INTERLEUKIN 3-DEPENDENT MEDIATOR RELEASE
IN BASOPHILS TRIGGERED BY C5a

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Basophils and mast cells are important effector cells of inflammation and in particular of acute allergic reactions. Basophils can be activated to release inflammatory mediators by IgE-dependent and IgE-independent stimuli (1-4). The mediator profile produced by basophils strongly depends upon the agonist used to activate the cells (4a). For example, crosslinking of high-affinity IgE-receptors by an antigen, anti–IgE or anti–receptor antibodies leads to the release of preformed mediators from granule stores, like histamine and proteolytic enzymes, and also results in the activation of enzymes (phospholipase[s] and 5-lipoxygenase) responsible for the generation of leukotriene C_4 (LTC_4),[1] a very potent lipid mediator and smooth muscle contractant (6). Conversely, the anaphylatoxin C5a, which at low concentrations is a potent inducer of basophil degranulation, does not activate basophils to produce bioactive lipids. These observations clearly indicate that degranulation and the generation of the arachidonic acid metabolite LTC_4 are dissociated events and therefore controlled by different mechanisms.

Granulocyte/macrophage colony-stimulating factor (GM-CSF) is a pluripotent growth factor for myeloid bone marrow precursors (7, 8), but more recent studies demonstrated that this cytokine also enhances the function (in particular cytotoxicity and oxygen radical release) of mature inflammatory effector cells, like monocytes, eosinophils, and neutrophils (PMN) (9-13). Studies from our laboratory demonstrated that PMN sequentially exposed to GM-CSF and the chemotactic peptides C5a or FMLP release relatively large amounts of LTB_4 and LTB_4 metabolites, while neither peptide alone triggers the formation of these lipid mediators (14). Preexposure of PMN to GM-CSF also results in a strong enhancement of chemotactic peptide–dependent generation of platelet-activating factor, another potent lipid mediator (15). From these experiments we concluded that GM-CSF not only enhances effector cell functions, but also qualitatively changes in the mediator profile of the neutrophils triggered by diverse agonists.

These observations suggested that other growth-promoting cytokines may also modify the mediator profile of mature leukocytes, and we thus studied the effects of IL-3 on blood basophils. IL-3 stimulates the growth of early progenitor cells of

[1] *Abbreviations used in this paper:* CML, chronic myeloid leukemia; LTC_4, leukotriene C_4; RT, room temperature; sLT, sulfidoleukotrienes.

all the hematopoietic lineages (reviewed in reference 16). In contrast to GM-CSF, there is little information about effects of IL-3 on mature white cells (16). It is conceivable that, upon maturation, leukocytes lose receptors for this cytokine. However, IL-3 is known to promote the growth of relatively mature histamine containing cells in bone marrow cultures, suggesting that circulating basophils could retain IL-3 receptors.

In this article we show that IL-3 strongly affects the function of mature basophils. It enhances the stimulus-dependent release of histamine. In particular, IL-3 enables basophils to produce large quantities of LTC$_4$ in response to C5a, while neither peptide alone triggers the generation of lipid mediators. This establishes a novel function of IL-3 and shows that the basophil mediator release profile can be modified by a cytokine. This IL-3 effect might be important in the pathogenesis of immediate-type hypersensitivity reactions, shock syndromes, and inflammation.

Materials and Methods

Blood Donor Selection. ˙ Normal (nonatopic) donors were defined as individuals without a history of immediate hypersensitivity reactions, normal IgE values, and negative skin tests to common allergies (pollen, molds, pet dander, house dust, mites). Atopic individuals had positive skin tests and RAST tests to at least one allergen, allergic symptomatology, and normal to high IgE levels.

Cell Preparation. Blood from informed volunteers, anticoagulated with 10 mM EDTA, was mixed with 0.25 volumes of 6% dextran (Pharmacia, Uppsala, Sweden), and erythrocytes were allowed to sediment at room temperature (RT). Leukocytes were pelleted by centrifugations (150 g, 20 min, RT) and suspended in HA buffer (20 mM Hepes, 125 mM NaCl, 5 mM KCl, 0.5 mM glucose, and 0.025% human serum albumin [Calbiochem Behring Corp., La Jolla, CA]). For most experiments, the cells were fractionated by Ficoll-Hypaque (Pharmacia) density centrifugation (400 g, 40 min, RT). The basophil-rich mononuclear cell layer was harvested, washed three times (400 g, 10 min, 4°C) in HA buffer, and finally suspended in HACM buffer (HA buffer supplemented with 1 mM CaCl$_2$ and 1 mM MgCl$_2$) at a cell density of 2.5 × 10^6 cells/ml.

In some experiments the leukocytes obtained after dextran sedimentation of erythrocytes were fractionated by discontinuous Percoll (Pharmacia) density gradients. Isotonic Percoll solutions with densities of 1.075, 1.070, 1.065, and 1.060 were made up by dilution of the Percoll solution with Hepes buffer (washing buffer without albumin) according to the manufacturer's specifications, and 2.5 ml of each was carefully layered into 15-ml plastic tubes. 1 ml of ~5 × 10^7 leukocytes in HA buffer was layered on the top of the Percoll gradient and centrifuged for 30 min, 500 g at RT. The bands formed at the density interfaces and at the top and the bottom of the gradient were harvested, washed three times (400 g, 10 min, 4°C), and resuspended in HACM buffer.

Each cell fraction was stained with anti-Leu-5b (T cells), anti-Leu-16 (B cells), anti-Leu-M3 (monocytes), and anti-Leu-11a (neutrophils/NK cells) (all from Becton Dickinson & Co., Mountain View, CA), and analyzed by FACS using an Ortho Cytofluorograph H50 (Ortho Diagnostic Systems Inc., Westwood, MA). The cell fractions were also differentiated microscopically (at least 400 cells per slide), after plating the cells on glass slides with a cytocentrifuge and staining with May-Grünwald.

Stimuli. C5a was purified from yeast-activated human serum as described (17, 18) and was homogeneous, as determined by amino acid analysis, SDS-PAGE, and microzone paper electrophoresis at pH 8.6. Human rIL-3 with bioactivity of 10^6 U/1.5 mg protein was a generous gift of Dr. M. Schreier, Sandoz, Switzerland. 1 U/ml of IL-3 is defined as the concentration giving a 50% of maximal [^{3}H]TdR incorporation in cultures of leukocytes from chronic myeloid leukemia (CML) patients. The peptides were stored in concentrated stock solutions at −70°C and diluted in HACM buffer before use. Stimuli were added to cells in a 1:100 volume ratio.

Mediator Release Assay. Cells (2.5 × 10^6/ml/batch) in HACM buffer were warmed up to 37°C for 10 min, before the addition of the peptide(s). The reaction was stopped after the time indicated by placing the tubes in an ice bath, except for kinetic experiments, where the cells were also cooled by the rapid addition of 1 vol of ice cold buffer. After centrifugation (400 g, 10 min, 4°C), 500 µl of the supernatant was added to 500 µl of HACM buffer and 1 ml of 0.8 M HClO$_4$ and centrifuged again (600 g, 15 min). This supernatant was either stored for up to 3 d at −30°C or immediately assayed for histamine using an automated fluorimetric method (19). The other 500 µl of the supernatants were stored for up to 1 wk at −70°C before determination of sulidoleukotriene release by RIA (LTC$_4$/LTD$_4$/LTE$_4$-RIA; Amersham Corp., Arlington, IL) performed according to the recommendations of the supplier.

To validate the RIA experiments, HPLC analysis of LTC$_4$, LTD$_4$, and LTE$_4$ was performed as described (20). Briefly, sulfidoleukotrienes formed by Ficoll-Hypaque isolated cells (2.5 × 10^7 cells/ml) were extracted (21) and analyzed by HPLC (20) using a U61K-injector, model 510 pumps and a 990-plus photo-diode-array-detector (all from Waters Associates, Milford, MA). The column was a 25 × 0.4 cm Nucleosil C18-100-5 µm and the lipids were eluted isocratically with methanol/3.5 mM ammonium acetate, pH 4.6, in H$_2$O (75:25 vol/vol) at a flow rate of 1 ml/min at RT. The HPLC analysis was calibrated with synthetic LTC$_4$, LTD$_4$, and LTE$_4$ (generous gift of Dr. Rockach, Merck-Frosst, Pointe Claire, Quebec, Canada). Elution times were: LTC$_4$, 5.3 min; LTD$_4$, 9.5 min; LTE$_4$, 11.7 min. Cell-derived LTC$_4$ was further identified by UV spectroscopy and RIA in the corresponding HPLC fraction. These experiments demonstrated that LTC$_4$ formed by basophils was but very slowly converted to LTE$_4$ under the incubation conditions used in this study (4a). Nevertheless, a RIA with an antibody with almost equal reactivity to LTC$_4$, LTD$_4$, and LTE$_4$ was used, in order not to underestimate sulfidoleukotriene generation due to conversion of LTC$_4$ to LTE$_4$.

Presentation of Data. Histamine release is expressed in percent of total histamine determined in cells lysed by perchloric acid (0.4 M final concentration). Total histamine release represents histamine release without corrections. Specific histamine release represents total histamine release minus the release obtained in the corresponding control samples incubated with buffer alone. Sulfidoleukotriene release was initially expressed as nanograms of sLT/2.5 × 10^6 cells. During the course of the studies, it was found that sLT release was related to the numbers of basophil in the leukocyte preparations. Values of sLT generation were therefore converted to picograms of sLT per nanogram of total cellular histamine, in order to correct for differences in basophil numbers in leukocytes obtained from different blood donors. All experiments were performed at least three times in triplicates. Significance was determined by the Student's *t*-test.

Results

IL-3 Changes the Mediator Profile of Basophils Stimulated by C5a. In preliminary experiments we determined the maximal effective concentration of C5a. Maximum histamine release was obtained at C5a concentrations between 10^{-9} M and 10^{-8} M with no further increase at higher C5a levels. In no instance did we observe the generation of LTC$_4$ in basophils stimulated with C5a, even at 10^{-7} M (data not shown). We then examined the effects of mediator release of IL-3 pretreatment. Fig. 1 shows the results of 24 experiments performed with leukocytes of normal and atopic donors. IL-3 alone did not significantly increase histamine release over that of spontaneous release. The small increase of the mean release induced by IL-3 (Fig. 1, *top*) was due to a very low continuous (>2 h) release, which was observed with the basophils from some atopic donors. However, in the normal and most atopic donors, IL-3 did not induce histamine release even at concentrations up to 1,000 ng/ml (data not shown). C5a alone is a potent inducer of basophil granule release in cells of all donors tested so far (mean of 57% histamine release). This effect is more pronounced and less variable when pure C5a rather than the partially purified C5 fragment (3, 4) is used.
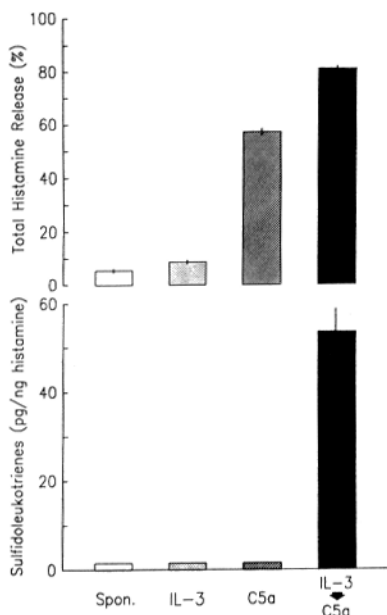
FIGURE 1.   Change of C5a-induced basophil mediator release profile by IL-3. In 24 experiments, leukocyte from different allergic and normal donors depleted of PMN by Ficoll-Hypaque were incubated after a warm-up period at 37°C for 10 min as follows: Spon: Cells were incubated in buffer for 30 min (*open columns*). IL-3: Cells were incubated with 10^{-9} M IL-3 for 30 min (*dotted columns*). C5a: After incubation of the cells for 10 min, they there challenged with 10^{-8} M C5a for 20 min (*dashed columns*). IL-3-C5a: The leukocytes were exposed to 10^{-9} M IL-3 for 10 min and then triggered with 10^{-8} M C5a for 20 min (*black columns*). The mean ± SEM of mediators found in the supernatants are shown. (*Top*) Histamine release (percent of total cell content; mean = 93 ± 3.9 ng/2.5 × 10^6 cells) measured fluorimetrically. (*Bottom*) sLT (LTC4/LTD4/LTE4) release measured by RIA. sLT generation is expressed as picograms sLT/nanogram total histamine content of the respective cell preparation, in order to correct for different basophil numbers in leukocytes samples from different donors. The three columns at the left represent the detection limit for sLT.

Preexposure of the cells to IL-3 significantly enhances ($p < 0.001$) the already marked effect of C5a (∼80% histamine release, corresponding to a 40% increase).

The effect of IL-3 was dramatic when the generation of sulfidoleukotrienes (sLT; LTC4/LTD4/LTE4) was determined. No production of LTC4 was observed with either IL-3 or C5a alone, up to 1,000 ng/ml and 10^{-7} M, respectively (not shown), with cells of normal and atopic donors (Fig. 1, *bottom*). However, cells exposed to IL-3 for 10 min and then stimulated with C5a produced very large quantities of LTC4 (Fig. 1). These results demonstrate that IL-3 not only enhances basophil releasability, but "primes" basophils to respond to C5a challenge with the synthesis of bioactive lipids. We believe that this qualitative change in the basophil mediator profile is pathophysiologically much more important than just an enhancement of a normally occurring degranulation response.

Concentration Dependence of the IL-3 Effect.   The cells were incubated without or with different concentrations of IL-3, and then either further incubated without addition of a second trigger (Fig. 2, *open symbols*), or stimulated with C5a (*closed symbols*). The results obtained from experiments performed with cells from an allergic (*circles*) and a normal donor (*triangles*) show that the enhancement of histamine release and the induction of sLT synthesis by IL-3 priming was concentration dependent (Fig. 2). From the curves, an ED50 of 0.3-0.6 ng/ml (2-4 × 10^{-11} M = 0.2-0.4 U/ml) can be estimated. A maximum effect was reached with 10 ng/ml, and the level of mediator release remained constant even when IL-3 was used at 100-1,000 ng/ml (data not shown). Again, IL-3 alone over the entire dose range did not trigger sLT synthesis nor did it induce histamine release substantially above that of controls (*open symbols*). The neglible, but statistically significant release of histamine by IL-3 alone observed with cells from one donor (*open circles*) is an example of the donor variability discussed above. The experiments also further document that preexposure
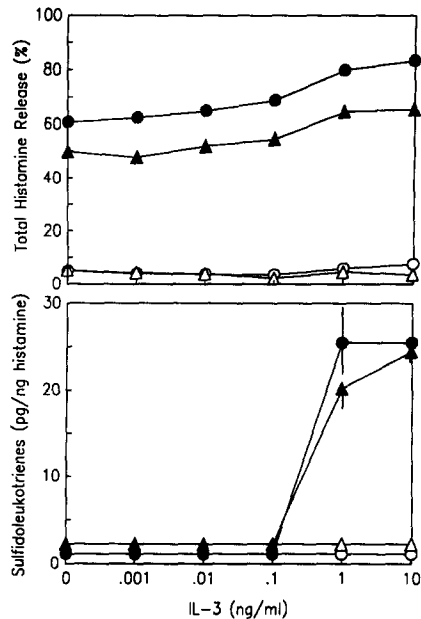
FIGURE 2. Dose-response of IL-3 priming. Cells were preincubated for 10 min with increasing doses of IL-3 and then stimulated with 10^{-8} M C5a for 20 min (*closed symbols*), or incubated with IL-3 alone for 30 min (*open symbols*). Each point is mean ± SD of triplicates. (*Circles*) atopic donor (total histamine = 112 ± 1.3 ng/2.5 × 10^6 cells). (*Triangles*) normal donor (total histamine = 56 ± 1.2 ng/2.5 × 10^6 cells). (*Top*) Histamine release (percent of total, without subtraction of spontaneous release). (*Bottom*) sLT generation (picograms sLT/nanogram histamine content of cells). No further increase of mediator release occurs at IL-3 concentration of >10 ng/ml. Where not shown, SD is smaller than the size of the symbol.

of basophils to IL-3 is absolutely required for C5a-induced sLT synthesis, while the effect of IL-3 upon basophil degranulation is much less striking, C5a being by itself a potent inducer of histamine release. As shown in these two representative experiments, there is no qualitative difference of the mediator profile or the IL-3 dose-response relationship in cells from allergic or normal donors.

Effect of IL-3 on the Time Course of C5a-induced Responses. In contrast to IgE-dependent basophil activation, C5a and other chemotactic agonists trigger a very rapid release of histamine (3, 4). Fig. 3 shows that IL-3 enhances the magnitude, but not the time course of C5a-induced histamine release (*top*). Interestingly, sLT generation of IL-3 treated basophils also occurs very rapidly in parallel to degranulation (Fig. 3, *bottom*). These experiments confirm that control cells do not generate sLT in response to C5a over a period of 0–40 min.

Effect of the Sequence of Addition of the Peptides and Dependence on Priming Time with IL-3. Fig. 4 shows how the sequence of addition of IL-3 and C5a influences mediator release. When IL-3 and C5a were added simultaneously, the enhancement of histamine release was 70% of that seen in primed cells. Addition of IL-3, even 10 min after C5a, still induced a small, but significant increase in histamine release. However, a short preincubation with IL-3 was definitely required for efficient induction of sLT synthesis (Fig. 4, *bottom*). Fig. 5 shows that IL-3 priming for C5a-induced leukotriene synthesis starts after a lag period of 1 min, becomes half-maximal at 2.5 min, and maximal after 5 min (*top*). Although this change in the mediator release pattern of the basophils is obviously a rapid process, priming for C5a-induced sLT synthesis persisted for an observation period of up to 2 h (data not shown). A preincubation period with IL-3 is not required for an enhancement of C5a-induced degranulation, but an optimal effect is reached also 5 min after priming with IL-3.

Mediator Release of Leukocytes Fractionated by Percoll Gradients. Histamine is a specific
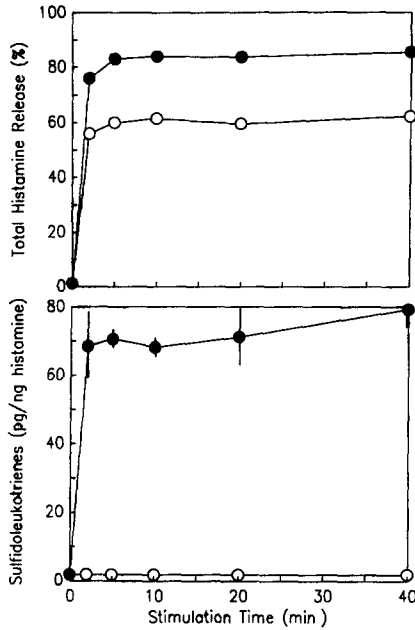
FIGURE 3. Time course of C5a-induced mediator release. Cells from an atopic donor were preincubated with (*closed circles*) or without (*open circles*) 10^{-9} M IL-3 for 10 min and then stimulated with C5a (10^{-8} M) for the time indicated. Mean ± SD (n = 3). (*Top*) percent histamine release (total histamine = 69 ± 1.1 ng/2.5 × 10^6 cells). (*Bottom*) sLT production. Open symbols indicate the detection limit for sLT when cells are exposed to IL-3 alone. Identical kinetics are obtained with cells from normals.

marker for basophil degranulation, since it is not present in other leukocytes. By contrast, sLT can also be generated by monocytes and eosinophils directly (6, 13), or through LTA₄ released by activated neutrophils (20). Since monocytes, neutrophils, and eosinophils, in addition to basophils, all possess C5a receptors, the possibility of an alternative source of the sLT had to be considered. Whole leukocytes
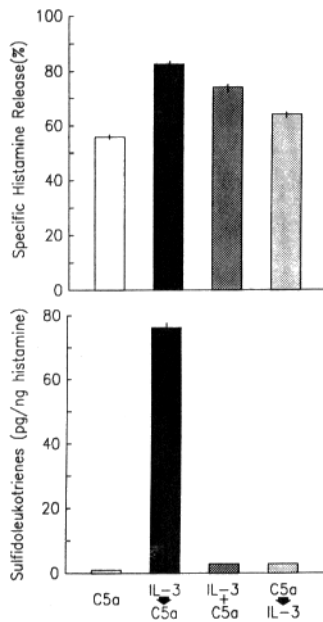

FIGURE 4. Role of sequence of addition of IL-3 and C5a. Cells are incubated as follows: (*Open*) control buffer 10 min, then C5a (10^{-8} M) 20 min. (*Filled*) IL-3 (10 ng/ml) 10 min, then C5a 20 min. (*Hatched*) Control buffer 10 min, then IL-3 and C5a simultaneously for 20 min. (*Dots*) C5a 10 min, then IL-3 20 min. (*Top*) Histamine release after subtraction of spontaneous release (total histamine = 133 ± 1.2 ng/2.5 × 10^6 cells). (*Bottom*) sLT production. The open column (C5a alone) represents the detection limit for sLT. Results of three experiments performed with cells from an atopic donor are shown (mean ± SD). Results of experiments with cells from both atopic and normals were similar, except that in some cases sLT generation was below detection limit for the latter two conditions.
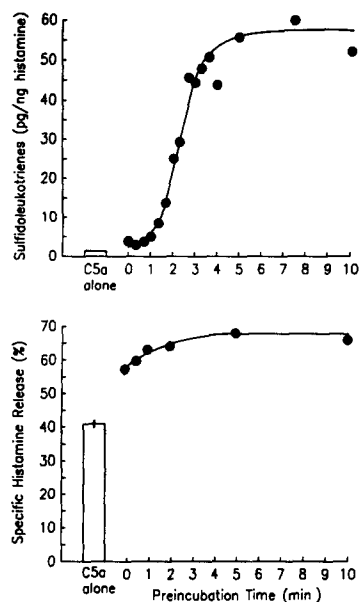
FIGURE 5. Effect of the preincubation time with IL-3 upon C5a-induced mediator release. The cells were exposed to IL-3 (1 nM) and the time before the addition of C5a (10^{-8} M) was varied as indicated in the abscissa. All the cells were incubated during 30 min after addition of IL-3, the only variable being the time between the addition of the two peptides. C5a alone is shown for comparison (columns). (*Top*) sLT generation. Each data point represents a single determination. The column for sLT generation with C5a alone represents the detection limit for sLT. (*Bottom*) Histamine release after subtraction of spontaneous release (total histamine = 124 ± 3.4 ng/2.5 × 10^6 cells). The mean of triplicates is shown.

obtained after dextran sedimentation of erythrocytes were fractionated by centrifugation through Percoll gradients and experiments as described in Fig. 1 were performed. Each leukocyte fraction was examined by FACS using monoclonal markers for T cells, B cells, monocytes, neutrophils, and NK cells and examined by microscopy in May-Grünwald stained cytocentrifuge samples. In addition, the total histamine content of the cell fractions was determined as a marker for basophils. The results of a typical experiment are shown in Table I. A very high amount of sLT was generated by fraction 4, which contained 40% unstained cells by FACS analysis corresponding to 35% basophils by morphological criteria, the remaining contaminating cells being exclusively small lymphocytes (mainly T cells). Fraction 1,

TABLE I

Total Histamine Content and sLT Generation of Leukocytes Fractionated by Percoll Gradient

Cell fractions	Percent monocytes*	Percent neutrophils[‡]	Histamine content[§] (ng/2.5 × 10^6 cells)	sLT[‖] (pg/2.5 × 10^6 cells)
Unfractionated cells	21	50	87	1,264
Fraction				
1 (<1.060)	74	<3	9	<100
2 (1.060/1.065)	36	<3	7	<100
3 (1.065/1.070)	7	<3	102	3,200
4 (1.070/1.075)	<2	<3	1,612	32,100
5 (>1.075)	<2	85	30	137

* Percent of Leu-M3$^+$ cells determined by FACS analysis.
[‡] Percent of neutrophils by FACS analysis (Leu-11a$^+$ in PMN right angle light scatter window).
[§] Histamine content of cell lysate.
[‖] sLT generated by sequential stimulation of leukocytes with IL-3 (1 nM) and C5a (10 nM).

which was highly enriched in monocytes and was contaminated exclusively by large lymphocytes, generated no sLT after stimulation with C5a with or without preincubation with IL-3, indicating that monocytes do not produce sLT under these conditions. Eosinophils, for which no mAb was avaiable, were only found in fraction 5 (7% by microscopy), and did not appear to contribute to the sLT yield. The extent of histamine release induced by C5a with or without IL-3 pretreatment was identical in unfractionated cells and in the different cell fractions (data not shown). Taken together these results indicate that the basophil was the source of sLT generated under the present experimental conditions, and that IL-3 effects the basophil response directly, presumably through specific receptors.

Discussion

This study demonstrates that the cytokine IL-3 at low concentrations profoundly modifies the mediator release profile of basophils stimulated with the anaphylatoxin C5a. IL-3 enhanced the responsiveness of basophils to C5a. An enhancement of histamine release was observed in all atopic or normal donors, while IL-3 alone did not induce degranulation in most cases. More remarkably, IL-3 pretreatment rendered the basophils capable of generating very large quantities of LTC_4 in response to C5a. Since neither IL-3 nor C5a, even at abnormally high concentrations, induced sLT production, it appears that the effect observed is not due to a synergism of threshold effects of two stimuli. Thus, IL-3 not only enhances the basophil response but qualitatively changes the response pattern to a second signal. The quantities of LTC_4 formed were remarkable since they averaged 50 pg LTC_4 per nanogram of cellular histamine content. This means that the few basophils normally present in 1 ml of blood generate nanogram quantities of sLT within a few minutes after triggering of primed cells with C5a. To our knowledge there is no other condition or cell type capable of generating sLT at such a high rate.

Priming for C5a-induced sLT synthesis occurs at low IL-3 concentrations. The ED_{50} of 0.2-0.4 U IL-3/ml is comparable to that required for the stimulation of proliferation of CML leukocytes (1 U is defined as the ED_{50} in this assay). It is remarkable that the priming of mature basophils occurs at 1/100th the concentration of IL-3 that is necessary to induce colony formation in bone marrow cultures. This bioactivity is detectable at 3-10 U/ml and reaches a maximum at 100-300 U/ml (Fagg, B., Sandoz, Switzerland, personal communication). It is therefore conceivable that the priming effect described here is the major physiological function of this cytokine.

Chemotactic factor-induced LTB_4 (14) and platelet-activating factor (PAF) (15) synthesis in neutrophils requires 60-90 min preincubation of the cells with granulocyte/macrophage CSF (GM-CSF). In contrast, C5a-induced sLT synthesis occurs very rapidly after exposure to IL-3, starting at 1 min, with optimal effects at 5 min. This change in responsiveness appears to be long lasting, as it is still apparent 2 h after IL-3 exposure. These observations allow some speculations about the mechanism of priming by IL-3. The rapid onset suggests that IL-3 priming does not involve gene expression and de novo synthesis of a regulatory protein of phospholipase(s)/lipoxygenase activation (22, 23). On the other hand, most known second messengers are formed only transiently after interaction of a ligand with its receptors. They

may be involved in signal transduction to IL-3 binding, but can not explain the maintenance of the primed state. Better candidates for the effect of IL-3 are a rapid but irreversible modification (i.e., phosphorylation), or translocation, of a regulatory protein necessary, but not sufficient for lipid mediator synthesis (24). Obviously, sLT synthesis and degranulation are separately regulated cell responses, but the second messengers responsible for each function remain to be defined. A study about the mechanism by which IL-3 "couples" the C5a response to the lipid mediator synthesis pathway should also give some insight into the regulation of the two cell responses.

Our work is related to two recent independent studies, which also demonstrate an effect of IL-3 upon histamine release (25, 26). From experiments on leukocytes incubated in 50% D_2O, Haak-Frendscho et al. (25) proposed that IL-3 is an histamine-releasing factor. In contrast, our study indicates that IL-3 is devoid of direct releasing activity. Preactivation of basophils in vivo, or e.g., as a consequence of complement activation during blood collection, may explain the borderline release induced by IL-3 alone in some cell batches (Figs. 1 and 2), since a small enhancement of histamine release occurs even when IL-3 is the second signal (Fig. 4). Consistent with our study, Hirai et al. (26) observed no histamine release by IL-3 alone, but an enhancement of degranulation was found after triggering with anti-IgE or FMLP. We also find an enhancement of histamine release induced by different IgE-dependent and -independent basophil agonists, but even for these stimuli, which by themselves are able to induce LTC_4 synthesis, IL-3 affects sLT synthesis much more strongly as compared with the modest increase in degranulation (Kurimoto, Y., et al., manuscript in preparation). Finally, there are several reports implicating mostly undefined cell derived factors in basophil histamine release (27–39). It remains to be better defined, which cytokines other than IL-3 affect basophil mediator release by themselves or in combination with other basophil agonists, and if only degranulation or the generation of lipid mediators, or both, are affected.

There are similarities between the effects of GM-CSF on PMN and of IL-3 on basophils. GM-CSF augments superoxide production in PMN (10, 11) and IL-3 enhances granule release in basophils. Thus, these two factors enhance the normal responses of the mature effector cells. More importantly, we show that they are both required for the induction of lipid mediator synthesis by C5a. This work extends our previous observations (14, 15) to the more general concept, that a major function of hematopoietic growth factors is to "prime" certain mature inflammatory cells for lipid mediator synthesis, after triggering with agonists that by themselves are insufficient to elicit such a response. As already indicated, however, the priming time needed for C5a-induced sLT production is short, and therefore the mechanisms of action of the cytokines in PMN and basophils may be different. The target cell profile of the two cytokines seems also different. There is an abundant recent literature about the effects of GM-CSF upon the function of a variety of mature leukocytes, including neutrophils, monocytes, macrophages, and eosinophils (e.g., 9–12), while almost nothing is known about an effect of IL-3 upon inflammatory effector cells (16). Our cell fractionation studies indicate that the basophil is the source sLT generated by IL-3 and C5a, and that other cell types like monocytes, eosinophils, or neutrophils do not produce sLT under these conditions. Also, IL-3 does not prime neutrophils for chemotactic factor–induced LTB_4 and PAF synthesis (Dahinden, C. A.,

unpublished observations), in contrast to GM-CSF (14, 15). Therefore, IL-3 seems to be relatively basophil specific, at least as far as C5a-induced mediator release is concerned.

In summary, we find that a product of the specific immune system (IL-3) profoundly affects the effector function of mature basophils. The in vivo relevance of this finding remains to be established. Possibly, the lipid mediators generated after triggering of IL-3-primed basophils are of importance in the pathogenesis of immediate-type hypersensitivity reactions, late-phase reactions, shock syndromes, and inflammation, while consequences of the enhancement of degranulation are less certain. The large quantities of sLT formed means that the biological effects of lipid mediators may be about 50 times stronger than that of all the histamines stored in the granules, since sLT are ~1,000 times more potent that histamine on most target tissues (6). Also, IL-3 priming of basophils may not only enhance but even qualitatively change the pathophysiological consequences of complement activation, since the biological effects of sLT and histamine do not totally overlap (6). Finally, the fact that low concentrations of IL-3 are sufficient to modify the basophil response indicates that such an effect could easily take place in the microenvironment of an inflammatory reaction. In contrast, higher concentrations are needed to induce proliferation of leukocyte progenitors, and it is not known if enough endogenous IL-3 can reach the target tissue by the blood stream, or if it can be generated locally in the bone marrow during an immune response. These arguments support the hypothesis that IL-3 is an important component in inflammatory reactions.

Summary

The anaphylatoxin C5a is a potent trigger for basophil degranulations, but in contrast to IgE-dependent basophil activation, it does not result in the synthesis of sulfidoleukotrienes (leukotriene $C_4/D_4/E_4$). Thus, degranulation and the generation of lipid mediators are separately regulated cellular responses. Exposure of human blood basophils to the cytokine IL-3 alone does not induce the release of histamine in cells from most donors and never leads to the generation of LTC_4, indicating that IL-3 is not a direct agonist for basophil mediator release. However, preincubation of basophils with IL-3 enhances the degranulation response to C5a. Most importantly, IL-3 "primes" basophils to release large amounts of leukotriene C_4 after challenge with C5a (mean of 50 gp LTC_4 per nanograms cellular histamine), while neither peptide alone is capable of inducing the formation of bioactive lipids. This effect is dose dependent, occurring at IL-3 concentrations considerably lower than are required to stimulate the growth of bone marrow progenitor cells. IL-3 affects the extent but not the time course of basophil degranulation, and leukotriene release of cells sequentially exposed to IL-3 and C5a occurs very rapidly concomitant with degranulation. A preincubation of the basophils with IL-3 is strictly required for C5a-induced LTC_4 synthesis, but not for an enhancement of degranulation. Priming for C5a-induced lipid mediator generation occurs rapidly after exposure of the cells to IL-3, starting at 1 min and reaching maximal effects at 5 min, but this altered state of responsiveness is relatively long lasting. Cell fractionation studies indicate that the basophil is the source of lipid mediators and that IL-3 affects the basophil response directly. This study demonstrates that IL-3 is a potent modifier

of effector functions of mature basophils; this is possibly of greater in vivo significance than its growth factor properties. The large amounts of LTC4 formed after triggering of IL-3-primed basophils may not only enhance but also qualitatively change the pathophysiological consequences of complement activation, and this might be important in the pathogenesis of immediate type hypersensitivity reactions, shock syndromes, and inflammation.

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