IL-4 production by human basophils found in the lung following segmental allergen challenge

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Background: Human blood basophils secrete high levels of IL-4 following activation with specific allergen, yet their role as cytokine-producing cells in allergic lesions has not been described.
Objective: Our objective was to investigate whether and under what conditions basophils infiltrating allergic lesions in the lung secrete IL-4 in vitro.
Methods: Bronchoalveolar lavage (BAL) cells were recovered 20 hours after segmental allergen challenge. Basophils were enriched with Percoll using a protocol commonly used for blood basophils. IL-4 and histamine were measured in culture supernatants following activation with a variety of stimuli. Two-color flow cytometry was performed to detect intracellular IL-4.
Results: IL-4 protein was detected in all basophil culture supernatants following a 4- to 5-hour incubation in medium alone; the levels obtained did not significantly increase with the addition of anti-IgE. BAL basophils failed to release histamine in response to specific allergen but showed nearly 60% histamine release with N-formyl-methionyl-leucyl-phenylalanine, suggesting that they were desensitized to IgE-mediated stimuli as a result of their activation in vivo. Using these same conditions, IL-4 was not detected in BAL cell fractions enriched for lymphocytes and eosinophils. Ionomycin induced IL-4 secretion by BAL basophils, and this response was reduced with the addition of phorbol myristate acetate. In contrast, phorbol myristate acetate promoted the secretion of IL-4 by BAL cells enriched for lymphocytes; both findings are identical to those reported for basophils and lymphocytes purified from blood. Flow cytometry confirmed the secretion of IL-4 by BAL basophils.
Conclusions: These data suggest that basophils migrating to the lung following allergen challenge represent a major source of IL-4. (J Allergy Clin Immunol 2001;107:265-71.)

Key words: *Basophil, cytokine, histamine, IgE, late phase, lung*

Abbreviations used
BAL: Bronchoalveolar lavage
FcεRI: High affinity IgE receptor
fMLP: N-formyl-methyonyl-leucyl-phenylalanine
LPR: Late phase reaction
PAG: Pipes/albumin/glucose
PE: Phycoerythrin
PIPES: Piperazine-N,N'-bis-2-ethanesulfonic acid
PMA: Phorbol myristate acetate
SAC: Segmental allergen challenge

The presence of inflammatory cells secreting T_H2 cytokines, in particular IL-4, has become accepted as a defining feature in sites of allergic inflammation. The increased generation of IL-4 and other T_H2 cytokines, such as IL-13 and IL-5, is thought to play an important role in the pathogenesis of asthma and other allergic diseases. Both IL-4 and IL-13 can provide the initial signal for promoting IgE secretion by B cells,[1] and each cytokine can up-regulate the endothelium adhesion molecule, vascular cell adhesion molecule 1, which promotes eosinophil migration.[2,3] Whereas the development of T_H2 cells is linked to IL-4 exposure, eosinophil development and effector function is dependent on the biologic activity of IL-5.[4] The primary cellular sources of these cytokines in asthmatic airways and other examples of allergic diseases remain controversial, as does the regulation of cytokine production. Most studies have used immunohistochemical and in situ hybridization techniques to assess cytokine generation in tissue taken from allergic lesions. Such studies indicate that $CD4^+$ T cells are the predominant cells expressing mRNA and protein for T_H2 cytokines, particularly IL-4 and IL-5.[5] The same techniques have been used more recently to demonstrate that eosinophils and mast cells, both of which constitute a part of the cellular infiltrate resulting from allergic reactions, also contribute in the production of these cytokines,[6,7] although the mast cell studies are controversial.

Although it has long been thought that IL-4 is derived solely from T lymphocytes, recent in vitro studies have clearly shown that human basophils account for most of the IL-4 (and IL-13) generated in cultures using peripheral blood leukocytes (reviewed in reference 8). This is true not only for cultures activated with stimuli that are specific for basophils, such as anti-IgE antibody, but it is also evident in cultures stimulated with allergen, which additionally activates allergen-specific T lymphocytes.[9]

The late-phase reaction (LPR) to allergen challenge is thought to be a clinically relevant model; it has provided a significant amount of information regarding the cells infiltrating allergic lesions. There is, in fact, a substantial

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Supported in part by grants HL62204 (LML), AI42221 (JTS), and AI07290 (LML) from the National Institutes of Health.
Received for publication September 8, 2000; revised November 1, 2000; accepted for publication November 8, 2000.
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0091-6749/2001 $35.00 + 0 **1/83/112846**
doi:10.1067/mai.2001.112846

amount of homology with the inflammation associated with allergic disease. In studies performed in the lung,[10] nose,[11] and skin,[12] we have shown that basophils selectively infiltrate late reactions along with eosinophils and lymphocytes. In the lung, evidence for basophil involvement was supported by the detection of a population of bronchoalveolar lavage (BAL) cells showing a variety of morphologic, phenotypic, and functional similarities to blood basophils.[10] Using monoclonal antibodies reportedly specific for basophils, others have more recently confirmed these reports by showing immunohistochemical evidence that basophils were found in tissue biopsy specimens taken from late reactions. In the skin, basophils were found at frequencies much higher than previously thought (ie, approaching ~50% of the number of eosinophils co-infiltrating these lesions).[13,14]

Our aim in this study was to address whether and under what conditions basophils recovered from the lung following allergen challenge secrete IL-4 in vitro. By using a density centrifugation protocol routinely used in our laboratory for the preparation of blood basophils, we were able to rapidly prepare BAL suspensions enriched for basophils, which were then suitable for experiments that simultaneously investigated both cytokine and histamine release. Both mononuclear cell and eosinophil suspensions were also generated and were similarly tested. To functionally delineate the secretion of IL-4 by basophils and lymphocytes, we investigated the effect of stimulation with phorbol myristate acetate (PMA), which differentiates the generation of this cytokine by the two cell types.[15] Finally, we have used flow cytometry methods that permit measurement of intracellular IL-4 in basophils defined by their high expression of IgE.

METHODS

Special reagents for in vitro studies

All reagents were purchased unless otherwise noted. Piperazine-N,N'-bis-2-ethanesulfonic acid (PIPES), ionomycin, N-formyl-methyonyl-leucyl-phenylalanine (f-MLP), PMA, FBS, saponin, BSA, and human IgG were purchased from Sigma Chemical Co, St Louis, Mo. PBS, Iscove's modified Dulbecco's medium with L-glutamine and containing 25 mmol/L HEPES, gentamicin, and nonessential amino acids (100×) were purchased from Life Technologies, Inc, Grand Island, NY. Percoll was purchased from Pharmacia, Piscataway, NJ. Phycoerythrin (PE)-conjugated rat anti-human IL-4 and its isotype control (IgG_1/PE) was purchased from BD PharMingen, San Diego, Calif. FITC-conjugated goat anti-human IgE was purchased from Kirkegaard Perry, Gaithersburg, Md. Carnation nonfat dry milk was also purchased, and monoclonal anti-FcεRI-α chain (22E7) was generously provided by J. Kochan, Roche Pharmaceuticals. All PIPES-containing buffers were made from stock 10X PIPES (250 mmol/L PIPES, 1.10 mol/L NaCl, and 50 mmol/L KCL, pH 7.3 and stored at 4°C). PIPES/albumin/glucose (PAG) contained one-tenth 10X PIPES, 0.003% human serum albumin, and 0.1% D-glucose.

Subjects

The study was reviewed and approved by the Institutional Review Board of The Johns Hopkins Bayview Medical Center and The Johns Hopkins Medical Institutions; all subjects participating had given informed consent before undergoing bronchoscopy. All subjects were classified as mildly asthmatic as determined by clinical history, pulmonary function testing, and bronchial provocation with methacholine using previously established criteria.[16] All subjects also had a positive reaction to either ragweed, timothy grass, or dust mite and were known to experience LPRs to these allergens.

Segmental allergen challenge and bronchoalveolar lavage

Bronchoscopy was performed as described[16,17] according to National Institutes of Health recommended guidelines. Subjects were premedicated with 0.6 mg intravenous atropine. After inhalation of nebulized 4% lidocaine, a fiberoptic bronchoscope was inserted into the lower airways, supplementing local anesthesia with 2% lidocaine. Nine persons underwent segmental allergen challenge (SAC) with either short ragweed antigen, timothy grass, or dust mite (Greer Laboratories, Lenoir, NC) by instilling a total dose of 100 or 500 protein nitrogen units in 5 mL of normal saline solution, depending on skin test sensitivity.[16] None of the subjects had asthmatic symptoms at the time SAC was performed. Bronchoalveolar lavage (BAL) was performed 18 to 24 hours after allergen challenge using five 20-mL aliquots of prewarmed (37°C) isotonic saline solution, with immediate aspiration after instillation of each aliquot. Recovered fluids were then pooled and centrifuged at 150*g* for 10 minutes before resuspending the cell pellet in PAG buffer to assess both the total cell count and the number of cells staining with Alcian blue.[18] Differential cell counts were also made by counting 500 cells on cytospin preparations (Shandon II, Pittsburg, Pa) stained using Diff-Quik (Harleco, Kansas City, Mo) to assess the percentages of the major leukocytes.

Preparation of BAL cells for in vitro culture

BAL cells were separated on double Percoll gradients (1.066/1.079 g/mL) using a protocol developed in our laboratory for isolating blood basophils from hemapheresis packs.[19] Briefly, up to 175×10^6 cells were resuspended in 1.5 mL of 1.079 Percoll solution in special polystyrene tubes (catalog No. 55-463, Sarstedt). Overlayed onto this suspension was 4 mL of 1.066 Percoll followed with 0.5 to 1.0 mL PAG buffer. The cells were then centrifuged (400*g*, 20 minutes) at room temperature (~23°C). Three cell fractions were collected and kept separated after density centrifugation. Cells floating on the 1.066 Percoll (mostly lymphocytes and monocytes) were carefully aspirated and immediately added to 10 mL of PAG buffer. The same was done for cells floating on the 1.079 Percoll, which consisted of Alcian blue positive cells (ie, basophils) and mononuclear cells. Eosinophils constituted up to 80% of the cells recovered in the BAL following SAC, which pelleted after density centrifugation along with a small percentage of neutrophils. All 3 cell suspensions were washed twice in PAG before counting with Alcian blue to determine the percentage of basophils in each.

An equal number of total cells from each suspension were cultured in C-Iscove's modified Dulbecco's medium alone and with various stimuli, as previously described.[20] Briefly, cultures were performed in microtiter wells (96-well plates, Costar, Corning, NY) by adding cells in a total volume of 0.125 mL. After equilibrating to 37°C, 5% CO_2 for 15 minutes, an equal volume of prewarmed stimulus (at twice the final concentration) was added. Total cell densities did not exceed 10×10^6/mL. Cell-free culture supernatants were harvested after 4 hours of incubation, and IL-4 measurements were made using an ultra-sensitive (<1 pg/mL) ELISA kit according to the manufacturer's protocol (Biosource, Camarillo, Calif). Automated fluorimetry was used in measuring histamine released in the basophil-enriched culture supernatants, as previously described.[20] This was done by taking 0.050 mL portions of the 4-hour cell-free supernatants and adding each to a 1 mL solution of perchloric acid

(1.6% in PAG). Total histamine content was determined by taking 0.025 mL of preculture cell suspension (an amount proportionate to the number found in 0.050 mL of culture) and lysing directly in 1 mL of the acid solution. Samples were assayed by the machine after an overnight precipitation at 4°C to remove protein. The amount of histamine released in the culture supernatants was reported as a percent of the total histamine content.

Flow cytometry

Two-color flow cytometry for intracellular staining of IL-4 in cells expressing IgE was performed, as previously described.[21] Briefly, BAL cells were enriched for basophils using density centrifugation. Cultures were also performed as above but, upon activation, received monensin (2 μmol/L) to prevent the secretion of IL-4. This helped to facilitate detection of intracellular cytokine during flow cytometry (see below). After being incubated for 4 hours, the cells were harvested, washed once with PBS, and then fixed with 4% buffered paraformaldehyde for 5 minutes at room temperature. After fixing, the cells were washed once with PBS, 0.1% BSA, resuspended in PBS, 10% dimethyl sulfoxide and frozen at -80°C.

In preparation for immunostaining, cells were thawed, washed once with cold PBS, and then incubated for 1 hour on ice in PBS containing 0.1% saponin (PBS-S), along with 5% nonfat dry milk and 1 mg/mL human IgG (up to 1×10^6 total cells in 0.05 mL). Without washing, the cell suspensions were aliquoted into 1.5 mL polypropylene microfuge tubes. Equal volumes of PBS-S, 5% nonfat dry milk containing twice the final concentrations of anti-IgE/FITC, and either anti-IL-4/PE or PE-conjugated isotype control (rat IgG1/PE) were then added. Anti-IgE/FITC was used at a ⅛ final dilution of stock, because this concentration had been previously shown to stain BAL basophils expressing IgE.[22] Anti-IL-4/PE and its isotype control were used at a final concentration of 10 μg/mL, as previously described for intracellular staining.[21] Cells were incubated with antibody for 30 minutes on ice before washing 3× with PBS-S (0.5 mL per wash). The detection and analysis of 2-color immunostaining was performed using an EPICS flow cytometer.

RESULTS

The total cell recovery in 8 BAL samples ranged from $43\text{-}182 \times 10^6$. Differential cell counts for the major leukocytes showed percentages averaging 25.2 ± 10.5% for macrophages, 10.7 ± 1.3% for lymphocytes, 16.2 ± 5.7% for neutrophils, and 47.8 ± 10.4% for eosinophils. Consistent with our previous findings,[10] we show in Fig 1 of this study that the percentage of basophils in unseparated BAL suspensions, as assessed by Alcian blue staining, ranged from 1% to 5% and averaged 2%. Density centrifugation enriched this percentage of BAL basophils to an average of 12% and was done to facilitate the detection of IL-4 and histamine released by these cells. It is important to note that lymphocytes accounted for nearly all of the contaminants found in the basophil-enriched suspensions (data not shown). Fig 1 shows that density centrifugation also resulted in mononuclear cell and eosinophil-enriched suspensions, which averaged less than 1% Alcian blue–positive cells. Although differentials were not routinely performed on these cell fractions, 2 preparations showed that they averaged purities of up to 90% for their respective cell types (data not shown).

Panel *A* of Fig 2 shows the IL-4 secreted by the 3 BAL cell fractions after a 4-hour incubation in medium alone and in response to the stimuli listed. The highest levels of

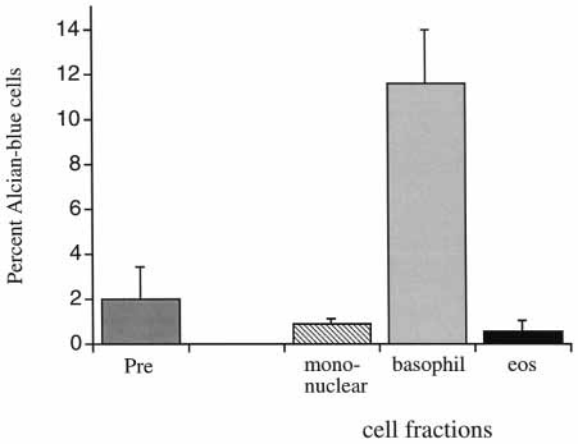

FIG 1. Percentage of Alcian blue–positive cells in bronchoalveolar lavage obtained following segmental allergen challenge before (Pre) and after (cell fractions) density centrifugation on Percoll (d = 1.066/1.079 g/mL). Values are the mean ± SEM, n = 8. *Eos,* Eosinophil.

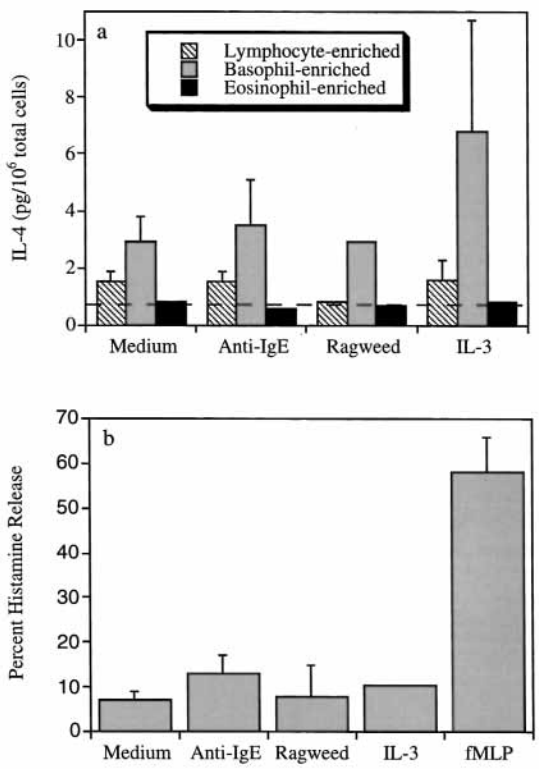

FIG 2. IL-4 secretion (panel *A*) by bronchoalveolar lavage cell fractions following a 4-hour culture in medium (n = 5), anti-IgE (10 ng/mL) (n = 4), ragweed allergen (10 ng/mL) (n = 2), or IL-3 (10 ng/mL) (n = 3). The percentage of histamine released by the same basophil-enriched cultures is shown in panel *B*, which also shows the histamine released in response to 1 μmol/L N-formyl-methyonyl-leucyl-phenylalanine (n = 3). Values are the mean ± SEM, when n ≥ 3.

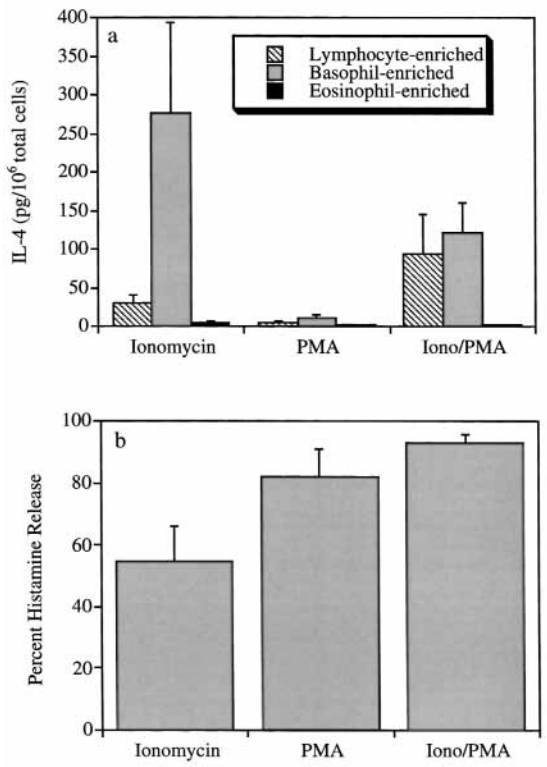

FIG 3. IL-4 secretion (panel *A*) by bronchoalveolar lavage cell fractions following 4-hour culture in medium containing ionomycin (500 ng/mL) (n = 5), phorbol myristate acetate (PMA) (10 ng/mL) (n = 4), or the combination of these stimuli (Iono/PMA) (n = 4). The percentage of histamine released by the same basophil-enriched cultures is shown in panel *B*. Values are the mean ± SEM.

IL-4 protein were detected only in the basophil-enriched suspensions and were measurable in all 5 experiments. Furthermore, a positive correlation ($r = 0.68$, n = 5) was observed between the IL-4 secreted and the percentage of basophils in these cultures (data not shown). However, this was not statistically significant because of low sample number. Only 1 of 5 mononuclear cell preparations resulted in detectable levels of IL-4. Most notably, IL-4 was not detected in any of the eosinophil preparations, indicating protein levels <1 pg/10^6 cells. In the basophil cultures, both anti-IgE antibody and the ragweed antigen that had been used to induce the LPR resulting in the BAL cells obtained produced IL-4 levels averaging approximately 3 pg/10^6 cells, which were not significantly different than those seen with cells cultured in medium alone. Although only 3 experiments were done testing IL-3–a non-IgE-dependent secretogogue for basophils—the highest levels of IL-4 (~7 pg/10^6 cells), in each, were seen using this stimulus. Panel *B* shows the average percentage of histamine released in the basophil cultures after 4 hours of incubation. As for IL-4 protein, little if any histamine was released in cultures receiving anti-IgE or antigen above that seen for cells cultured in medium alone. Higher and lower concentrations of these stimuli did not result in any greater histamine release (data not shown). In contrast, the bacterial-derived peptide fMLP (10^{-6} M) induced high levels of histamine in 3/3 preparations, averaging 58 ± 8% release, suggesting that the basophils were functional but desensitized to the cross-linking stimuli as a result of their activation in vivo. With respect to this finding, it is important to note that the average content of histamine from 6 BAL basophil preparations averaged just 0.8 pg/cell, which was significantly lower than the theoretical 1.0 to 1.4 pg/cell typically found in blood basophils (data not shown).

In previous studies comparing the generation of IL-4 by basophils and lymphocytes isolated from blood, we showed that protein kinase C activation, as induced by PMA, differentially regulated the secretion of this cytokine by the two cell types.[15] In these experiments, the IL-4 produced by basophils activated with ionomycin was inhibited by some 70% with the addition of PMA, whereas the combination of the two stimuli promoted the secretion of this cytokine by lymphocytes. We therefore used PMA co-stimulation in the present study to determine whether the same effect was seen for BAL cells. As shown in panel *A* of Fig 3, ionomycin alone induced high levels of IL-4 from the BAL cell suspensions enriched for basophils, which averaged 277 ± 117 pg/10^6 total cells; this was decreased by an average of some 60% with the addition of PMA. In contrast, the combination of these stimuli was necessary for optimal IL-4 secretion in the mononuclear cell fractions. Ionomycin alone caused only 29 ± 12 pg/10^6 cells to be secreted, whereas the two together caused the secretion of 94 ± 51 pg/10^6 cells. Little to no IL-4 was induced by PMA alone, and this cytokine was not detected in the eosinophil cultures activated with either the phorbol ester, ionomycin, or the combination of these stimuli. Shown in panel *B* of this figure is the histamine released in the basophil cultures activated with these stimuli. As expected, BAL basophils released high percentages (~90%) of histamine in response to ionomycin and PMA, much like basophils isolated from blood.

To further support the evidence that BAL basophils secreted IL-4 detected in the bulk culture assays, we used 2-color flow cytometry to detect intracellular IL-4. Using gating parameters previously established for detecting these cells when labeled with FITC-conjugated anti-IgE antibody, we were able to detect a subpopulation of cells expressing IgE (ie, basophils) that closely correlated with the percentage of Alcian blue–staining cells following the enrichment step using density centrifugation (data not shown). Co-expression for IL-4 was then determined using a PE-conjugated anti-IL-4 antibody. Fig 4 shows the results from a representative experiment, with the average values from additional experiments shown in parentheses. As predicted from the low levels of IL-4 detected in bulk culture supernatants, a low percentage (2 ± 1%, n = 3) of basophils co-expressed IL-4 when cultured in medium (panel *A*), and this increased slightly to some 4% (n = 2) with anti-FcεRI-mediated activation (panel *B*). Note that cross-linking was induced with anti-receptor antibody rather

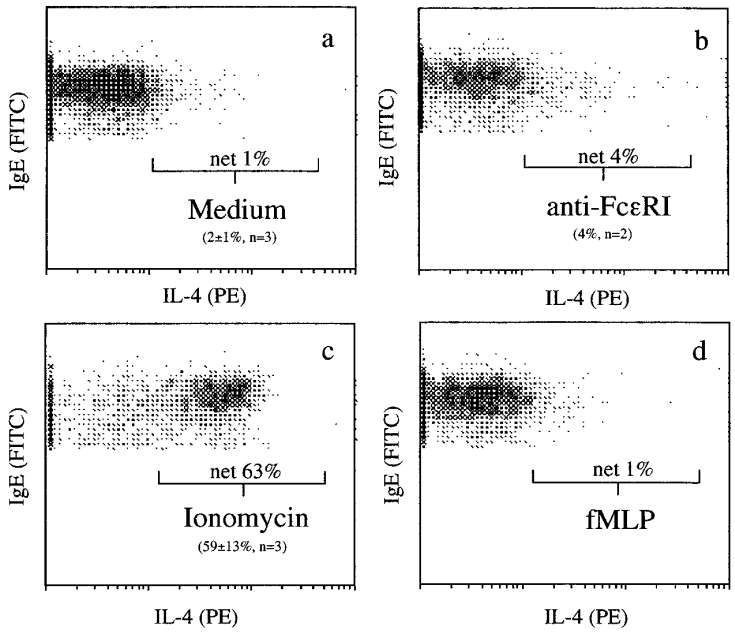

FIG 4. Detection of intracellular IL-4 in bronchoalveolar lavage (BAL) basophils. BAL basophils were enriched on double-Percoll gradients. Cells were cultured in *A,* medium alone, *B,* anti–high affinity IgE receptor (FcεRI) (22E7, 10 ng/mL), *C,* ionomycin (500 ng/mL), or *D,* N-formyl-methyonyl-leucyl-phenylalanine (fMLP) (1 μmol/L) in the presence of 2 μmol/L monensin. After a 4-hour incubation, the cultures were harvested and fixed. Two-color flow cytometry was performed to assess intracellular IL-4 in IgE-bearing cells (ie, basophils). The scatter graphs are from a representative experiment with the net percentages shown being those above the isotype/phycoerythrin (PE) control. The values shown in parentheses are the mean ± SEM from additional experiments.

than with anti-IgE to allow detection of basophils with the anti-IgE/FITC antibody. A high percentage (59 ± 13%, n = 3) of IgE-positive cells expressed IL-4 following activation with ionomycin (panel *C*). In this representative experiment, fMLP, which activated histamine release in the culture supernatants, did not promote IL-4 staining above that seen with medium alone (panel *D*), as has been reported previously.

DISCUSSION

In this article we show evidence for the first time that basophils infiltrating into the airways during the LPR to allergen challenge, when cultured in vitro, produce detectable levels of IL-4. These BAL basophils, like basophils isolated from blood, appear to represent a significant source of this cytokine, secreting greater levels than any other cell type recovered 18 to 24 hours after segmental allergen challenge using the parameters tested. Although this finding does not exclude lymphocytes and eosinophils from producing IL-4, it does challenge the belief that lymphocytes are the predominant source of this cytokine in allergic lesions. In contrast, other investigators have shown by immunohistochemistry in bronchial biopsy specimens that lymphocytes, eosinophils, and mast cells all co-express either mRNA and/or protein for IL-4, as well as other cytokines.[5-7] These differences probably reflect the nature of the specimens and/or detection assays used. Our focus in this study was on cells infiltrating the airway and alveolar lumen, which were recovered for ex vivo studies. Whereas it is thought that cells recovered in BAL represent those found in lung tissue, it remains possible that some lymphocytes, including those that produce IL-4, remain in the tissue and do not migrate into the lumen. Although this seems less likely for specimens recovered 20 hours after segmental allergen challenge (a time that is thought to allow for maximal infiltration of cells), it remains a possibility. Eosinophils, which represented up to 80% of the cells recovered in the BAL, did not secrete IL-4 with any of the stimuli tested. This finding is in sharp contrast to reports showing spontaneous secretion of IL-4 by blood eosinophils[23,24] and immunohistochemistry evidence of IL-4 found in bronchial eosinophils.[6,25] As a result of these differences, the belief that this cell type contributes to the secretion of IL-4 remains somewhat controversial.

The BAL basophils recovered after segmental allergen challenge were desensitized to IgE-mediated activation, reflecting their activation in vivo. The evidence to support this belief was best demonstrated by their lack of response to cross-linking stimuli. In fact, the BAL basophils were completely unresponsive to the allergen used to provoke the LPR and were only marginally responsive to activation with anti-IgE. This so-called specific desensitization to IgE-mediated stimulation has also been demonstrated experimentally in vitro.[26] In contrast,

fMLP, which causes histamine release from blood basophils by using signal transduction pathways unrelated to those associated with FcεRI crosslinking, was quite effective at inducing histamine release from BAL basophils and indicated that the cells were functional.

The IL-4 levels detected in the BAL basophil cultures stimulated with anti-IgE were much lower than expected compared with those typically produced by blood basophils prepared under similar conditions.[20] This probably reflects, in part, the time course of IL-4 secretion in these cells. For instance, IgE-mediated secretion of IL-4 by normal basophils isolated from blood occurs during a 4- to 6-hour time period.[19,27] In this study, BAL was performed 18 to 24 hours after allergen challenge to allow enough time for cells to infiltrate the airway lumen and to maximize the number of cells recovered for ex vivo experiments. As a result, these basophils have likely long been activated and have essentially completed their secretion of IL-4. The highest levels of IL-4 were those detected when BAL basophils were cultured with IL-3. This finding was unexpected because basophils isolated from blood normally do not secrete IL-4 in response to IL-3 stimulation alone. However, others have shown in vitro that basophils, when first activated with cross-linking stimuli and then cultured in the presence of IL-3, will continue to secrete IL-4 beyond the 4- to 6-hour time range normally maximal for secretion.[28] If so, then our finding is consistent with the belief that the BAL basophils recovered were, indeed, activated in vivo.

We used PMA stimulation to help delineate the role of BAL basophils versus lymphocytes in the production of IL-4. As predicted, the phorbol ester, while not directly stimulating IL-4, greatly reduced the secretion of this cytokine in BAL basophil cultures stimulated with ionomycin, consistent with our previous results with basophils purified from blood.[15] It is surprising that this effect of PMA was seen even though the BAL suspensions were only enriched for basophils and contained many mononuclear cells, including lymphocytes. If lymphocytes accounted for the IL-4 produced in these cultures, then the combination of PMA and ionomycin would have resulted in greater amounts of cytokine. Instead, the production of IL-4 in BAL suspensions enriched for lymphocytes, as expected, required the combination of PMA and ionomycin, exactly as we had shown for blood lymphocytes. Finally, these stimuli, when used either alone or in combination, are known to be secretogogues for many cell types. They can also activate cells by bypassing receptor-mediated signaling events that may be otherwise desensitized. In fact, this probably accounted for their ability to induce histamine and IL-4 release from the BAL basophils in this study, even though cross-linking stimuli (eg, anti-IgE and specific antigen) failed to do so. Interestingly, both PMA and/or ionomycin were unable to induce detectable levels of IL-4 in cultures containing nearly pure BAL eosinophils, even though blood eosinophils have been reported to secrete low levels of this cytokine. As noted, this finding suggests that eosinophils infiltrating allergic lesions do not represent an important source of this cytokine.

To confirm that basophils were producing IL-4, we used flow cytometry to detect intracellular cytokine in IgE^+ cells (ie, basophils). Whereas mast cells and possibly other cell types, such as monocytes and eosinophils, may also express low numbers of IgE receptors, it has been our experience that these cell types are not readily detected by flow cytometry when identification is made based on IgE expression. Furthermore, our previous studies clearly show that the IgE-expressing cells recovered in BAL are phenotypically identical to blood basophils.[10] Finally, the percentages of IgE-positive cells detected by flow cytometry also correlated with the percentages of Alcian blue cells counted following density centrifugation—an additional observation that makes it unlikely that eosinophils and monocytes were detected in this assay.

In conclusion, it seems probable that IL-4 produced by the basophils found in these LPR lesions play an important role in amplifying allergic inflammation by promoting T_H2 development, eosinophil migration, and IgE synthesis. Indeed, studies have shown that basophil-derived IL-4 supports in vitro IgE synthesis.[29] Whether antigen-specific lymphocytes become T_H2-like cells when coexposed to the IL-4 produced by basophils remains to be seen. A better understanding regarding the relationship between the secretion of this cytokine by these two cell types is worthy of further investigation.

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