Human Peripheral Blood Basophils Primed by Interleukin 3 (IL-3) Produce IL-4 in Response to Immunoglobulin E Receptor Stimulation

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Summary

In contrast to most cytokines, interleukin 4 (IL-4) expression is restricted to T lymphocytes, with the exception of mast cell lines and mast cells, as more recently demonstrated in rodents. Little is known, however, about the capacity of human nonlymphoid cells to produce IL-4. In this study we show that mature human basophils are capable of expressing IL-4 and examine the regulation of IL-4 production in comparison with the lipid mediator leukotriene C4. IL-4 was produced upon immunoglobulin E receptor (IgER) activation of basophils cultured with IL-3, a cytokine previously shown to prime these cells for enhanced release of inflammatory mediators. In some experiments, IL-3 or IgER activation alone also induced IL-4 production close to the detection limit. The effect of IL-3 on IgER-dependent IL-4 expression was dose and time dependent: maximal IL-4 production occurred between 18 and 48 h preexposure of basophils to 3–10 ng/ml IL-3. IgER-induced IL-4 synthesis and release by basophils cultured with IL-3 was rapid and complete after 6 h. In contrast to IL-3, other cytokines (IL-5, granulocyte/macrophage colony–stimulating factor, and nerve growth factor) that also prime basophils for enhanced histamine and leukotriene C4 release did not promote IgER-induced IL-4 synthesis. Basophils appear to secrete a "TH2-like" cytokine profile since no detectable IL-2 or interferon γ was produced upon IgER activation. Mononuclear cells (depleted of basophils), cultured in parallel, did not release IL-4 in response to IL-3 and/or IgER activation, and produced approximately ten times less IL-4 than basophils upon nonspecific activation by phorbol ester and calcium ionophore. Thus, human basophils are an important cellular source of IL-4, and may, therefore, in addition to their inflammatory effector functions, also regulate the differentiation of T helper cells and B cells, in particular in allergic diseases.

IL-4 is an immunoregulatory cytokine with a relatively restricted action on cells of the specific immune system, in contrast to most other cytokines with more pleiotrophic bioactivities. In most systems, the effects of IL-4 are antagonized by IFN-γ, and vice versa. IL-4 acts on T cells inducing T cell proliferation and differentiation into a TH2 phenotype (1), and is of particular importance for the induction of IgE synthesis by providing an essential signal for isotype switching to IgE in human B cells (2, 3). Furthermore, IL-4 has some inhibitory activities on monocyte function such as oxygen radical release and the synthesis of proinflammatory cytokines (4), and may be involved in the emigration of cells expressing very late antigen 4 (lymphocytes, eosinophils, and basophils) by inducing vascular cell adhesion molecule expression on endothelial cells (5). Thus, the regulation of IL-4 synthesis appears to be a key element in the initiation and progression of allergic diseases.

In contrast to proinflammatory cytokines (e.g., IL-1, TNF, IL-8) which are produced by many cell types including tissue cells, the expression of cytokines with predominantly immunomodulatory bioactivities, such as IL-4, IFN-γ, and IL-2, is largely restricted to T cells. In rodents, IL-4 is also expressed (among other cytokines) by crosslinking of the immunoglobulin E receptor (IgER)[1] of immature bone marrow–derived mast cells and mast cell lines (6–8, for review see reference 9). In the human system, IgER-bearing bone marrow non-B, non-T cells (10) and most recently lung mast cells (10a) have been found capable of expressing IL-4. However, no blood leukocyte type other than lymphocytes has yet been identified to produce IL-4.

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[1] Abbreviations used in this paper: αIgER, anti-IgER mAb; IgER, high-affinity IgE receptor (FcεRI); LTC4, leukotriene C4; MNC, mononuclear cells; NGF, nerve growth factor.

Mature human basophils are the only blood leukocyte type known to express the high-affinity IgER (FcεRI). The function of this cell type is strongly regulated by certain cytokines, IL-3, IL-5, GM-CSF, and nerve growth factor (NGF). A short preincubation (5–10 min) with one of these cytokines strongly enhances the release of inflammatory mediators in response to diverse agonists (11–13). They also prime the basophils to produce leukotriene C4 (LTC4) in response to several agonists (e.g., C5a, monocyte-chemotactic peptide 1, IL-8, C3a, platelet-activating factor) which are otherwise inactive in this aspect (11–15). Thus, lymphocyte products can qualitatively alter the function of these myeloid effector cells. Recently established methods for purifying this rare leukocyte type to near homogeneity have allowed us to examine direct long-term effects of cytokines on basophil functions. In particular, we investigated whether IgER crosslinking of mature human basophils can induce the expression of IL-4 and whether this expression is under the control of certain cytokines.

Materials and Methods

Reagents and Media. The following were used: Hepes (Calbiochem-Behring Corp., La Jolla, CA); EDTA (Fluka AG, Buchs, Switzerland); Percoll and dextran (Pharmacia, Uppsala, Sweden); fatty acid-free BSA (Boehringer Mannheim, Mannheim, Germany); and actinomycin D and cycloheximide (Sigma Immunochemicals, St. Louis, MO). All other reagents were of the highest purity available. Hepes buffer contained 20 mM Hepes, 125 mM NaCl, 5 mM KCl, and 0.5 mM glucose. HA buffer was Hepes buffer containing 0.25 mg/ml BSA. Culture medium was RPMI 1640 supplemented with 10% FCS, 25 mM Hepes, 100 U/ml penicillin, and 100 μg/ml streptomycin, respectively, 2 mM nonessential amino acids, and 2 mM L-glutamine (Gibco, Paisley, Scotland).

Preparation of Basophils and Mononuclear Cells. Blood of unselected healthy volunteers was anticoagulated with 10 mM EDTA and mixed with 0.25 volumes of 6% dextran in 0.9% NaCl, and erythrocytes were allowed to sediment at room temperature. Leukocytes were pelleted by centrifugation (150 g, for 20 min at room temperature) and resuspended in isotonic Percoll solution with a density of 1.065 at ~2 × 10⁷ cells/ml. 3 ml of cell suspension were layered on the top of a discontinuous Percoll gradient consisting of 3 ml each of Percoll solutions with the densities 1.079 and 1.070 g/ml, respectively. Cells were centrifuged (400 g, for 30 min at room temperature) and basophil-containing interphases between the densities 1.079 and 1.070 were harvested. For control experiments, the upper cell layers, containing lymphocytes, monocytes, and generally <0.5% basophils (mononuclear cells [MNC]), were harvested as well and used in parallel under identical experimental conditions. Basophil-enriched fractions (10–70% basophils) were washed twice in HA buffer and resuspended in 150 μl HA buffer containing 1 mg/ml BSA at a cell density of 70–200 × 10⁶ cells/ml. Basophils were then further purified by negative selection using antibody-coated paramagnetic beads (depending on the composition of contaminating cell types: anti-CD4, 10–18 μl; anti-CD8, 8–15 μl; anti-CD19, 3–7 μl; anti-CD14, 3–7 μl; anti-CD16, 3–7 μl; MACS® system [Miltenyi Biotec, Bergisch Gladbach, Germany]) (16). Basophil purity, as monitored by Giemsa-stained cytospin smears, generally ranged between 80 and 97%, the contaminating cells consisting mainly of small lymphocytes and occasionally few monocytes. Since the degree of contamination by monocytes and lymphocytes did not appear to influence IL-4 production under the experimental conditions of this study (see results), less pure basophil preparations of 50–80% were also used, and the results obtained were not excluded from data analysis.

Culture Conditions. Basophil preparations, and MNC (cultured in parallel for comparison), were resuspended at a cell density of 1.0–1.5 × 10⁶ cells/ml in culture medium, incubated in sterile round bottomed 96-well microtiter plates (200 μl/well) (Becton Dickinson & Co., Lincoln Park, NJ) at 37°C in a humidified atmosphere with 5% CO₂. Cells were exposed to the different reagents (added at a 0.5–1:100 vol/vol ratio). Cell-free supernatants were then harvested after the time indicated and stored at −20°C until measurements of cytokine production by ELISA and sulfidoleukotriene synthesis by RIA. In some experiments, cell pellets were lysed with 0.5% Nonidet in the original volume of culture medium and two freeze/thaw cycles after which cell debris were removed by high-speed centrifugation.

Measurement of Cytokines and Leukotriene C4/D4/E4. IL-4 was measured by ELISA as described (17, 18). Briefly, microtiter plates were coated with mouse anti-hIL-4 antibody 8F-12 and after incubation with samples or purified rhIL-4 plates were developed with the biotinylated mouse anti-hIL-4 antibody 3H-4 followed by avidine–alkaline phosphatase conjugate (Calbiochem-Behring Corp.) which was revealed by p-nitrophenylphosphate disodium salt (Merck, Darmstadt, Germany). The assay had a sensitivity of 30 pg/ml with a dynamic range of up to 3 ng/ml. In some experiments, IL-4 was also measured using the kit supplied by R&D Systems (British Biotechnology Ltd., Oxford, UK) with similar results. IL-2 (R&D Systems) and IFN-γ (Genzyme Corp., Cambridge, MA) were measured with ELISA kits according to the manufacturer's protocols. Sulfidoleukotrienes were determined in a RIA as described (13).

Cell Stimuli. rhIL-3 and rhGM-CSF were a kind gift of Sandoz (Basel, Switzerland), and rhIL-5 was from Amgen Biologicals (Thousand Oaks, CA) (11). rhNGF was kindly provided by Genentech Inc. (South San Francisco, CA) (13). Purified mAb 29C6, directed against the non-IgE–binding epitope of the high-affinity IgER α chain (FcεRI), was a generous gift from Drs. Hakimi and Chizzonite (Hoffmann-La Roche, Nutley, NJ) (19). PMA and ionomycin were from Sigma Immunochemicals. For all cytokines and stimuli, stock solutions were made in HA buffer containing 1 mg/ml BSA and 1–2 μl were added to cell cultures. Ionomycin and PMA were solved in DMSO (final DMSO concentration ≤0.1%).

Results

Mature Human Basophils Produce IL-4. Fig. 1 demonstrates that purified basophils cultured during 18 h with IL-3 produced IL-4 to variable degrees in response to IgER crosslinking in all experiments performed. No spontaneous IL-4 production was detected. In some experiments, IL-4 was also produced in amounts close to the detection limit of the assay after exposure to IL-3 alone or, less frequently, by IgER stimulation of unprimed cells. IgER-dependent IL-4 production by IL-3 primed cells was not affected by the percentage of contaminating cells within the basophil preparations since no correlation was found between basophil purity and IL-4 production (percent basophils vs. pg IL-4/10⁶ basophils, $r^2 = 0.047$). Furthermore, in four experiments with highly purified basophil preparations (90–97%) IL-4 production was 252 ± 24 (mean ± SEM) pg/10⁶ basophils in response to

sequential exposure to IL-3 and anti-IgER mAb (αIgER). Mononuclear cells from the same donors that were cultured under identical conditions did not release detectable amounts of IL-4 (data not shown). LTC4 released into the supernatants was measured in parallel. As shown in Fig. 1, LTC4 formation in response to IgER crosslinking was also strongly enhanced (mean = 6.7 times more) by culturing the basophils overnight with IL-3. LTC4 in supernatants of unstimulated basophils or cells cultured with IL-3 alone was generally not detectable or minimal. IL-4 release under all experimental conditions, as well as the enhancement of leukotriene synthesis by IL-3, but not LTC4 synthesis induced by IgER crosslinking alone, was abolished by treating the cells with 0.1 μg/ml actinomycin D or 0.1 μg/ml cycloheximide (data not shown). The culture time needed for IL-3 to promote IgER-dependent IL-4 expression was examined by varying the time intervals between IL-3 and αIgER addition from 10 min to 65 h. These experiments showed that a minimum of 9 h culture with IL-3 was needed for detectable IL-4 release (data not shown). The IL-3 effect became optimal between 18 and 41 h (199 ± 10 and 291 ± 65 pg IL-4/10⁶ basophils after 18 and 41 h, respectively; mean ± SEM, n = 8).

Dose Response for Priming and Triggering Human Basophils to Express IL-4. Fig. 2 demonstrates that IL-3 primes basophils to secrete IL-4 in a dose-dependent manner. Consistent with Fig. 1, zero to minimal amounts of IL-4 were detected after stimulation with αIgER alone. Only very low concentrations of IL-3 (1–3 ng/ml) were needed to allow optimal IgER-dependent IL-4 production. The enhancement of αIgER-induced LTC4 synthesis after overnight culture was also dose dependent, occurring over a similar concentration range of IL-3 (Fig. 2). In another set of experiments, variable doses of αIgER mAb were used to trigger basophils cultured during 18 h without or with a constant amount of IL-3. Again, de

novo synthesis of lipid mediators and production of IL-4 occurred dose-dependently over an identical concentration range of αIgER for both basophil effector functions (Fig. 3). In these experiments, low levels of IL-4 expression were also detected after exposure to IL-3 alone, however in the absence of LTC4 generation. No IL-4 release was detected in unprimed basophils in response to αIgER at 0.1–1,000 ng/ml (data not shown). Figs. 2 and 3 also demonstrate the inability of MNC prepared from the same blood donors to produce IL-4 under these experimental conditions.

Time Course of αIgER-induced IL-4 Synthesis. Fig. 4 shows the kinetic of αIgER-induced IL-4 production by human basophils. IL-4 synthesis and release by basophils cultured with IL-3 was very rapid and occurred within 2–6 h. No further increase of IL-4 release was observed between 6 and 24 h after αIgER addition. By contrast, the kinetic of LTC4 synthesis (Fig. 4) and degranulation (12; data not shown) was clearly distinct since the release reaction of these inflammatory mediators was already complete 30 min after stimulation. These data indicate that IL-4 release in response to αIgER is not

Figure 2. Dose response of the IL-3 effect upon IL-4 synthesis and leukotriene C4 generation. Basophils (●) or MNC controls (◆), were cultured for 18 h with variable concentrations of IL-3 and subsequently stimulated with 250 ng/ml αIgER mAb for 6 h more. (*Unfilled symbols*) LTC4 generation; (*filled symbols*) IL-4 synthesis. Mean values ± SEM of three different experiments performed in duplicates. No IL-4 or LTC4 was detected in the supernatants of basophils treated with IL-3 alone over the concentration 0.1–100 ng/ml.
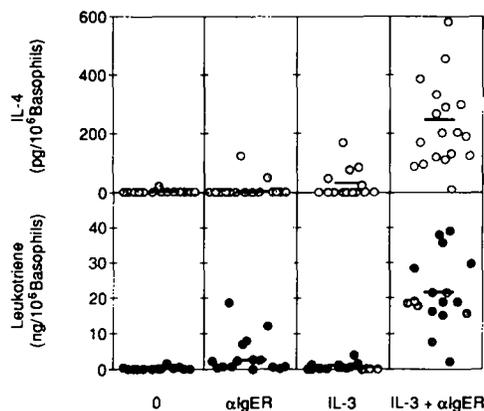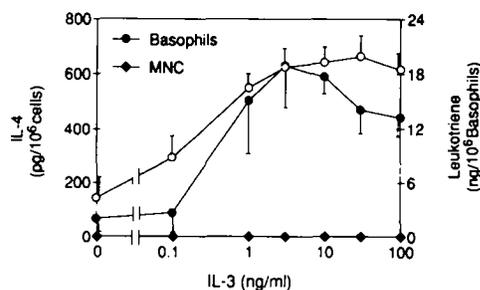
Figure 1. Mature human basophils produce IL-4: donor variability of IL-4 and LTC4 synthesis. Purified human basophils were preincubated for 18 h with medium alone or with 50 ng/ml IL-3, and then exposed to buffer control or to 250 ng/ml αIgER mAb for 6 h. IL-4 (*top*) and LTC4 (*bottom*) were measured in culture supernatants. Each data point represents the mean value of duplicates. The results of 18 experiments using cells isolated from different unselected donors are shown. Bars, mean values of all data.
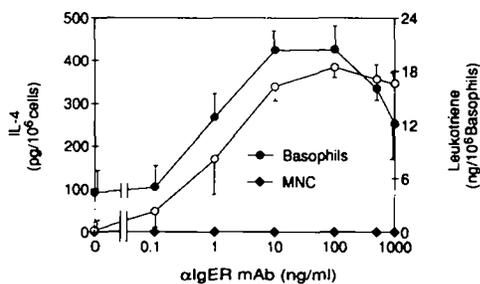
Figure 3. Dose response of αIgER mAb-induced IL-4 production and leukotriene C4 synthesis by basophils cultured with IL-3. Basophils (●) or MNC (◆) were incubated for 18 h with 50 ng/ml IL-3 and then stimulated with variable concentrations of monoclonal αIgER for 6 h more. (*Unfilled symbols*) LTC4 (ng/10⁶ basophils); (*filled symbols*) IL-4 (pg/10⁶ basophils or MNC, respectively). Mean values ± SEM, n = 6) are shown. No IL-4 was detected after αIgER stimulation of basophils cultured without IL-3 in these experiments.
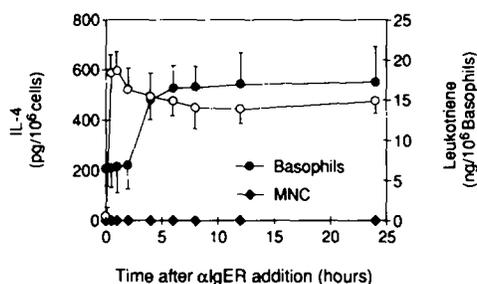
Figure 4. Kinetic of αIgER mAb-induced IL-4 production and leukotriene C4 synthesis. Basophils (●) and control MNC (◆) were cultured for 18 h in 50 ng/ml IL-3 before exposing them to 250 ng/ml αIgER mAb. (x-axis) Time (h) after αIgER mAb addition. Time-point zero indicates the IL-4 production induced by IL-3 alone that was not increased during further incubation (data not shown). (*Filled symbols*) IL-4; (*unfilled symbols*) LTC4. Mean values are shown ± SEM, *n* = 6).

due to a release reaction of preformed and granule-stored IL-4 that may have been synthesized during culture with IL-3. To further address this question, we examined IL-4 in culture supernatants as well as in lysed cell pellets of basophils treated with either buffer control, IL-3 or αIgER alone, or the sequential combination of both. Cell-associated IL-4 was only detected in IL-3 primed basophils exposed to αIgER stimulation, possibly representing IL-4 in a process of being secreted, but not in cell lysates under the other experimental conditions (data not shown). Furthermore, actinomycin D or cycloheximide (5 μg/ml) added to IL-3 primed cells just before IgER stimulation strongly suppressed IL-4 release (data not shown). Taken together, these results indicate that IL-4 secretion in response to IgER crosslinking of IL-3 primed basophils is due to de novo synthesis of IL-4.

Human Basophils Produce a TH2-like Profile of Cytokines. Recent studies in vitro and ex vivo have shown that Th cells can differentiate into subsets with a distinct capacity for producing different cytokines, in particular with regard to the expression of either IL-4 or IFN-γ and IL-2, respectively (1, 20–22). Therefore, culture supernatants from representative experiments shown above were tested for the presence of IFN-γ or IL-2. Under none of the experimental conditions, including those resulting in IL-4 release, did we detect any IFN-γ or IL-2, whereas in culture supernatants of control MNC stimulated with PMA/ionomycin, large amounts of these cytokines were measured (data not shown).

Effect of Basophil-priming Cytokines on IgER-induced IL-4 Production. Apart from IL-3, two other hematopoietic growth factors, IL-5 and GM-CSF, as well as the neurotrophic cytokines NGF have been shown to rapidly modify basophil mediator release in a way similar to IL-3. These cytokines were therefore tested for their ability to promote IgER-induced IL-4 production. Basophils were cultured overnight with these cytokines at concentrations maximally effective with regard to their priming activity for basophil mediator release, and then stimulated by IgER crosslinking for 6 h. IL-4 production was measured and compared with that of IL-3–treated basophils. Fig. 5 shows that IL-3 strongly primes basophils
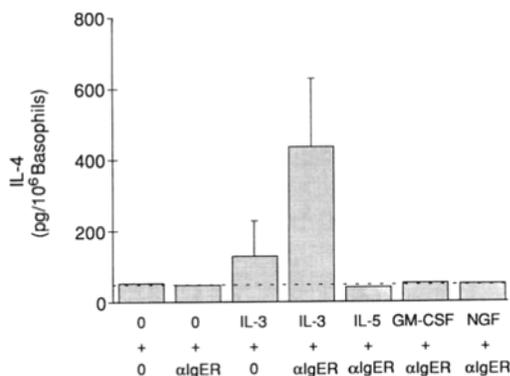

Figure 5. Effect of other cytokines capable of enhancing basophil mediator release upon αIgER mAb-induced IL-4 synthesis. Basophils were cultured for 18 h in medium alone or with IL-3 (50 ng/ml), IL-5 (30 ng/ml), GM-CSF (50 ng/ml), or NGF (30 ng/ml), before exposure to buffer control (O) or to αIgER mAb (250 ng/ml) for 6 h more. Mean values of three different experiments are shown. Bars, SD. (- - - -) Detection limit of the assay.

for IgER-induced IL-4 synthesis, whereas IL-5 and GM-CSF, as well as NGF were absolutely inactive in this respect.

Comparison of the Capacity to Produce IL-4 in Response to Nonspecific Activation between Human Peripheral Blood Basophils and MNC. Since lymphocytes are a well-established source of IL-4, it was interesting to compare the general capacity of basophils and MNC to produce IL-4. Basophils, as well as MNC consisting of lymphocytes and monocytes and depleted of basophils (<0.5%), purified from the same blood specimens of different donors, were stimulated with PMA and ionomycin, a combination known to be a particular potent mode to induce cytokine expression in most cell types. Under these conditions, basophils produced IL-4 in amounts clearly exceeding that induced by IgER crosslinking of IL-3 primed basophils. It is striking that basophils produced nearly one order of magnitude more IL-4 than MNC under identical conditions, further emphasizing that basophils may represent a particularly important source of IL-4 among different
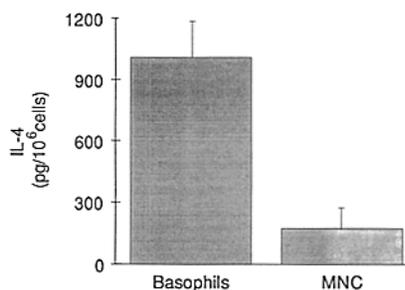

Figure 6. Comparison of the capacity to produce IL-4 between basophils and MNC. Basophils and MNC, isolated from the same blood specimen of different donors were stimulated for 24 h with a combination of PMA (5 ng/ml) and ionomycin (1 μM) and IL-4 released in the supernatant was measured. Mean values ± SD of four experiments performed in duplicates are shown.

blood leukocyte types (Fig. 6). Furthermore, stimulation of basophils with ionomycin alone resulted in IL-4 production as well, comparable to that induced by sequential stimulation by IL-3 and αIgER, whereas that produced by MNC was low and close to the detection limit (data not shown).

Discussion

Basophils are an important source of vasoactive and proinflammatory mediators that are released from granule stores (e.g., histamine) or de novo synthesized (e.g., leukotrienes) upon stimulation with IgE-dependent or -independent agonists. This study shows that mature human peripheral blood basophils cultured with IL-3 are also capable of producing substantial amounts of IL-4 upon crosslinking of the high-affinity IgER. Although basophil preparations were not purified to 100% homogeneity, several lines of evidence indicate that indeed mature basophils were the source of IL-4 released under the experimental conditions of this study. Basophils are the only PBL known to express the high-affinity IgER, and IL-4 was produced in response to stimulation with a mAb specific to the α chain of the FcεRI. IgER-dependent IL-4 production was only observed in Percoll fractions enriched in basophils, independent of the degree of contamination with leukocyte types other than basophils, and never in MNC depleted of basophils. Although we cannot exclude the existence of IgER-bearing nongranulated "non-B, non-T cells" in the peripheral blood capable of producing IL-4, such cells would be expected to accumulate in the Percoll fraction of lower density rather than in the fraction containing granulated basophils. Mature human basophils consistently produced IL-4 after culture with IL-3 for 18–42 h upon crosslinking of the IgER. In some experiments, IL-4 was also detected in supernatants of cells cultured with either IL-3, and less frequently, with αIgER alone, possibly because both ligands by themselves can induce low levels of IL-4 expression near the detection limit of the assay. Alternatively, cells of certain individuals may need only one of the two signals.

IL-3 primed the human basophils for IgER-dependent IL-4 synthesis dose-dependently at low concentrations (0.1–10 ng/ml). This dose range is similar to that previously found to enhance basophil mediator release and prime the cells for LTC4 synthesis in response to appropriate agonists (11, 12). Two other hematopoietic growth factors, GM-CSF and IL-5, as well as a neurotrophic cytokine, NGF, have been found capable of rapidly modifying the basophil response in an identical manner to IL-3 with regard to histamine release and leukotriene generation (11–13). Basophils carry high-affinity receptors for IL-3, IL-5, and GM-CSF with variable degrees of competition of binding among these three growth factors (23, and our own unpublished results), due to a common high-affinity converter/signal transducer (KH97/gp120) receptor β chain for IL-3, IL-5, and GM-CSF (24, 25). It is interesting that only cells cultured with IL-3, but not with GM-CSF, IL-5, or NGF, were able to synthesize and release IL-4 in response to IgER crosslinking. This difference in the cytokine profile capable of rapidly priming basophils for LTC4

synthesis on the one hand, and priming the cells for IL-4 production after prolonged culture on the other, suggests that in addition to the common β chain, the cytokine-specific α chain may also participate in signal transduction for these hematopoietic growth factors, particularly regarding the later occurring downstream events regulating gene expression. Alternatively, basophil priming for IL-4 synthesis may require a critical amount of occupied receptors, higher than that necessary for enhancing the release of inflammatory mediators, and thus the unique property of IL-3 may be due to different receptor densities for these hematopoietic growth factors (23).

The fact that IL-3, apart from potentiating basophil mediator release, also regulates the expression of the immunomodulatory cytokine IL-4 in this cell type, suggests that IL-3 may have an important function in the pathogenesis of immediate-type hypersensitivity diseases. Indeed, recent in situ hybridization studies of allergic late-phase reactions show that IL-3 is expressed possibly by activated TH2 helper cells (26). Furthermore, eosinophils have been shown to express, at least upon calcium ionophore stimulation, hematopoietic growth factors (e.g., IL-3, IL-5, GM-CSF) which may act in an autocrine manner (27, 28). The capacity of eosinophils to express IL-3 that regulates basophil functions may also represent a link of interaction between these effector cell types in allergic inflammatory exudates.

The regulation of IL-4 expression in human basophils was not investigated in detail. Our data indicate that IgER activation of IL-3 primed basophils induces the release of IL-4 by de novo synthesis. It is unlikely that IL-3 by itself induces the synthesis of IL-4 that is stored in the cell granules and released upon receptor stimulation. Cell-associated IL-4 was detected only in IL-3 primed cells after IgER crosslinking, presumably representing the fraction of IL-4 in the process of being secreted, but not in basophils exposed to IL-3 alone, and the protein synthesis inhibitor cycloheximide or the RNA synthesis inhibitor actinomycin D added after IL-3 culture and just before IgER stimulation abolished IL-4 release without affecting leukotriene synthesis. However, it is unclear whether IL-3 and αIgER act synergistically by an identical mechanism on transcription, RNA stability and/or translation, or whether these events are regulated separately by these two signals.

IgER-induced IL-4 production by IL-3 primed basophils occurred rapidly and was completed within 6 h. IL-4 release was, however, clearly slower and distinct from the very rapid degranulation response and synthesis of LTC4, further dissociating these cellular responses. The amount of IL-4 produced by basophil within 6 h after IgER stimulation was comparable to that produced by PHA-activated MNC within 24 h (our unpublished observations). It is also interesting to note that activation of basophils by a combination of phorbol ester and calcium ionophore, a particularly potent mode of cytokine expression in most cell types, results in a considerably higher IL-4 release than stimulation of the MNC fraction under identical conditions, further emphasizing the high potential of human basophils to express IL-4.

Whereas most cytokines can be produced by many different cell types, the expression of IL-4, IL-2, and IFN-γ is more

restricted to the lymphocyte lineage. In the rodent system, certain mast cell lines, IL-3–dependent cell lines, and bone marrow–derived mast cells have been shown to express IL-4, among other less restricted cytokines (6–9), mainly on the level of gene expression with little information about product release. Even less is known about the capacity of human mucosal and connective tissue–type mast cells to express and release cytokines. Skin mast cells have been shown to express TNF-α (29) and a recent paper indicates that human lung mast cells can produce IL-4 (10a). The profile of cytokines regulating mediator release by human basophils and mucosal lung mast cells suggests that the human basophils are functionally more related to mouse mucosal mast cells than they are to human mucosal mast cells (30). Thus, further studies are needed to define the importance of basophils or mast cells, respectively, as a source of IL-4 or other cytokines in the human system.

An increasing number of studies, including our own, indicate that the cells of the adaptive immune system strongly regulate the effector functions of granulocytic cells of the in-

nate immune system in an antigen-independent manner by the release of different cytokine profiles (e.g., IL-3, IL-5 of TH2 cells regulating eosinophil and basophil functions). On the other hand, recent studies indicate that myeloid inflammatory effector cells can express several cytokines as well, and may thus influence the function of the adaptive immune system. With regard to IL-4 expression, however, the basophil appears to be the only yet identified nonlymphoid leukocyte type capable of producing this important immunoregulatory cytokine. The capacity of basophils to rapidly produce IL-4, in the absence of detectable IL-2 and IFN-γ expression, indicates that this cell type, apart from its inflammatory effector function, also regulates the maturation and differentiation of T cells (differentiation into TH2 phenotype) (1, 31) and B cells (IgE switch) (2). Thus, basophils may also represent immunomodulatory cells involved in the propagation and possibly even the initiation of immediate-type hypersensitivity disease, or also in the pathogenesis of infectious diseases with inadequate TH1 cell-mediated protective immunity (31).

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