristic oval nucleus (original magnification 1000×, oil). Note the smaller diameter and the different granular pattern of the granulated myeloid cell (C) compared with the lung mast cell (D). **E**, Summary data for the total population of IgE⁺ nucleated cells. *P < .001 for presence of myeloid cells compared with other cell types.

We next analyzed early IL-4 mRNA expression in lung tissues from sensitized mast cell-deficient mice (W/W^v and SI/SI^d). Both mast cell-deficient mouse strains produced IL-4 mRNA at 100 minutes after antigen challenge, with similar IL-4 mRNA expression as that found in congenic littermates (data not shown), and this is consistent with the conclusion that an IgE⁺ cell other than a mast cell appears responsible for the majority of early IL-4 production.

Demonstration of IgE/FcR-dependent IL-4 production by IgE⁺ cells

After detecting antigen-specific IL-4 production in IgE⁺ cells, we next evaluated whether this process was

IgE dependent. BALB/c mice were passively sensitized with IgE anti-DNP and challenged with DNP antigen.²¹ By using this passive sensitization method, greater than 70% of the IgE⁺ cells at 100 minutes produced IL-4 in response to specific DNP challenge versus 5% to 10% in PBS challenge alone (P < .001; Fig 2, B). Thus IgE-bearing cells are able to produce IL-4 protein by 100 minutes in an IgE-dependent manner.

We next determined whether IL-4⁺ lung cells could be identified at 100 minutes in FcR γ-chain knockout mice, which lack functional FcεRI, FcγRI, and FcγRIII.²⁶ As can be seen in Fig 2, C, antigen-specific IL-4⁺ lung cells are observed at 100 minutes in B6129 F2/J littermate

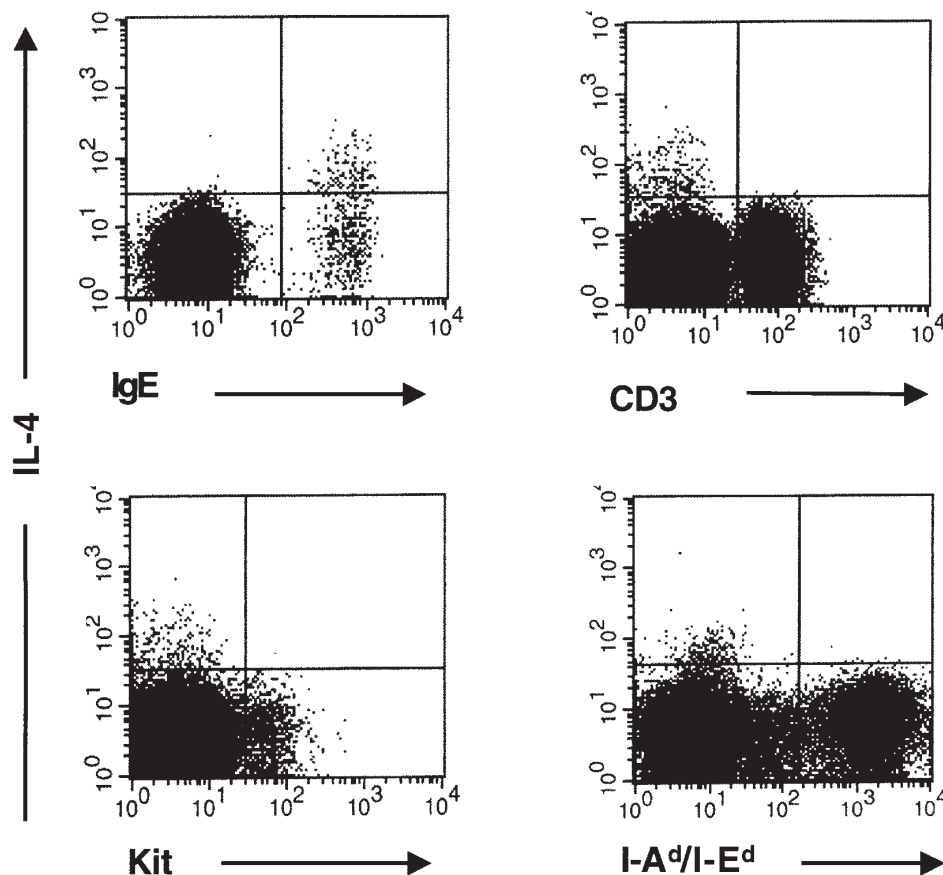


FIG 4. Flow cytometry of IL-4-producing, lung-derived cells. BALB/c mice were sensitized and challenged with OVA, and lung cell suspensions were stained with antibody to IL-4 and to a variety of lineage-specific markers: anti-IgE (FcεRI⁺ cells), anti-CD3 (naïve, γδ⁺, and natural killer T cells), anti-Kit (mast cells), or anti-I-A^d/I-E^d (MHC class II-bearing cells, such as B cells, macrophages, and dendritic cells). Data from one representative lung sample are shown.

control animals but not in FcR γ-chain knockout mice in either active (OVA/OVA) or passive (IgE/DNP) sensitization models. Results in the B6129 F2/J mice were similar to those seen in BALB/c mice (data not shown). This observation is consistent with the conclusion that the source of early IL-4 production is a cell that requires expression of the γ-chain for initiation of IL-4 production and that the absence of a signaling γ-chain negates both active and passive IgE-mediated IL-4 production.

ISH of IL-4-producing cells in the lung

In an attempt to characterize the distribution of cells that produce IL-4 mRNA at 100 minutes, lungs were examined by means of ISH with a radiolabeled IL-4 probe. Cells hybridizing for IL-4 (Fig 3, A) were randomly distributed throughout the lung field and were not consistently associated with serosal surfaces, respiratory epithelium, nerves, or lymphoid tissue, as is the distribution of mouse lung mast cells.

Characterization of IgE⁺ cells by means of FACS

Because IL-4 production was determined to be from an

IgE⁺ lung cell population, we sought to further characterize this population morphologically. To preserve cell morphology, we performed flow cytometry at 100 minutes on live OVA-sensitized and OVA-challenged whole lung cells and sorted on the IgE⁺ population. Fig 3, B, is a representative view (400×) of results from 4 independent IgE⁺ sorting experiments, which yielded a dominant population of myeloid cells exhibiting a ring-shaped nucleus. The mean differential of nucleated cells from these IgE⁺ sorts included 83.7% myeloid cells with few to no granules (Fig 3, C), 11.4% myeloid cells with granules that were metachromatic (Fig 3, C), 2.9% mast cells (Fig 3, D), 0.7% eosinophilic myeloid cells, and 1.5% monocytes (Fig 3, E). The IgE⁻ sorted cells were predominantly macrophages (data not shown). This sorting experiment was also performed on mice passively sensitized with IgE, with similar results. Thus, the IgE⁺ cells were predominantly myeloid cells, with 1 in 10 shown to have distinct metachromatic granules by means of light microscopy.

IL-4-producing cells were further characterized by means of analysis of lineage-specific marker expression from whole lungs by using flow cytometry. This approach revealed that antigen-specific IL-4 production

was exclusive to IgE⁺ cells that did not express CD3, Kit, or MHC class II (Fig 4). These results are consistent with the conclusion that the early IL-4-producing cell is not a mast cell, T cell, natural killer T cell, B cell, monocyte-macrophage, or dendritic cell. A mouse IgE⁺ myeloid cell that is negative for Kit, as the above results confirm, meets the definition of a basophil.^{25,27}

DISCUSSION

By using an SINC model of allergic pulmonary inflammation designed to elicit immediate antigen-mediated responses, we found IL-4 mRNA, blockade of which resulted in attenuation of eosinophilic BAL infiltration (Fig 1, *D*), to be expressed rapidly after specific antigen challenge (Fig 1, *A* and *B*), and we found that its expression was elicited in lung cells through an IgE-mediated mechanism (Fig 2, *B*). Cells producing IL-4 were randomly distributed throughout the lung parenchyma (Fig 3, *A*). By using flow cytometry, the lineage of the IL-4-producing cell in question was an IgE⁺ non-B, non-T cell (Figs 2 and 4).¹² Studies in mast cell-deficient mice and the observation that IL-4-producing cells were Kit[−] (Fig 4) eliminated the mast cell as the principal source of this IL-4. Use of lineage-specific antibodies further eliminated T lymphocytes, natural killer T cells, dendritic cells, monocytes-macrophages, and B cells from consideration (Fig 4). The morphology of these IgE⁺ cells after sorting revealed that 95% were myeloid cells, with 10% to 15% containing metachromatic granules (Fig 3). Because 30% to 80% of these IgE⁺ cells produced IL-4 after antigen-specific challenge, it can be deduced that the IL-4-producing cell is from this IgE⁺, Kit[−], I-A/I-E[−] myeloid cell majority, a population of cells most consistent with basophils.

Allergen-induced eosinophilic infiltration of the airways is one of the pathologic hallmarks of asthma. Although IL-5 has a known role in this process, IL-4 is both sufficient and necessary to induce lung recruitment of eosinophils.^{3,28} This effect of IL-4 is believed to be mediated through expression of VCAM-1,⁶ an endothelial adherence molecule for both eosinophils and lymphocytes. In our study blockade with anti-IL-4 mAb before challenge resulted in downregulation of both VCAM-1 mRNA and IL-5 mRNA levels in the lung (Fig 1, *C*) and attenuated eosinophil recruitment (Fig 1, *D*). With time, however, eosinophil infiltration does increase to levels resembling those seen in untreated animals. This occurs in association with lymphocyte infiltration, in which the lymphocytes produce IL-4. There are also data to suggest a role for IL-4-independent eosinophil recruitment by cytokines such as TNF- α and IL-13, which have not been examined in this study.²⁹

The temporal importance of IL-4 in this model of allergic pulmonary inflammation is emphasized by the production of IL-4 within 100 minutes after IgE-dependent mechanisms in a population of IgE⁺ lung cells (Fig 2, *B*). This result confirmed findings seen with active OVA sensitization (Fig 2, *A*) and strongly suggested an *in vivo*

dependency of IgE-mediated mechanisms for production of IL-4. Although the importance of IgE in allergic pulmonary inflammation has been questioned by findings in IgE^{−/−} mice,³⁰ it is known that IgE-bearing cells, namely mast cells and basophils, are able to elaborate IL-4 in response to specific antigen *in vitro*,^{11,12} *in vivo*, and in an IgE-dependent fashion.¹⁰ Moreover, basophils isolated from BAL in human subjects after segmental allergen challenge have been shown to produce IL-4,³¹ and peripheral blood basophils rapidly express IL-4, which peaks 2 hours after antigen activation.³² These observations, coupled with the rapidity of the specific response seen and the finding that IL-4 production is γ -chain dependent (Fig 2, *C*), add to the importance of IgE-mediated IL-4 production in influencing early inflammatory events in the models used in this study. This IL-4 production by IgE⁺ cells might also be a mechanism to sustain and amplify local IgE responses in the lung.³³ A model with hyper-IgE responder mice³⁴ was also shown to generate immediate inflammatory parameters after a single sensitization and challenge, and an early infiltration of IgE-bearing myeloid cells into the lung was detected. The authors noted that blockade of IgE-mediated processes by a nonanaphylactic anti-IgE mAb resulted in decreased recruitment of these IgE⁺ cells, as well as a decreased production of IL-4 mRNA in the BAL.

Basophils are clearly present early during the genesis of allergic asthma and have the capability to elaborate IL-4 in specific response to antigen. Basophils expressing Fc ϵ RI have been shown, by means of histochemistry, to be recruited to the lungs as early as 3 hours after antigen challenge in human subjects,^{35,36} and a nonneutrophil IgE-bearing myeloid cell was recruited to the lungs of challenged mice.³⁴ Moreover, IgE-staining non-B, non-T cells have been shown to be rapid and prolific producers of IL-4 in a number of model systems.^{13,37,38}

Mouse basophils were reported by means of light microscopy in the early 1980s³⁹ and were subsequently characterized by means of electron microscopy.⁴⁰ Mouse basophils from the peritoneal cavity have been identified as CD3[−], IgE⁺, Kit[−] cells that stain positive for alcian blue,²⁵ identical to the cells currently identified. As mentioned, both mouse mast cells and basophils are IgE⁺.^{10,19,41} The finding that these cells are not mast cells supports studies in mast cell-deficient mice in which it has been shown that mast cells are not important in certain models of allergic inflammation.^{42,43} Additionally, neutrophils, a somewhat similar-appearing myeloid cell, isolated from mice transgenic for human Fc ϵ RI do not express the high-affinity IgE receptor,⁴⁴ suggesting that they lack the cellular machinery to do so. Also, specific staining for eosinophils with Wright-Giemsa or Kwik-diff stains was negative in the IgE⁺ population despite positive staining in the IgE[−] population. Taken together, the myeloid cells producing IL-4 early during inflammation in this mouse model of asthma, and which are IgE⁺, Kit[−], CD3[−], and I-A^d/I-E^d−, fulfill the criteria for mouse basophils. The lack of visible metachromatic granules within most of these myeloid cells might be either a con-

sequence of degranulation after the challenge process or because of cellular immaturity.

Recognition of the importance of cytokines in allergic inflammation has increased our understanding of the pathogenesis of asthma and has led to novel approaches to modulating cytokine activity. On the basis of the results presented in this article, it also appears important to implicate and target specific cytokine-producing cells, in this case IgE⁺, Kit⁺, CD3⁺, I-A^d/I-E^d- myeloid cells. Clearly, these cells appear to be an important source of propagation of IgE-dependent signals. Therapies targeted to such cells and to inhibition of their IgE-mediated activation should be considered in the treatment of inflammation of the lung.

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