First publ. in: Blood ; 86 (1995), 11. - pp. 4039-4049

IgE-Independent Interleukin-4 Expression and Induction of a Late Phase of Leukotriene C4 Formation in Human Blood Basophils

By Brigitte Ochensberger, Silvia Rihs, Thomas Brunner, and Clemens A. Dahinden

T-helper cells can differentiate into at least two subtypes secreting distinct profiles of cytokines, Th1 and Th2, regulating immunoprotection and different immunopathologies. Interleukin-4 (IL-4) is both the product and the inducer of Th2 cells, raising the question whether IL-4 can be produced in response to antigen-independent stimuli. Here we show that human basophils produce IL-4 on stimulation with IL-3 and C5a or $C5a_{desarg}$ in similar amounts as induced by IgE-receptor–cross-linking. C5a-induced IL-4 production requires the presence of IL-3, with little effect of the sequence of stimuli addition. No "Th1-cytokines" (interferon-γ and IL-2) and even no "Th2-cytokines" (IL-3, IL-5, IL-10, and granulocyte-macrophage colony-stimulating factor) are produced by basophils in response to either IgE-dependent or IgE-independent activation. The generation of leukotriene C_4 (LTC_4) is regulated in a similar manner. However, C5a induces a rapid, transient burst of leukotriene formation only if added after IL-3. Interestingly, upon prolonged culture, a late phase of continuous LTC_4 production is observed, which also requires two signals (IL-3 and C5a), but rather depends on their continuous presence than on their sequence of action. These data describe an antigen-independent pathway of very restricted IL-4 expression. Thus, basophils must be considered as central immunoregulatory cells of the innate immune system. Furthermore, the results show that LTC_4 can also be generated more continuously for many hours, a phenomenon that may be of particular importance in chronic allergic inflammation, such as asthma.

INTERLEUKIN-4 (IL-4) plays an important role in the regulation of differentiation of T and B lymphocytes. In B cells IL-4 provides an essential signal for isotype switching and the production of IgE and IgG1.[1-3] More importantly, the presence of IL-4 is critical in the differentiation of $CD4^+$ T cells toward a phenotype, T helper 2 (Th2), producing a restricted and distinctive pattern of cytokines (eg, IL-4, IL-5, but no interferon-γ [IFN-γ]). More recently IL-4 has also been shown to influence $CD8^+$ cells leading to a T-cell subset of reduced cytotoxicity and a change in cytokine secretion (eg, reduced IFN-γ and increased IL-4 and IL-5).[8,9] This key immunoregulatory role of IL-4 has been demonstrated in vitro as well as in vivo in the murine system, in diverse experimental models.[4-12] For IL-4 to have this effect, it must be present early in a immune response and early during T-cell activation. However, the cellular source of IL-4 in this process is still unclear and has been the subject of considerable debate, because IL-4 is both a product of Th2 cells, but is also needed for the induction of a Th2 type immune response.[4-7]

In contrast to most other cytokines, the expression of IL-4 appears to be restricted to a few specialized cell types.[3] Beside its established production by T lymphocytes, alternative IL-4 sources by non-T cells have received increasing attention. In the murine system, mast cell lines and certain IgE receptor (IgER)-positive non-B non-T cells have been shown to transcribe IL-4 message and, in some cases, also to produce IL-4 upon IgER activation.[13-15] We and others have shown that mature human basophils are capable of producing IL-4 after activation of the IgER in synergy with the hematopoietic growth factor IL-3.[16-20] However, the induction of IL-4 in $IgER^+$ cells depends on cross-linking of cell bound IgE by antigen. No antigen-independent activation pathway for IL-4 production has yet been described in any cell type. Antigen-independent induction of IL-4 synthesis, in the absence of IFN-γ expression, by cells of the innate immune system at an early stage of an inflammatory response may be crucial for the initiation of a Th2 response. The most potent IgE-independent basophil agonist for mediator release are the complement fragment C5a[21-24] and its degradation product $C5a_{desarg}$.[25] We now examined whether and under what conditions C5a/$C5a_{desarg}$ are also capable of inducing the expression of IL-4. Indeed, our results indicate that basophils may not only have an amplifying (by IgE-dependent IL-4 production) but also an initiating role in the development of a Th2 response.

Leukotrienes (LTs) are important inflammatory lipid mediators, formed by 5-lipoxygenase-catalyzed oxidation of free arachidonic acid. The epoxid leukotriene A_4 is further metabolized to leukotriene B_4 or C_4 depending on the cell type and the enzymes they express. In contrast to prostaglandins, leukotrienes are only generated by myeloid leukocytes and mast cells. Neutrophils and monocytes produce LTB_4, a chemoattractant, whereas mast cells, basophils, and eosinophils generate LTC_4, a mediator inducing smooth muscle contraction and an increase in vascular permeability.[26] The production of leukotrienes can be induced by cross-linking of Ig receptors, particularly IgE receptors of mast cells and basophils.[26-28] However, leukotrienes are involved in many inflammatory conditions without the participation of Ig-antigen complexes. Particular high levels of leukotriene C_4 are found in the exudate of allergic late phase reactions and at chronic inflammatory sites in allergic or "intrinsic" types of asthma.[26,29] Thus, antigen-independent pathways for leukotriene formation in response to soluble endogenous stimuli

From the Institute of Immunology and Allergology, Inselspital, Bern, Switzerland.

Submitted September 1, 1995; accepted September 14, 1995.

Supported in part by the Swiss National Science Foundation (Grant No. 3100-041906.94).

Address reprint requests to Clemens A. Dahinden, MD, Institute of Immunology and Allergology, University Hospital, Inselspital, CH-3010 Bern, Switzerland.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

must exist in vivo. Yet, a physiologic mode of leukocyte activation could not be found for years, until it was discovered that two sequential signals are required for leukotriene formation.[21-30] However, leukotrienes are always formed in a very rapid and transient burst that is rather unlikely to occur in chronic inflammatory processes. In our study of IgE-independent IL-4 expression we observed that LTC_4 can also be produced more slowly and continuously for many hours, reaching very high levels of this important lipid mediator.

MATERIALS AND METHODS

Reagents and media. Reagents used were HEPES (Calbiochem-Behring Corp, La Jolla, CA); EDTA (Fluka AG, Buchs, Switzerland); Percoll and dextran (Pharmacia, Uppsala, Sweden); and bovine serum albumin fatty acid-free (BSA; Boehringer Mannheim Inc, Mannheim, Germany). All other reagents were of the highest purity available. HA buffer contained 20 mmol/L HEPES, 125 mmol/L NaCl, 5 mmol/L KCl, and 0.5 mmol/L glucose and 0.25 mg/mL BSA. Culture medium was RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 25 mmol/L HEPES, 100 U/mL penicillin, and 100 mg/mL streptomycin, 2 mmol/L nonessential amino acids, and 2 mmol/L L-glutamine (GIBCO, Paisley, Scotland).

Preparation of basophils and mononuclear cells. Highly purified basophils and control mononuclear cells were prepared as previously described.[16] Briefly, blood of unselected healthy volunteers was anticoagulated with 10 mmol/L EDTA and mixed with 0.25 vol of 6% dextran in 0.9% NaCl, and erythrocytes were allowed to sediment at room temperature (RT). Leukocytes were pelleted by centrifugation (150*g*, 20 minutes RT) and separated over three-step discontinuous Percoll gradients (1.0795/1.070/1.065 g/mL isotonic Percoll solution, respectively) at 400*g* for 30 minutes at RT. Basophil-enriched interphases between the densities 1.0795 and 1.070, and for control experiments the upper cell layers, containing lymphocytes, monocytes, and generally less than 0.5% basophils (MNC), were obtained and used in parallel under identical experimental conditions. Basophil-enriched fractions, containing 10% to 40% basophils (with lymphocytes and variable proportions of neutrophils), were washed twice in HA buffer and then further purified by negative selection using antibody-coated paramagnetic beads (MACS system; Miltenyi Biotec, Bergisch Gladbach, Germany). Basophil purity was examined by Giemsa-stained cytospin smears, and generally ranged between 70% and 95%, the contaminating cells consisting mainly of small lymphocytes and occasionally few monocytes.

In some experiments basophils enriched to 30% to 40% by Percoll gradients, the contaminating cell population consisting almost exclusively of lymphocytes (<1% monocytes, <5% neutrophils), was directly used for culture without negative selection. Basophils were also purified to near homogeneity (<0.5% lymphocytes, monocytes, neutrophils, eosinophils) by incubation with a cocktail of IgG1 monoclonal antibodies (MoAbs) (αCD2, αCD3, αCD4, αCD8, αCD14, αCD16, αCD19, αCD20, αCD21, and αCD56) and negative selection with rat-antimouse IgG1 paramagnetic beads.

Culture conditions. Basophil preparations, and MNC from the same blood specimen (cultured in parallel for comparison), were resuspended at a cell density of 1.0×10^6 cells/mL in culture medium, incubated in sterile round-bottom 96-well microtiter plates (100 μL/well) (Becton Dickinson, Lincoln Park, NJ) at 37°C in a humidified atmosphere with 5% CO_2. Reagents were added at a 1:100 vol:vol ratio. After the time indicated, cell-free supernatants were procured and stored at −70°C until measurements of cytokine production by enzyme-linked immunosorbent assay (ELISA) and sulfidoleukotriene synthesis by radioimmunoassay (RIA).

Measurement of cytokines and leukotriene C4/D4/E4. IL-4 and IL-3 was measured with an ELISA-assay (kindly provided by Sandoz, Vienna, Austria) as described.[31] Both assays had a sensitivity of 10 pg/mL with a dynamic range up to 1 ng/mL. In some experiments, IL-4 was also measured using the kit supplied by Genzyme Corp (Cambridge, MA), according to manufacturer's protocols or by an ELISA using anti–IL-4 MoAb pairs obtained by Pharmingen (San Diego, CA) with identical results. IL-2, IL-5, IL-10, and granulocyte-macrophage colony-stimulating factor (GM-CSF) were measured using antibody pairs from Pharmingen (San Diego). IFN-γ was measured with ELISA (detection limit 30 pg/mL) as previously described.[32] Sulfidoleukotrienes were determined in an RIA-assay as described.[23]

Cell stimuli. rhIL-3 was a kind gift of Dr M. Schreier (Sandoz, Basel, Switzerland).[21] The complement products C5a and $C5a_{desarg}$ were purified from yeast-activated human serum as previously described,[21,25,33] and were found to be homogenous as determined by amino acid analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and microzone paper electrophoresis at pH 8.6. Purified MoAb 29C6 (αIgER), directed against the non–IgE-binding epitope of the high-affinity IgER α-chain (FcϵRI), was a generous gift from Drs J. Hakimi and R. Chizzonite (Hoffmann-La Roche, Nutley, NJ).[34]

Statistical analysis. Statistical analysis was performed using linear regression analysis. Differences associated with probability values of $P < .05$ were considered significant.

RESULTS

C5a or $C5a_{desarg}$ induce IL-4 synthesis in mature human basophils cultured with IL-3. It is now well established that human basophils produce IL-4 in response to IgER activation in synergy with IL-3.[16-20] We now examined whether the complement product C5a is also capable of inducing IL-4 expression, under the experimental conditions used in our previous study.[16] Figure 1 shows that the IgE-independent chemotactic agonist C5a promotes IL-4 production in cells cultured with IL-3 in amounts overall similar to that induced by IgER cross-linking, but to variable degrees depending on the donor. Stimulation of basophils cultured in medium alone with C5a does not lead to IL-4 production. Lipid mediator formation is regulated in a similar manner with an absolute requirement of IL-3 for C5a-induced LTC_4 generation (Fig 1). A logarithmic scale was used to better visualize the fact that C5a strongly enhanced IL-4 generation consistently over that induced by IL-3 alone and that IL-4 was always detected after C5a stimulation of cells cultured with IL-3, even in experiments in which IL-3 alone was an ineffective stimulus. In vivo the half-life and, therefore, the radius of action of C5a within an inflammatory site is very short due to rapid cleavage of the C-terminal arginine by carboxypeptidase(s) resulting in the generation of $C5a_{desarg}$. $C5a_{desarg}$ is still a potent neutrophil chemotaxin, but has lost most of the anaphylactic and cell-activating properties of C5a. Consistent with our recent finding that $C5a_{desarg}$ retains its capacity to induce basophil mediator release,[25] $C5a_{desarg}$ also promotes IL-4 generation with an efficacy identical to C5a (Fig 1), but with somewhat lower potency at a molar basis (Fig 2). Priming for C5a-induced IL-4 expression occurs over the same concentration range of IL-3 as reported for the enhancement of the IgE-dependent response, reaching optimal effects at 1 to 10 ng/mL (data not shown). Contami-

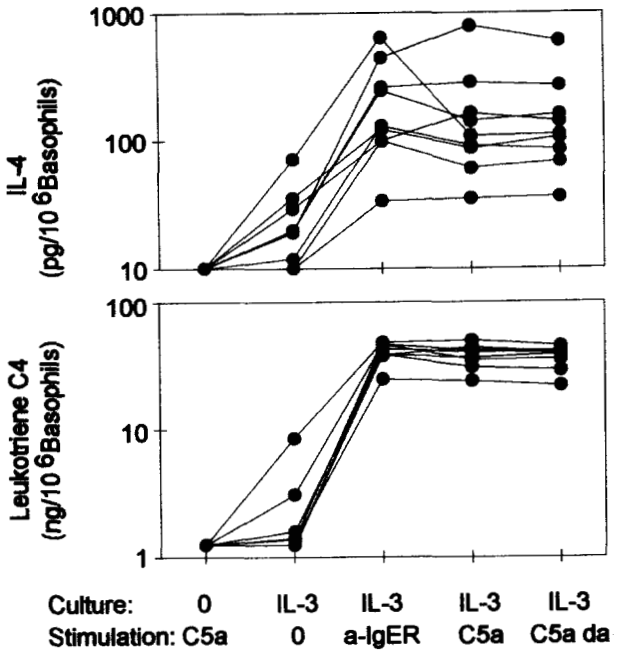

Fig 1. IL-4 and LTC_4 production of human basophils cultured with or without IL-3. Purified basophils from different donors were cultured during 18 hours with or without 10 ng/mL IL-3 before addition of buffer control, αIgER (100 ng/mL), C5a (10 nmol/L) or $C5a_{desarg}$ (100 nmol/L) and further cultured for 10 hours. The top panel shows IL-4 synthesis, the bottom shows LTC_4 generation. Values obtained with cells from the same donor are connected by lines. Mean values of duplicates from nine different experiments are shown. Note the logarithmic scale.

nating lymphocytes do not appear to influence IL-4 release by basophils, because (1) there is no correlation between IL-4 release and the degree of lymphocyte contamination, and (2) basophils purified to near homogeneity (<0.5 lymphocytes, monocytes, neutrophils, eosinophils) and basophils purified (30% to 40%) without negative selection produce identical amounts of IL-4 in response to IL-3 and C5a (data not shown). No IL-4 is detected in parallel experiments with control MNC depleted of basophils (data not shown).

Effect of time interval between IL-3 and C5a for IL-4 and LTC_4 production. To investigate to what extent the time of exposure to IL-3 influences the responsiveness of the cells to C5a, the time interval between maximally effective concentrations of IL-3 and C5a was varied between 10 minutes and 18 hours. Figure 3 shows that neither LTC_4 generation nor IL-4 expression is influenced in an important manner by the preincubation time with IL-3.

Two signals are required for antigen-independent IL-4 production. Basophils are capable of expressing IL-4 in response to IgER activation alone, although IL-4 production is more consistent and pronounced after culture with IL-3.[16] Our further studies showed that, under optimal isolation conditions, freshly isolated unprimed basophils can produce IL-4 in response to IgER cross-linking in variable amounts, depending on the blood donor. Although undetectable in cells of some donors, IL-4 release can be quite substantial and reach up to 800 pg IL-4 per million basophils (unpublished observations, January 1994), consistent with data from a study with basophil enriched mixed leukocyte cultures.[19] Therefore, we examined the response of freshly isolated basophils to C5a stimulation with and without pretreatment with IL-3 for 15 minutes in cells isolated from several donors differing in their responsiveness to IgER stimulation. From the data shown in Fig 4 it is evident that even cells from donors which produce relatively high levels of IL-4 in response to αIgER alone, IL-4 release upon stimulation with C5a is undetectable or minimal. However, after preincubation with IL-3 for 15 minutes, large amounts of IL-4 are produced in response to both IgE-dependent and IgE-independent stimulation. These data also demonstrate that after a short preincubation with IL-3, αIgER-induced IL-4 generation is enhanced in nearly all experiments, with a mean enhancement of approximately twofold. The formation of LTC_4 measured in the same cell supernatants follows a similar pattern.

Correlations between IgE-dependent and IgE-independent induction of IL-4 and of LTC_4 release. The data shown in Fig 4 indicate that basophils from donors with a particularly high responsiveness for the production of IL-4 upon combined stimulation with IL-3 and C5a do not necessarily produce high levels of IL-4 in response to αIgER stimulation, and vice versa. To investigate whether the marked donor variability of IL-4 and LTC_4 release is due to a difference

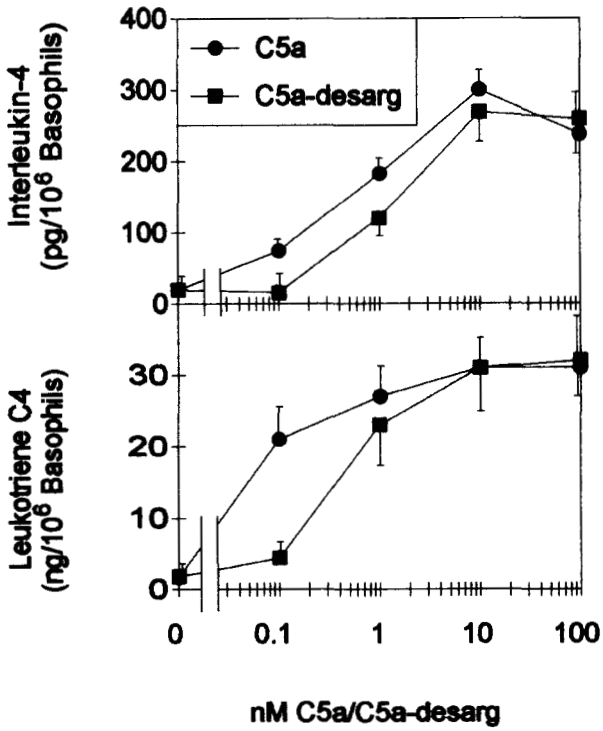

Fig 2. Dose response of C5a- and $C5a_{desarg}$-induced IL-4 synthesis. Basophils were incubated for 18 hours with 10 ng/mL IL-3 before exposure to either control buffer (effect of IL-3 alone) or to increasing concentrations of C5a (●) and $C5a_{desarg}$ (■), respectively, for 10 hours. IL-4, top panel; LTC_4, bottom panel. Mean values ± SEM; n = 6.

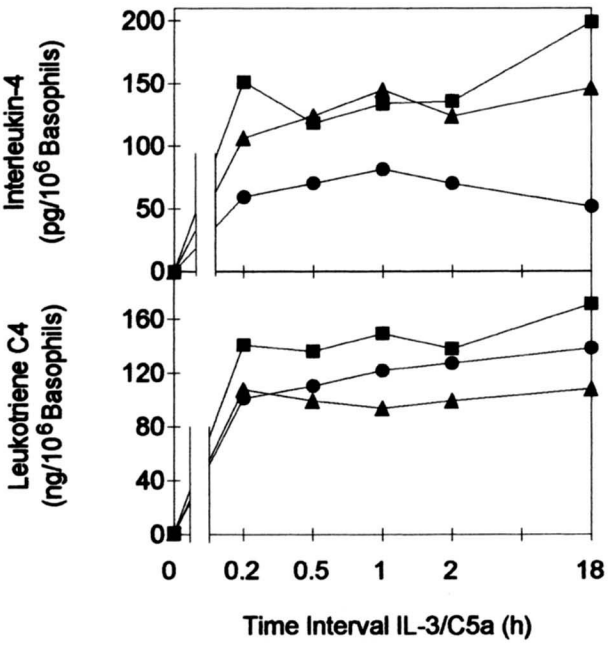

Fig 3. Effect of the time of preexposure with IL-3 on the C5a-induced IL-4. Purified basophils were primed with IL-3 (10 ng/mL) for different times indicated in the X-axis, and then stimulated with C5a (10^{-8} mol/L) for 18 hours. Basophils pretreated with IL-3 during 18 hours before C5a addition were cultured during 28 hours. 0, Basophils stimulated with C5a alone. Kinetic studies showed that under all conditions IL-4 release was complete and optimal. The top panel shows the IL-4 synthesis (picograms/10^6 basophils), the bottom shows LTC_4 production (nanograms/10^6 basophils). Three independent experiments (mean of duplicate determination) are shown.

in the general capacity of the basophils to express this cytokine or to produce lipid mediator, respectively, or rather to a different responsiveness of the basophils toward the stimuli used, we examined the correlations of product generation after different modes of activation. A significant correlation is found for both IL-4 and LTC_4 generation in response to IgER cross-linking of untreated versus IL-3 primed cells. The correlation is particularly strong for LTC_4 release but weaker for IL-4 expression (Fig 5). By contrast, IL-4 produced in response to IgE-dependent versus IgE-independent stimulation does not correlate at all (Fig 5, Table 1). Correlations of LTC_4 generation between the different modes of activation are generally higher than those of IL-4 expression. Our previous study and the present study have shown that IL-3, at least in the basophils of certain donors, can by itself promote IL-4 production. It was most interesting to find that IL-4 release in response to IL-3 alone is highly correlated to that induced by stimulation of IL-3 primed cells with C5a, but not to that observed after IgE-dependent activation (αIgER alone or αIgER after IL-3 priming) (Table 1). This may indicate that the donor variability of IgE-independent IL-4 expression (IL-3 or IL-3/C5a) could be caused by differences in the cellular responsiveness to IL-3, although the lack of correlation for IL-4 production between IL-3/C5a versus IL-3/αIgER and the strong correlation of αIgER versus IL-3/αIgER argues against this hypothesis. Alternatively, a component of the FCS used in the culture medium may provide a necessary second signal, such as some bovine $C5a_{desarg}$ formed during clotting.

Correlation between IL-4 expression and LTC_4 generation. Stimulation conditions leading to IL-4 expression consistently promote LTC_4 generation. However, although the correlation between IL-4 and LTC_4 release upon stimulation with IL-3 and C5a reached statistical significance, the association is weak and the cells from donors secreting large amounts of IL-4 are not necessary the most efficient in producing LTC_4 and vice versa (Table 1, see also Fig 6). Similarly, no correlation between cytokine expression and lipid mediator formation is found after stimulating the cells by IgER cross-linking regardless of whether or not cells have been pretreated with IL-3. Thus, the donor variability of IL-4 release in response to either IgE-dependent or IgE-independent activation cannot be simply explained by a variability of the general responsiveness of the cells toward the stimuli used.

Requirement for the persistence of the stimuli inducing IL-4 and LTC_4 production. Mediator release is a very rapid process[21-25,27] whereas IL-4 production occurs more slowly,[16-20] regardless of the mode of activation. Thus, we examined whether one of the two signals, or both, have to be present for prolonged periods of time to promote IgE-independent IL-4 expression. For this purpose, basophils exposed to IL-3 and C5a were washed after an incubation period of 30 minutes, and recultured with medium with or without addition of one or of the two stimuli for further 18 hours. Figure 6 shows, that both IL-3 and C5a must be continuously present for optimal IL-4 secretion. During the initial stimulation of 30 minutes, large amounts of

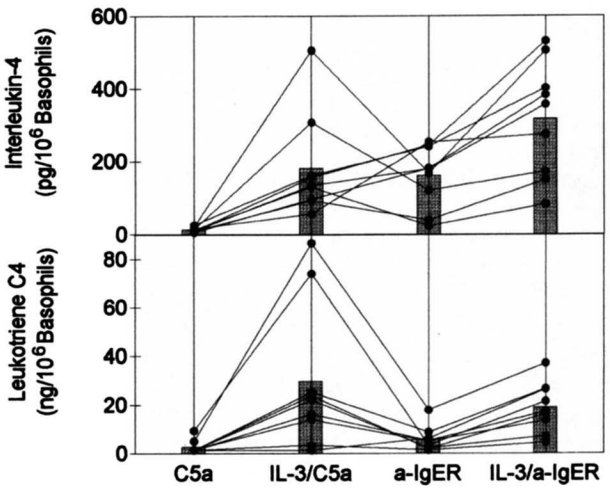

Fig 4. IL-4 and LTC_4 synthesis by freshly isolated human basophils in response to IgE-dependent or IgE-independent activation. Basophils purified from nine different donors were preincubated with buffer or IL-3 (10 ng/mL) for 15 minutes followed by a stimulation with either C5a (10^{-8} mol/L) or αIgER (100 ng/mL) for 18 hours. The top panel shows IL-4 synthesis, the bottom shows the LTC_4 generation. Each data point represents the mean value of duplicates or triplicates. The data from each of nine different experiments with cells from distinct donors are connected by lines. Columns represent the mean values of all experiments.

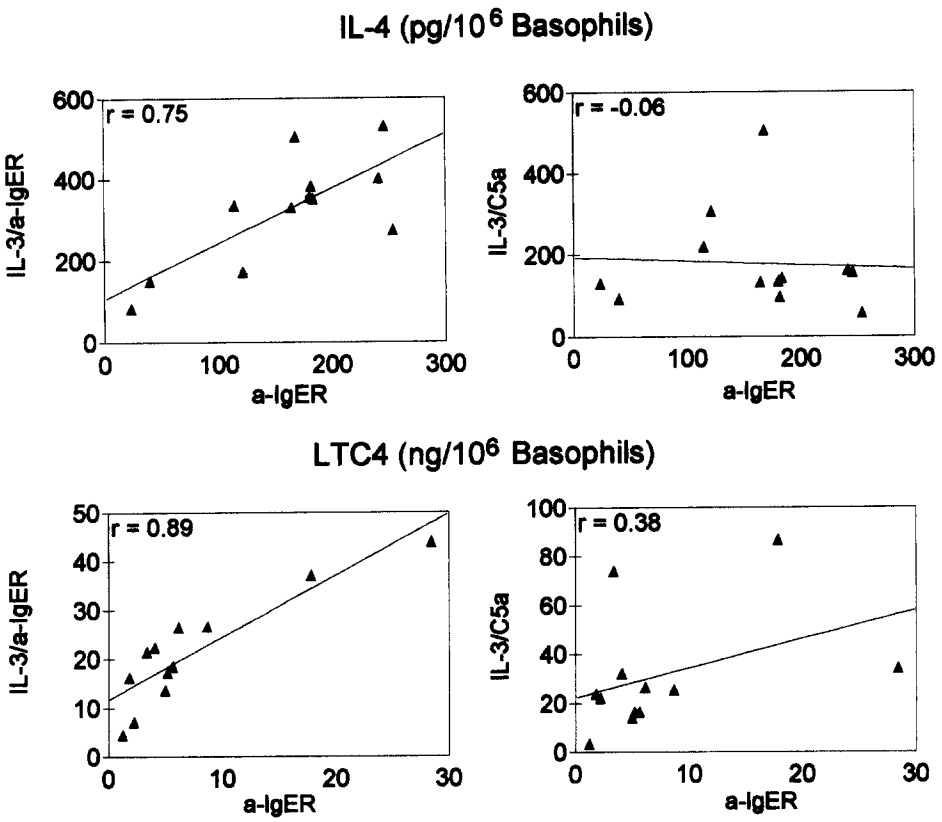

Fig 5. Correlations of IL-4 and LTC_4 formation in response to different modes of activation. The figure shows the correlations of the products released by basophils in response to αIgER versus IL-3 + αIgER (left panels) or to αIgER versus IL-3 + C5a (right panels). (Top) Correlations of the IL-4 expression. (Bottom) LTC_4 formation. Curve fit was done by linear regression analysis. Each symbol represents a datapair of the mean of duplicates or triplicates from one separate experiment. All experiments were performed with basophils purified from different donors. The experimental conditions were as in Fig 4.

Table 1. Correlations of IL-4 and of LTC_4 Formation Between Different Modes of Activation (A) and Correlations Between IL-4 and LTC_4 Production (B)

	IL-4			LTC_4		
Stimulation	*r*	n	*P*	*r*	n	*P*
A.						
IL-3 *v* IL-3/C5a	.91	25	<.0001	ND		
IL-3 *v* αIgER	.65	13	.015	ND		
IL-3 *v* IL-3/αIgER	.23	16	.386	ND		
IL-3/C5a *v* αIgER	−.06	12	.857	.38	12	.217
IL-3/C5a *v* IL-3/αIgER	.36	15	.184	.67	15	.006
αIgER *v* IL-3/αIgER	.75	12	.005	.89	12	.0001

	IL-4 versus LTC_4		
Stimulation	*r*	n	*P*
B.			
IL-3/C5a	.45	25	.023
0/αIgER	.0013	12	.997
IL-3/αIgER	.11	16	.698

Individual data pairs are the mean of duplicate or triplicate determinations and were derived from seperate experiments. Experimental conditions were as described in Figure legends 4 and 5.

Abbreviations: r, correlation coefficient, calculated by linear regression analysis; n, number of experiments with basophils from different unselected donors; *P*, probability; ND, not determined because in many experiments LTC_4 release in response to IL-3 alone was not detected.

LTC_4 are formed, consistent with our previous studies,[21,25] whereas IL-4 release is minimal. Most interestingly, during further culture for 18 hours, a second phase of pronounced lipid mediator release was observed under conditions of optimal IL-4 expression. For this late phase of LTC_4 generation the re-addition of both stimuli is required. For comparison, a similar experimental set-up was adapted using αIgER as a trigger. Again LTC_4 is formed very rapidly as compared to IL-4 expression. In contrast to antigen-independent activation, the basophils efficiently produce IL-4 after washing and culturing the cells with medium alone, possibly because of irreversible IgER cross-linking by the antibody. However, IL-3 has to be present for an optimal response. No second phase of LTC_4 generation during the time of IL-4 release is observed, in contrast to cells cultured with IL-3 and C5a.

Importance of the sequence of the two signals. Several previous studies have clearly shown that the rapid burst of antigen-independent lipid mediator formation is critically dependent on the sequential action of an appropriate priming cytokine followed by triggering with a chemotactic agonist.[21-25,30] The need for a more prolonged presence of IL-3 and C5a for cytokine expression could indicate that IL-4 production is regulated differently. Indeed, Fig 7 shows that IL-4 is also produced when IL-3 and C5a are added in a reversed order, in amounts that are even significantly higher. During culture of 18 hours the basophils also produce large amounts of LTC_4 even if C5a is added 15 minutes before IL-3, but not in response to C5a alone.

Time course of IL-4 and LTC_4 production. IL-4 release

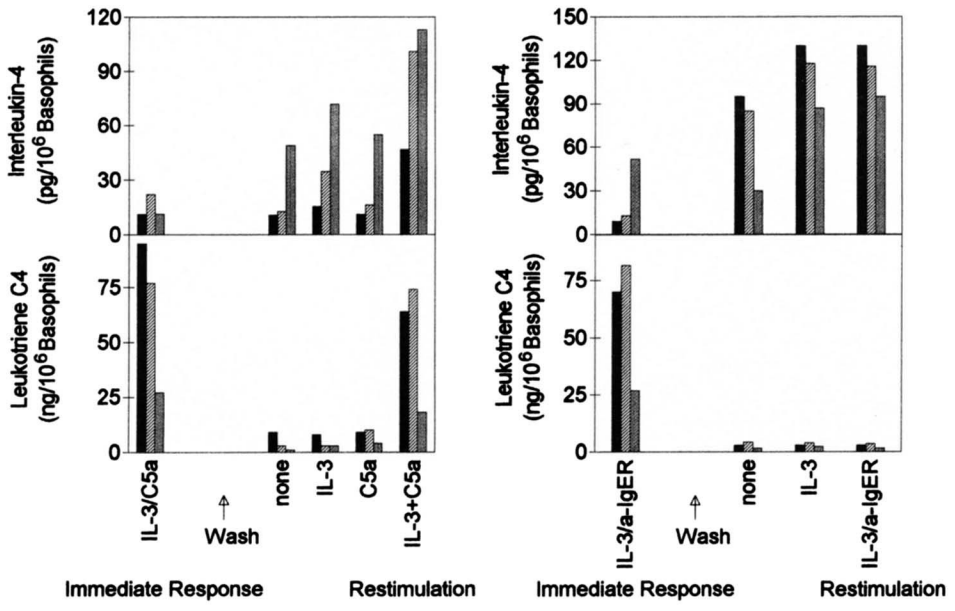

Fig 6. Requirement for the persistence of the stimuli. Basophils were stimulated with IL-3 (10 ng/mL) and C5a (10^{-8} mol/L) or αIgER (100 ng/mL), respectively. After 30 minutes the supernatant was obtained (Immediate Response) and the cells were washed with medium and exposed to the stimuli indicated in the figure for further 18 hours (Restimulation). Each column represents the mean value of duplicates, three experiments are shown (presented with different shadings).

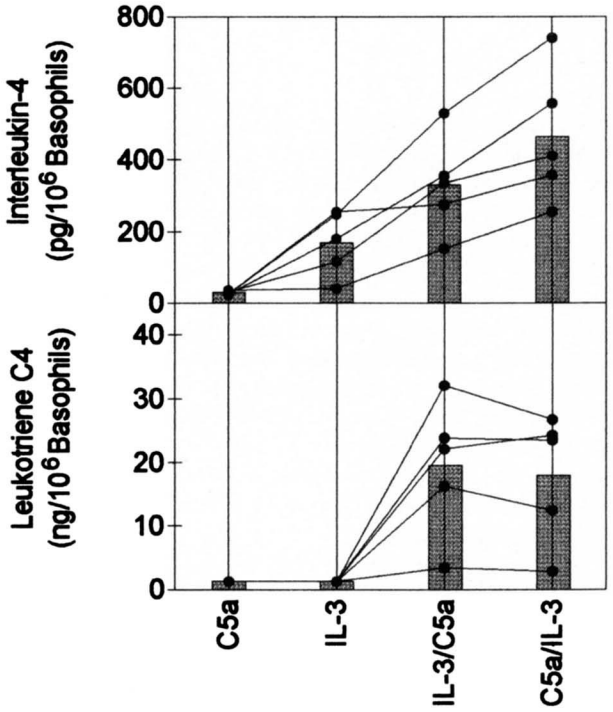

Fig 7. IL-4 and LTC_4 production by basophils in response to C5a and IL-3. Effect of the sequence of stimuli addition. Freshly purified basophils from five different donors were stimulated with C5a (10^{-8} mol/L), IL-3 (10 ng/mL), or both. IL-3 was added 15 minutes before (IL-3/C5a) or after (C5a/IL-3) C5a. IL-4 (top) and LTC_4 (bottom) were measured in the cell-free supernatant after 18 hours of culture. The data from the same donor (mean of duplicates indicated by circles) are connected by lines. The mean value of five experiments is shown in columns.

is induced rapidly and continues during 16 hours after stimulation, with identical kinetics regardless of the sequence of IL-3 and C5a addition (Fig 8). However, marked differences in the kinetics of LTC_4 production are observed depending on whether C5a is added before or after IL-3. In contrast to the rapid burst of LTC_4 formation triggered by C5a in IL-3 primed cells,[21,25] no LTC_4 is formed early after stimulation if the order of the stimuli is inversed. After 2 hours, however, and in parallel with IL-4 release, large amounts of LTC_4 are continuously generated, resulting in levels comparable to those after C5a stimulation of primed cells (Fig 8). The kinetics of LTC_4 accumulation in the supernatant of IL-3 primed basophils exposed to C5a may be explained by some degradation of sulfido-leukotrienes during the first 4 hours after the rapid burst which lasts only a few minutes.[21,25] The second phase of LTC_4 increase may reflect a balance between leukotriene production and degradation, a hypothesis consistent with the data shown in Fig 6.

Effect of the sequence of the two signals upon the dose response of C5a. Figure 9 shows that about one order of magnitude higher concentrations of C5a are needed for LTC_4 production if C5a is added before IL-3, as determined by measurements of LTC_4 after culture of 18 hours. This difference is almost certainly due to the fact that lower amounts of C5a are needed for the rapid burst of mediator formation by IL-3 primed cells[21,25,27] compared with the second, late phase of LTC_4 generation accompanying IL-4 expression. The dose response of IL-4 release was affected to a lesser extent by the order of the stimuli (Fig 9).

Restricted expression of IL-4 by human basophils. We have previously shown that basophils cultured with IL-3 and stimulated by IgER cross-linking produce IL-4 without detectable amounts of the "Th1 cytokines," IL-2 and IFN-γ. Thus, human basophils may represent a kind of innate Th2-like cells. We now determined whether basophils can also produce other cytokines expressed by T-helper 2

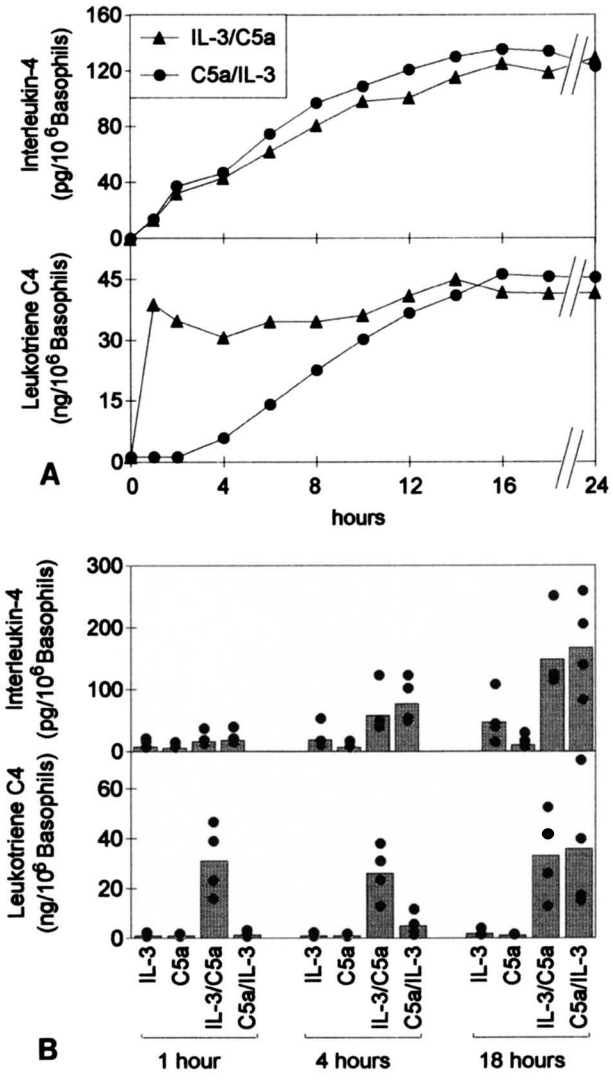

Fig 8. Time course of IgE-independent IL-4 and LTC_4 production. Effect of the sequence of IL-3 and C5a. (A) Basophils were stimulated with C5a (10^{-8} mol/L) added 15 minutes before (●) or after (▲) IL-3 (10 ng/mL) and the supernatant was obtained at different times indicated in the X-axis. One representative experiment out of three is shown. In all experiments, the time course of product release was virtually identical, but the amount of IL-4 and LTC_4 differed markedly. (B) Basophils were exposed to IL-3 (10 ng/mL) C5a (10^{-8} mol/L), or both. C5a was added either 15 minutes before (C5a/IL-3) or after (IL-3/C5a) IL-3. Supernatants were obtained 1, 4, and 18 hours after stimulation. Four experiments ([●] mean of triplicates) and the mean of all the data (columns) are shown. No IL-4 and LTC_4 were detected in basophils cultured in medium alone.

clones,[4-7] and whether the cytokine profile may depend on the mode of activation of the cells. For this purpose, larger amounts of supernatants from basophils stimulated with C5a or αIgER with or without pretreatment with IL-3 were produced in several experiments with cells from different donors. Table 2 shows that no Th1 cytokines IL-2 and IFN-γ can be detected, regardless of the mode of activation and the amount of IL-4 produced. Even more interesting is the finding that basophils stimulated by either an IgE-dependent

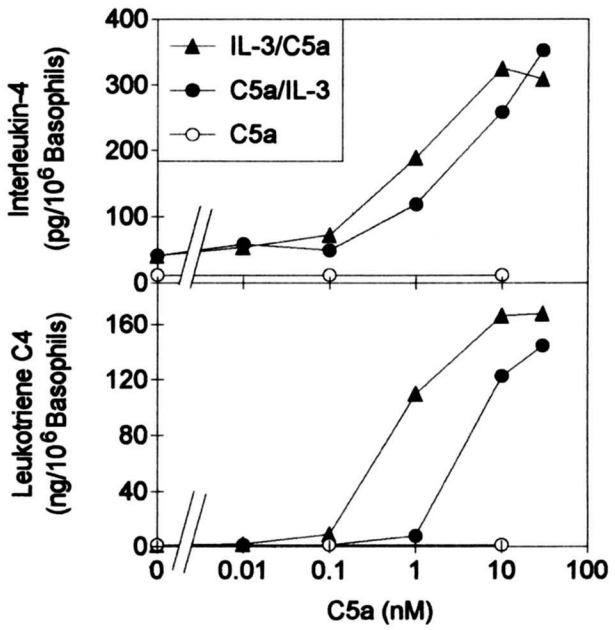

Fig 9. Dose response of C5a-induced IL-4 and LTC_4 synthesis added before or after IL-3. Purified human basophils were stimulated with different concentrations of C5a with (closed symbols) or without (open symbols) IL-3 (10 ng/mL) given either 15 minutes before (●) or after (▲) C5a. Each point represents the mean value of triplicates from one representative experiment out of three.

or IgE-independent mechanism are unable to produce any detectable amounts of GM-CSF, IL-3, IL-5, and IL-10 (Table 2). Therefore, it appears that basophils express IL-4 in a very restricted manner and that the nature of the stimuli does not influence the cytokine profile produced, at least under the experimental conditions of this study.

Table 2. Cytokine Profile of Stimulated Human Basophils

	Th 2 Cytokines					Th 1 Cytokines	
	IL-4	GM-CSF	IL-3	IL-5	IL-10	IL-2	γIFN
C5a	15	<30	<10	<10	<30	<20	<30
	10	<30	<10	<10	<30	<20	<30
	0	<30	<10	<10	<30	<20	<30
IL-3/C5a	506	<30	ND	<10	<30	<20	<30
	520	<30	ND	<10	<30	<20	<30
	322	<30	ND	<10	<30	<20	<30
αIgER	594	<30	<10	<10	<30	<20	<30
	440	<30	<10	<10	<30	<20	<30
	0	<30	<10	<10	<30	<20	<30
IL-3/αIgER	850	<30	ND	<10	<30	<20	<30
	604	<30	ND	<10	<30	<20	<30
	372	<30	ND	<10	<30	<20	<30

Purified basophils stimulated with C5a (10^{-8} mmol/L) or αIgER (100 ng/mL) with or without a preincubation with IL-3 (10 ng/mL) for 15 minutes were cultured for 18 hours. The different cytokines were measured in the cell-free supernatants. The data (mean of duplicates) of three representative experiments are shown. All data are in pg/ml.

Abbreviation: ND, not determined.

DISCUSSION

Protective immunity of the host to different pathogens and immune-mediated diseases does not only depend on the recognition of antigens but also on the activation of distinct effector systems. The differentiation of T-helper cells, and possibly also $CD8^+$ cells, into at least two subsets, "Th1" and "Th2," producing distinct sets of cytokines, leads to the different types of immune responses.[4-9] There is increasing evidence that cytokines themselves determine whether an immune response will be dominated by Th1 or Th2 cells.[4-12,35,36] The importance of the innate immune system in this process has been clearly demonstrated for the induction of a Th1 response (IL-12 production by macrophages with the participation of natural killer [NK] cells, IFN-γ, and tumor necrosis factor-α [TNF-α]).[7,11,35,36] How a Th2 response is initiated is more controversial because IL-4 is both the inducer and the product.[7-12] The capacity of basophils to produce large amounts of IL-4 in response to IgE-independent activation, as shown here, now provides a potential mechanism for the induction of a Th2 type immune response. Other mechanisms regulating T-cell differentiation into Th2 that have recently been proposed in the murine system are the type of costimulatory molecules on antigen-presenting cells[37] and the activation of a T-cell subpopulation of $CD4^+$, $NK1.1^+$ phenotype.[38] However, it is rather unlikely that the skewing of the immune response toward Th1 or Th2 is under all conditions controlled by the same mechanism or cell type.

The present study shows that basophils produce large amounts of IL-4 in response to the combination of low concentrations of IL-3 and C5a or $C5a_{desarg}$. The time interval between IL-3 and C5a addition is not critical for the degree of IL-4 expression. However, the amount of the product released varies strongly, raising the question of whether the isolation procedure of this study may have affected the function of this rare cell type. We do not believe this to be the case because the variability was only seen between cells from different donors and not between separate experiments with cells from the same donor (data not shown). The lack of correlation for IL-4 formation in response to IgE-dependent versus IgE-independent stimulation shown here also argues against an isolation-artefact, because this is expected to affect the basophil responsiveness in general. Nevertheless, the isolation of basophils to high purity and with preserved functionality remains a problem, and this may explain some discrepant findings in the literature with regard to the optimal conditions for IgE-dependent IL-4 production.[16,18-20]

In marked contrast to IgE receptor activation, the presence of IL-3 is essential for C5a-induced IL-4 production. Thus, IL-4 production seems to be regulated in a manner similar to lipid mediator formation. Although IgER activation is by itself sufficient to promote LTC_4 formation, two sequential signals are necessary for all IgE-independent endogenous agonists.[21-25] The first is provided by a growth factor interacting with a tyrosine kinase associated receptor[21-25,39] and the second by a chemotactic agonist interacting with a G-protein coupled receptor leading to phospholipase C (PLC) activation and a transient elevation of intracellular calcium.[39-42] This has been found to be a basic principle for triggering leukotriene formation not only in basophils but in other myeloid cells as well.[43-45] Although the rapid and transient burst of LTC_4 formation strictly depends on the priming cytokine preceding the triggering agent,[30] no such dependence of the sequence of the stimuli was found with regard to IL-4 expression. In fact, even somewhat larger amounts of IL-4 are produced when the sequence of the two stimuli is reversed, at least at higher C5a concentrations. Furthermore, both stimuli need to be present for longer periods of time, as shown by removing the agonists from the medium. IL-4 release is induced rapidly upon C5a addition and continues for further 16 hours, in contrast to the response after IgER activation, which is complete within 4 hours under all experimental conditions.[16,18] This difference in the duration of IL-4 release may be one of the reasons why in a previous study IL-4 could in general not be detected in response to IgE-independent stimuli, because IL-4 was measured 4 hours after cell activation.[19]

In some experiments, basophils also produced IL-4 upon culture with IL-3 without further stimulation. At present it is unclear whether IL-3 alone is sufficient or whether two signals are always required to induce IL-4 release for an IgE-independent response. The very strong correlation between IL-3 versus IL-3 + C5a induced IL-4 release may indicate that a second signal acting similarly to C5a is provided by the serum in the medium. Indeed, we found that under serum-free culture only the combination of IL-3 and C5a, but not IL-3 alone, promoted IL-4 expression, even in cells of donors responding well to IL-3. However, these results are preliminary because we could not yet find serum-free culture conditions allowing IL-4 expression in amounts comparable to those induced in media containing FCS.

Most interesting and unexpected was the finding that IgE-independent IL-4 production was accompanied by a sustained generation of LTs that accumulate in large amounts after 18 hours of culture. In contrast to the rapid burst observed after triggering primed cells, which is completed in a few minutes, this late phase is, like the IL-4 expression, independent of the sequence of the stimuli but requires their more continuous presence. This observation represents a novel pathway of LT production by endogenous agonists. In contrast to prostaglandins, which can be generated slowly and continuously,[46] only a rapid and transient burst of LT release has been described up to now in all cell types in response to any effective stimuli.[16-28,30,39-45] The slow phase of LTC_4 formation described here may be of much greater pathophysiologic importance than the rapid burst requiring stimulation of primed myeloid effector cells by a bolus of chemotactic agonist, hitherto the only available model for lipid mediator formation in response to endogenous agonists. LTs are found to accumulate in allergic late-phase reactions in amounts even exceeding that formed during the immediate reaction, presumably through the action of host-derived factors on infiltrating leukocytes.[26,29] The importance of LTs in this process is now supported by the increasing evidence for the efficacy of LT antagonists in the treatment of chronic allergic inflammation such as asthma.[29] However, it is unlikely that in vivo LTC_4 is formed only in a rapid and transient burst. Furthermore, both the chemotactic agonist C5a

and the cytokine IL-3 may rather be generated continuously and concomitantly at inflammatory sites.

Although LTC_4 can be produced in the absence of IL-4 expression (unpublished observation, October 1993), all conditions leading to IL-4 release also induce lipid mediator formation. Mitogen activated protein (MAP) kinase was recently proposed to regulate cytosolic PLA_2 activity, and thus activation of MAP kinase may be a necessary common step in regulating gene expression and mediator formation.[47,48] It will also be interesting to examine whether the signaling mechanisms differ for the rapid and the slow phase of LTC_4 generation. In this respect it is intriguing that both $cPLA_2$ and the 5-lipoxygenase, in contrast to the cyclooxygenase, require high concentrations of calcium for activity and that chemotactic agonists are thought to induce only a rapid and transient elevation of intracellular free calcium.[45]

The marked donor variability of IL-4 release cannot be explained solely by differences in the general capacity of basophils to express IL-4. This observation may be related to the complexity in the genetic predisposition for allergic disease. Differences in the responsiveness of basophils to IL-3, C5a, IgER cross-linking, and in the corresponding signal transduction pathways may be separately inherited components. The lack of correlation of IL-4 release induced by IgE-independent and IgE-dependent activation could also indicate that initiation and amplification of Th2 type immune responses are separately regulated. In this regard it is interesting that up to now two gene loci involved in atopy have been proposed: one was localized to the cytokine gene cluster on chromosome 5 and the other to the of the β-chain gene of the high affinity IgER.[49-51]

Basophils appear to produce IL-4 in a very exclusive manner, because no IL-2, IFN-γ, IL-3, IL-5, IL-10, and GM-CSF could be detected in supernatants containing large amounts of IL-4, regardless of the mode of activation. Thus, the cytokine profile of basophils is even more restricted than that of Th2 lymphocytes, which produce also IL-3, IL-5, IL-10, and GM-CSF.[4-7] Furthermore, IL-5, for example, is generally secreted in much larger amounts than IL-4 by Th2 cells. "Th2 type cytokines" such as IL-5, IL-10, and, in particular, GM-CSF, are also produced by a number of cell types incapable of producing IL-4, whereas IL-4 expression appears to be more restricted (T cells and $IgER^+$ cells). Thus, IL-4 expression in the absence of GM-CSF and IL-5 was a rather unexpected finding, indicating that IL-4 expression in basophils and T cells is regulated differently.[52]

By the capacity to produce large amounts of IL-4 in a very restricted manner, basophils could play a prominent immunoregulatory role not shared by other cell types. Basophils differ from human mast cells, which express other cytokines such as TNF-α, IL-5, and IL-6.[53,54] To which extent human mast cells are capable of secreting IL-4 is, however, still controversial,[55] in contrast to the capacity of basophils to produce large amounts of this cytokine, which has now been confirmed by independent groups.[16-20] Therefore, we propose the hypothesis that in the human system mast cells play mainly a proinflammatory role by secreting cytokines such as TNF-α, IL-5, and chemokines, whereas basophils are the primary $IgER^+$ immunoregulatory cells.

The role of complement as an important humoral effector arm in host defense of the innate immune system and in inflammation is well established.[56] However, whether complement is also involved in immunoregulation of the adaptive immune system is less clear. This study indicates that, depending on the microenvironment in the presence of IL-3 and basophils, complement activation can lead to IL-4 synthesis with all its possible implications for the regulation of the immune system. The requirement of IL-3 for antigen-independent IL-4 expression in response to $C5a/C5a_{desarg}$ suggests that this cytokine may play a yet unexplored important role in the development of a Th2 response. In addition, IL-3 is a growth and differentiation factor of basophils in humans and promotes mast cell hyperplasia in the murine system, expanding the pool of $IgER^+$ cells capable of expressing IL-4. Thus, IL-3 may act very early in a Th2 response, similar to the recently proposed role of IL-12 in initiating cell-mediated immunity.[36]

In conclusion, we find that basophils secrete large amounts of IL-4 in a very restricted manner under a variety of experimental conditions by antigen-dependent as well as antigen-independent activation, implying a key immunoregulatory function of this effector cell of the innate immune system. Thus, basophils may represent a kind of counterpart of NK cells that generate IFN-γ but no IL-4. Furthermore, the data provide a novel IgE-independent pathway of continuous and prolonged lipid mediator formation that may be of particular relevance in the pathogenesis of chronic allergic processes.

ACKNOWLEDGMENT

We express our sincerest thanks to J. Engeli-Zingg and A. Urwyler for technical assistance. We also thank Drs F. Kalthoff and E. Liehl (Sandoz, Vienna, Austria), Dr H. Galati (Hoffmann-La Roche, Basel, Switzerland), and Drs J. Hakimi and R. Chizzonite (Hoffmann-La Roche, Nutley, NJ) for providing us with MoAbs.

REFERENCES

1. Del Prete G, Maggi E, Parronchi P, Chretien I, Tiri A, Macchia D, Ricci M, Banchereau J, de Vries JE, Romagnani S: IL-4 is an essential factor in the IgE synthesis induced in vitro by human T cell clones and their supernatants. J Immunol 140:4193, 1988

2. Péne J, Chrétien I, Rousset F, Brière F, Bonnefoy YF, Spits H, Yakota Y, Arai N, Banchereau J, de Vries J: IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferon γ and α and prostaglandin E2. Proc Natl Acad Sci USA 85:6880, 1988

3. Paul WE: Interleukin-4: A prototypic immunoregulatory lymphokine. Blood 77:1859, 1991

4. Mosmann TR, Coffman RL: TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 7:145, 1989

5. Kapsenberg ML, Jansen HM, Bos JD, Wierenga EA: Role of type 1 and type 2 T helper cells in allergic diseases. Curr Opin Immunol 4:788, 1992

6. Romagnani S: Lymphokine production by human T cells in disease states. Annu Rev Immunol 12:227, 1994

7. Seder RA, Paul WE: Acquisition of lymphokine-producing phenotype by $CD4^+$ T cells. Annu Rev Immunol 12:635, 1994

8. Croft M, Carter L, Swain SL, Dutton RW: Generation of polarised antigen-specific CD8 effector populations: reciprocal action of interleukin (IL)-4 and IL-12 in promoting type 2 versus type 1 cytokine profiles. J Exp Med 180:1715, 1994

9. Seder RA, Le Gros GG: The functional role of $CD8^+$ T helper type 2 cells. J Exp Med 181:5, 1995

10. Swain SL, Weinberg AD, English M, Huston G: IL-4 directs the development of different subsets of helper T cells. J Immunol 145:3796, 1990

11. Maggi E, Parronchi P, Manetti R, Simonelli C, Piccinni MP, Santoni Rugiu F, de Carli M, Ricci M, Romagnani S: Reciprocal regulatory effects of IFN-γ and IL-4 on the in vitro development of human Th1 and Th2 clones. J Immunol 148:2142, 1992

12. Kopf M, Le Gros G, Bachmann M, Lamers MC, Bluethmann H, Köhler G: Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature 362:245, 1993

13. Brown MA, Pierce JH, Watson CJ, Falco J, Ihle JN, Paul WE: B cell stimulatory factor-1/interleukin-4 mRNA is expressed by normal and transformed mast cells. Cell 50:809, 1987

14. Ben-Sasson SZ, Le Gros G, Conrad DH, Finkelman FD, Paul WE: Crosslinking Fc receptors stimulates splenic non-B, non-T cells to secrete IL-4 and other lymphokines. Proc Natl Acad Sci USA 88:8656, 1990

15. Galli SJ, Gordon JR, Wershil BK: Cytokine production by mast cells and basophils. Curr Opin Immunol 3:865, 1991

16. Brunner T, Heusser CH, Dahinden CA: Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. J Exp Med 177:605, 1993

17. Arock M, Merle-Béral H, Dugas B, Ouaaz F, Le Goff L, Vouldoukis I, Mencia-Huerta JM, Schmitt C, Leblond-Missenard V, Debre P, Mossalayi MD: IL-4 release by human leukemic and activated normal basophils. J Immunol 151:1441, 1993

18. MacGlashan DW Jr, White JM, Huang SK, Ono SJ, Schroeder JT, Lichtenstein LM: Secretion of IL-4 from human basophils. J Immunol 152:3006, 1994

19. Schroeder JT, MacGlashan DW Jr, Kagey-Sobotka A, White JM, Lichtenstein LM: IgE-dependent IL-4 secretion by human basophils. J Immunol 153:1808, 1994

20. Mueller R, Heusser CH, Rihs S, Brunner T, Bullock GR, Dahinden CA: Immunolocalisation of intracellular interleukin-4 in normal human peripheral blood basophils. Eur J Immunol 24:2935, 1994

21. Kurimoto Y, de Weck AL, Dahinden CA: Interleukin-3 dependent mediator release in basophils triggered by C5a. J Exp Med 170:467, 1989

22. Bischoff SC, Brunner T, de Weck AL, Dahinden CA: Interleukin 5 modifies histamine release and leukotriene generation by human basophils in response to diverse agonists. J Exp Med 172:1577, 1990

23. Bischoff SC, Dahinden CA: Effect of nerve growth factor on the release of inflammatory mediators by mature human basophils. Blood 79:2662, 1992

24. MacGlashan DW Jr, Hubbard WC: IL-3 alters free arachidonic acid generation in C5a stimulated human basophils. J Immunol 151:6358, 1993

25. Bürgi B, Brunner T, Dahinden CA: The degradation product of the anaphylatoxin $C5a_{desarg}$ retains basophil-activating properties. Eur J Immunol 24:1583, 1994

26. Lewis RA, Austen KF, Soberman RJ: Leukotrienes and other products of the 5-lipoxygenase pathway. Biochemistry and relation to pathobiology in human diseases. N Engl J Med 323:645, 1990

27. Kurimoto Y, de Weck AL, Dahinden CA: The effect of interleukin 3 upon IgE-dependent and IgE-independent basophil degranulation and leukotriene generation. Eur J Immunol 21:361, 1991

28. Bischoff SC, Dahinden CA: C-kit ligand: A unique potentiator of mediator release by human lung mast cells. J Exp Med 175:237, 1992

29. Israel E: Moderating the inflammation of asthma: Inhibiting the production or action of products of the 5-lipoxygenase pathway. Ann Allergy 72:279, 1994

30. Dahinden CA, Bischoff SC, Brunner T, Krieger M, Taktfuji S, de Weck AL: Regulation of mediator release by human basophils: Importance of the sequence and time of addition in the combined action of different agonists. Int Arch Allergy Appl Immunol 94:161, 1991

31. Van Reijsen FC, Bruijnzeel-Koomen CAFM, Kalthoff FS, Maggi E, Romagnani S, Westland JKT: Skin-derived aeroallergen-specific T-cell clones of Th2 phenotype in patients with atopic dermatitis. J Allergy Clin Immunol 90:184, 1992

32. Gallati H: Interferon. Wesentlich vereinfachte, enzymimmunologische Bestimmung mit zwei monoklonalen Antikörpern. J Clin Chem Clin Biochem 20:907, 1982

33. Hugli TE, Gerard C, Kawahara M, Scheetz ME II, Barton R, Briggs S, Koppel G, Russell S: Isolation of three separate anaphylatoxins from complement-activated human serum. Mol Cell Biochem 41:59, 1981

34. Riske F, Hakimi J, Mallamaci M, Griffin M, Pilson B, Tobkes N, Lin P, Danho W, Kochan J, Chizzonite R: High affinity human IgE receptor (FcϵRI). J Biol Chem 266:11245, 1991

35. Manetti R, Gerosa F, Giudizi MG, Biagiotti R, Parronchi P, Piccini MP, Sampognaro S, Maggi E, Romagnani S, Trinchieri G: Interleukin 12 induces interferon γ production during differentiation of human T helper cells and transient IFN-γ production in established Th2 cells clones. J Exp Med 179:1273, 1994

36. Trinchieri G: Interleukin-12 and its role in the generation of TH1 cells. Immunol Today 14:335, 1993

37. Kuchroo VK, Das MP, Brown JA, Ranger AM, Zamvil SS, Sobel RA, Weiner HL, Nabavi N, Glimcher H: B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. Cell 80:707, 1995

38. Yoshimoto T, Paul WE: $CD4^{pos}$, $NK1.1^{pos}$ T cells promptly produce interleukin 4 in response to in vivo challenge with anti-CD3. J Exp Med 179:1285, 1994

39. Krieger M, von Tscharner V, Dahinden CA: Signal transduction for interleukin 3 dependent leukotriene synthesis in normal human basophils: Opposing role of tyrosine kinase and protein kinase C. Eur J Immunol 22:2907, 1992

40. Bischoff SC, Krieger M, Brunner T, Dahinden CA: Monocyte chemotactic protein-1 is a potent activator of human basophils. J Exp Med 175:1271, 1992

41. Bischoff SC, Krieger M, Brunner T, Rot A, von Tscharner V, Baggiolini M, Dahinden CA: RANTES and related chemokines activate human basophil granulocytes through different G protein coupled receptors. J Exp Med 177:605, 1993

42. Dahinden CA, Geisser T, Brunner T, von Tscharner V, Caput D, Ferrara P, Minty A, Baggiolini M: Monocyte chemotactic protein 3 is a most effective basophil- and eosinophil-activating chemokine. J Exp Med 179:751, 1994

43. Dahinden CA, Zingg J, Maly FE, de Weck AL: Leukotriene production in human neutrophils primed by recombinant human granulocyte/macrophage colony-stimulating factor and stimulated with the complement component C5a and fMLP as second signals. J Exp Med 167:1281, 1988

44. Takafuji S, Bischoff SC, de Weck AL, Dahinden CA: Interleukin 3 and interleukin 5 prime normal human eosinophils to produce leukotriene C4 in response to soluble agonists. J Immunol 147:3855, 1991

45. Dahinden CA: Regulation of leukotriene production by cytokines, in Dahlén SE (ed): Advances in Prostaglandin, Thromboxane, and Leukotriene Research. New York, NY, Raven, 1994, p 327

46. Murakami M, Matsumoto R, Austen KF, Arm JP: Prostaglandin endeoperoxide synthase-1 and -2 couple to different transmem-

brane stimuli to generate prostaglandin D2 in mouse bone marrow-derived mast cells. J Biol Chem 269:22269, 1994

47. Nemenoff, RA, Winitz S, Qian NX, Van Putten V, Johnson GL, Heasley LE: Phosphorylation and activation of a high molecular weight form of phospholipase A_2 by p42 microtubule-associated protein 2 kinase and protein kinase C. J Biol Chem 268:1960, 1993

48. Lin LL, Wartmann M, Lin AY, Knopf JL, Seth A, Davis RJ: $cPLA_2$ is phosphorylated and activated by MAP kinase. Cell 72:269, 1993

49. Standford AJ, Shirakawa T, Moffatt MF, Daniels SE, Ra C, Faux JA, Young RP, Nakamura Y, Lanthrop GM, Cookson WOCM, Hopkin JM: Localisation of atopy and β subunit of high-affinity IgE receptor (FcϵRI) on chromosome 11q. Lancet 341:332, 1993

50. Hizawa N, Yamaguchi E, Ohe M, Itoh K, Furuya K, Ohnuma N, Kawakami Y: Lack of linkage between atopy and locus 11q13. Clin Exp Allergy 22:1065, 1992

51. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Friedhoff LR, Ehrlich-Kautzky E, Schou C, Krishnaswamy G, Beaty TH: Linkage analysis of IL-4 and other chromosomal 5q 31.1 markers and total serum immunoglobulin E concentrations. Science 264:1152, 1994

52. Henkel G, Brown MA: PU.1 and GATA: Components of a mast cell-specific interleukin 4 intronic enhancer. Proc Natl Acad Sci USA 91:7737, 1994

53. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, Heusser CH, Howarth PH, Holgate ST: Interleukin-4, -5, -6 and tumor necrosis factor-alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. Am J Respir Cell Mol Biol 10:471, 1994

54. Church MK, Okayama Y, Bradding P: The role of the mast cell in acute and chronic allergic inflammation. Ann NY Acad Sci 725:69, 1994

55. Bradding P, Feather IH, Howarth PH, Mueller R, Roberts JA, Britten K, Bews JPA, Hunt TG, Okayama Y, Heusser CH: Interleukin 4 is localized to and released by human mast cells. J Exp Med 176:1381, 1992

56. Frank MM, Fries LF: The role of complement in inflammation and phagocytosis. Immunol Today 12:322, 1991