Human Blood Basophils Produce Interleukin-13 in Response to IgE-Receptor–Dependent and –Independent Activation

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Interleukin-13 (IL-13) is a recently discovered immunoregulatory cytokine. The cellular sources of IL-13 and the regulation of its expression are largely unknown. Here we show that human basophils produce IL-13 in response to IgE-receptor (IgER) crosslinking, IL-3, IL-3 plus CSa, but not CSa alone. Human basophils express IL-13 in a restricted manner since, apart from IL-4, no other cytokines encoded on the cytokine gene cluster (IL-3, IL-5, and granulocyte macrophage-colony-stimulating factor [GM-CSF]), are induced. Highest levels of IL-13 are formed after IgE-independent activation leading to a prolonged secretion of IL-13. The response to IgER-crosslinking is more transient preferentially inducing IL-4. IL-3 is a unique cytokine regulating IL-13 production by human basophils: Among a large number of cytokines tested, only IL-3 is capable of directly inducing IL-13 expression. Furthermore, although some IL-13 is produced in response to CSa in the presence of IL-5, GM-CSF, IGF-1 or IL-1β, IL-3 is by far the most effective. IL-13 production was blocked by actinomycin D and cycloheximide and conditions leading to IL-13 release also lead to the induction of IL-13 mRNA. This study supports an important immunoregulatory role of human blood basophils, owing to their capacity to simultaneously express IL-13 and IL-4 in a restricted manner.

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Reagents and media. HEPES was from Calbiochem-Behring Corp (La Jolla, CA); EDTA was from Fluka AG (Buchs, Switzerland); Pepsin and dextran were from Pharmacia (Uppsala, Sweden); fatty acid-free bovine serum albumin (BSA) was from Boehringer Mannheim Inc (Mannheim, Germany); cycloheximide and actinomycin D were from Sigma Immunochemicals (St Louis, MO); all other reagents were of the highest purity available. HA buffer contained 20 mmol/L HEPES, 125 mmol/L NaCl, 5 mol/L KCl, and 0.5 mmol/L glucose and 0.25 mg/mL BSA. Culture medium was...
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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 (200 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 harvested and stored at −70°C until measurements of cytokine production by ELISA and sulfidoleukotriene synthesis by RIA.

Measurement of cytokines and leukotriene C4/D4/E4. IL-4 and IL-13 were measured with specific sandwich ELISA’s using two noncompetitive antibody pairs, the detecting antibody being biotinylated, followed by streptavidine alkaline phosphatase conjugate. Anti–IL-4 MoAb were provided by Sandoz (Vienna, Austria) or obtained by Pharmingen (San Diego, CA) with identical results. Anti–IL-13 MoAb pairs and IL-13 standards were from Diaclone (Besançon, France). Both assays had a sensitivity of 10 to 30 pg/mL with a dynamic range of up to 1 ng/mL. IL-13 standard curves were also generated using CHO derived purified recombinant human IL-13 (rhIL-13) kindly provided by Dr A. Minty (Sanoﬁ Research, France). The standard curves for the two standards were parallel in the dose response, but differed in that 1,000 pg/mL of the standard from A. Minty corresponds to 683 ± 24 (SD) pg/mL of the standard from Diaclone. All the data shown are from measurements using the standard from Diaclone, which would have to be multiplied by a factor of 1.46 in order to be normalized to the standard provided by Dr A. Minty. All the other cytokines were measured using either antibody pairs from Pharmingen or kits provided by R&D (Minneapolis, MN) as described. Sulfidoleukotrienes were determined in a radioimmunoassay (RIA).

Isolation of cellular RNA. Basophils and mononuclear cells were isolated and stimulated, as described above and then washed three times in 20 mL HEPES, 125 mM NaCl, 5 mM KCl, and 0.5 mM/L glucose. The total cellular RNA (cell RNA) was extracted with TRIzol Reagent (Life Technologies, Gaithersburg, MD) following the manufacturer’s protocol.

Synthesis of internal standard RNA. cRNA was prepared from the plasmid pQB-3, kindly provided by Dr D. Shire, after linearization with EcoRI (Boehringer) by transcription with T7 RNA polymerase. The transcription reaction contained 0.5 μg of linearized plasmid DNA template, 500 μmol/L of each NTPs (Perkin Elmer, Branchburg, NJ), 16 U RNasin (Perkin Elmer), and 10 U of T7 polymerase (Promega, Madison, WI) in 40 mM/L Tris-HCl, pH 7.9, 10 mM/L NaCl, 10 mM/L dithiothreitol, 6 mM/L MgCl_2, 2 mM/L spermidine, and 50 μmol/L EDTA. The reaction was carried out in a final volume of 20 μL at 37°C for 1 hour. The template DNA was degraded using 1 U of RNase-free DNase (Promega) at 37°C for 15 min. The cRNA was purified by phenol/chloroform extraction and precipitated with ethanol.

Oligonucleotides. The following primers were a kind gift of Dr D. Shire: β2–microglobulin sense primer, 5′CCACGAGAGAATGGAAAATGCTC3′; β2–microglobulin antisense primer, 5′GATGCTGCTCTACATGTCGG3′; IL-4 sense primer, 5′TGCTCTGAGAGAACAACACTG3′; IL-4 antisense primer, 5′AACACTCTCTGTTGGCTC3′; IL-13 sense primer, 5′TGCAATGGGGCAAGGATG3′; IL-13 antisense primer, 5′GCAGGCTTCTTAAACCTG3′.

Semi-quantitative competitive RT-PCR. RT-PCR was carried out with the GeneAmp RNA PCR Kit (Perkin Elmer NJ) using all the solutions provided by the kit and following the manufacturer’s protocol. cDNA was synthesized in a solution (3.5 μL/PCR reaction) containing approximately 50 ng total cellular RNA, 100 pg standard RNA, 5 mM/L MgCl_2, 1X PCR buffer II, 1 mM/L NTPs, 1 U/μL RNase inhibitor, and 2.5 μL/buffer Oligo d(T)_12. The total reaction volumes and the amounts of the internal standard RNA and the cellular RNA were multiplied by the numbers of PCR reactions to

Fig. 1. IL-13 production by human blood basophils. After a warm-up period of 15 minutes basophils were cultured in medium alone (C); or exposed to αgER MoAb 29C6 (αgER) (100 ng/mL); IL-3 (10 ng/mL); C5a (10–8 mol/L); or IL-3 followed 15 minutes later by C5a. IL-13 was measured in the supernatant after an optimal culture period of 18 hours. The mean values of 12 independent experiments performed in duplicates with basophils from different donors are shown in columns (range of all experiments in brackets).
be performed. The samples were heated to 70°C for 5 minutes and cooled to 4°C before adding 2.5 U/µL MuLV reverse transcriptase. After a 15-minute incubation at 42°C, samples were quickly chilled on ice. The cDNA was split into equal aliquots and DNA amplification was carried out after adding a solution containing 2 mmol/L MgCl₂, 1× PCR buffer II, and 2.5 U/100 µL AmpliTaq DNA Polymerase. A total of 100 ng of appropriate sense and antisense primer pairs was added to a final volume of 50 µL and the DNA was amplified in 30 sequential cycles at 95°C for 15 seconds and 60°C for 15 seconds with a Perkin Gene Amp PCR System 9600 thermal cycler. The reaction was completed at 60°C for 7 minutes before cooling to 4°C. All amplification products were analyzed on a 2.5% agarose gel (NuSieve 3:1, FMC, Rockland, ME) containing 10 µg/mL ethidium bromide in Tris acetat EDTA buffer.

Cell stimuli. The complement product C5a was purified from yeast-activated human serum as previously described, and was found to be homogenous as determined by amino acid analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and microzone paper electrophoresis at pH 8.6.³⁰ Purified MoAb 29C6 (αIGER), directed against the non-α binding epitope of the high-affinity IGER α-chain (FcεRI), was a generous gift from Drs Hakimi and Chizzonite, Hoffmann-La Roche (Nutley, NJ).⁴⁰ rhIL-3 and rhGM-CSF was provided by Sandor (Basel, Switzerland and Vienna, Austria)⁷ and rhFN-γ by Hoffmann-La Roche (Basel, Switzerland). rhTNFα was a kind gift from Dr G.R. Adolf (Ernst-Bohringer Institut, Vienna, Austria); insulin-like growth factor 1 (IGF1) was kindly provided by Dr J. Zapf, University Hospital Zürich (Switzerland) and hIL-10 by Dr J. de Vries, DNAX (Palo Alto, CA); IL-2, IL-5, and IL-6 were obtained from Amersham (Buckinghamshire, UK) and IL-1β, IL-9, IL-10, and IL-12 from R&D.

**RESULTS**

Human blood basophils produce IL-13 in response to IGER-dependent as well as IgE-independent activation. Recent studies have shown that basophils are a prominent source of IL-4 implying that there is an important immunoregulatory function of this effector cell type.¹⁰⁻¹⁵ IL-4 synthesis and secretion can be induced in basophils by crosslinking their high affinity IGER with antibodies against the IGER,¹⁰ against (receptor bound) IgE,¹²,²³ or in cells of sensitized individuals, with the appropriate antigen (our unpublished observation). IL-4 expression is also regulated by IL-3, the major hematopoietic growth factor for the basophil lineage, which also acts on mature basophils. IL-3 does not, in general, directly activate basophils, but enhances their responsiveness toward different stimuli. In the presence of IL-3 chemotaxis and exocytosis are enhanced and large amounts of leukotrienes are formed in response to several IgE-independent agonists, which by themselves cannot promote lipid mediator formation. IL-3 is an ineffective or only very weak stimulus of IL-4 expression, but enhances the response to IGER activation. More importantly, in the presence of IL-3 the complement product C5a induces IL-4 secretion at levels similar to that observed after IGER activation providing a first antigen independent pathway for the expression of this key immunoregulatory cytokine.

Figure 1 shows that under experimental conditions previously found optimal for IL-4 expression,³⁵ basophils also produce IL-13. Particularly high levels of IL-13 are secreted on IGER-independent activation in response to combined stimulation with IL-3 and C5a, but not at all in response to C5a alone. Furthermore, culture of basophils with IL-3 in the absence of a second signal resulted in IL-13 levels even exceeding those induced by IGER crosslinking. In the experiments shown in Fig 1, IL-3 was added 15 minutes before stimulation with C5a. However, if the order of the stimuli (IL-3 plus C5a) was inverted, IL-13 expression was equally effective (data not shown), as previously found for IL-4 production.³⁵ No IL-13 was detected in supernatants of the mononuclear cell preparations depleted of basophils, which were cultured in parallel under identical experimental conditions (data not shown). IL-4 and IL-13 was derived exclusively from blood basophils, without the participation of mononuclear cells, since the amounts of IL-4 or IL-13 produced and expressed per basophil numbers were not affected by the purity of the basophil preparation, and since basophils purified to near homogeneity (<1% lymphocytes or mono-
cytes) efficiently expressed both IL-13 and IL-4 (data not shown). Thus, all the data including experiments with less pure cell preparation (minimally 50% basophil purity), were used for data analysis in order not to bias results for donors with higher basophil counts for which high purity of basophils is achieved more consistently.

IL-13 appears to be expressed in a very restricted fashion since no (<30 pg/mL) IL-2, IL-3, IL-5, IL-10, GM-CSF, and IFN-γ was detected in the same cell supernatants, consistent with previous observations. Furthermore, in supernatants of basophil preparations purified to near homogeneity (<1% lymphocytes, monocytes) IL-6 and TNFα was also not detectable (<30 pg/mL).

Time course of IgE-dependent and IgE-independent cytokine expression by basophils. While IL-13 release in response to IgE crosslinking was quite transient and complete within 4 hours, cells exposed to IL-3 alone or IL-3 plus C5a continuously generated IL-13 for up to 18 hours. Thus the time-course of IgE-dependent and IgE-independent IL-13 expression (Fig 2, upper panel) is quite similar to that found for IL-4 generation (Fig 2, lower panel), with the exception of generally only marginal IL-4 expression in response to IL-3 alone. When the cells were cultured for additional 24 to 36 hours we sometimes observed a further continuing release of IL-13, but not of IL-4 (in two of ten experiments) in response to IL-3 or IL-3 and C5a. Overall, these results were, however, not statistically significant. IL-13 was formed by de novo synthesis after both, IgE-dependent as well as IgE-independent activation, since the production of IL-13 (but not of LTC4, measured in parallel) was totally blocked by 10 minutes preincubation with the transcription inhibitor actinomycin D (10 μg/mL) or the translation inhibitor cycloheximid (10 μg/mL) (data not shown).

Dose response of the agonists for inducing cytokine expression. Because of the marked induction of IL-13 production in response to IgE-independent stimulation the dose range of IL-3 required for IL-13 expression was examined. Figure 3 shows that IL-13 and IL-4 are both induced dose dependently at IL-3 concentrations between 0.1 to 10 ng/mL. Optimal effects were reached at ≥10 ng/mL IL-3 regardless of whether C5a was present. The results also demonstrate that the synergistic stimulation with IL-3 and C5a did not lead to a higher sensitivity to low IL-3 concentrations but resulted rather in an enhancement of IL-13 release, and that high concentrations of IL-3 could not mimic the effect of the combination of the two stimuli. Priming for C5a-induced LTC4 formation occurs over a similar concentration range of IL-3 (Fig 4, lower panel). Experiments in which the concentration of C5a was varied in the presence of an optimally effective IL-3 concentration of 10 ng/mL revealed that IL-13 release was enhanced concentration dependently with a threshold of 0.1 nmol/L C5a and a plateau reached at ≥10 nmol/L C5a, as recently reported for IL-4 expression. Dose response studies with the αILER MoAb in the absence or the presence of IL-3 showed that again IL-13 was co-ordinately expressed with IL-4 reaching a maximal response at ~10 ng/mL αILER MoAb and that supraoptimal concentrations of the antibody did not result in a decrease of cytokine expression (data not shown). Thus, no bell-shaped dose response curves are observed when using αILER MoAb for IgE activation, as reported for IL-4 production in response to polyclonal anti-IgE antibodies. Particularly prominent induction of IL-13 expression after IgE-independent activation. The data in Figs 1 and 2 already indicate that IL-13 is induced particularly efficiently after culture with IL-3, or IL-3 and C5a, while the response to IgE crosslinking is rather weak. Because of the known donor variability for IL-4 expression by basophils in response to different agonists, this issue was further examined in several experiments with different unselected donors in which IL-13 and IL-4 were measured in the same cell supernatants after exposure to the different stimuli and culture for either 4 or 18 hours. Indeed, levels of IL-4 were
significantly higher than of IL-13 after IgER crosslinking, in marked contrast to the consistently higher levels of IL-13 as compared with IL-4 found 18 hours after culture with IL-3 alone or IL-3 plus C5a (Fig 4). The slightly, but significant ($P = .014$), higher levels of IL-4 produced within 4 hours even after IgE-independent activation (Fig 4, upper panel) may indicate a generally more rapid, but less sustained, induction of IL-4 expression compared with that of IL-13.

Regulation of IgER-dependent and IgE-independent IL-13 and IL-4 expression by different cytokines. Among a large number of different cytokines, IL-3 was by far the most effective at priming the cells for C5a induced IL-13 expression and at enhancing the IgE-dependent response (Fig 5). Among all the cytokines tested, IL-3 was also the only stimulus capable of directly inducing IL-13 expression. Small amounts of IL-13 were also produced in response to C5a in the presence of IL-5, GM-CSF, IGF-1, and IL-1β. These cytokines also enhanced IL-13 expression induced by IgER crosslinking although the effect of IL-1β was only marginal and was not studied in more detail. IL-2, IL-6, IL-10, IL-12, TNF, and IFN-γ neither primed human basophils for C5a-induced cytokine expression nor enhanced IL-13 production in response to IgER activation. IL-4 production measured in the same cell supernatants was regulated in a qualitatively identical manner (Fig 5, lower panels).

Previous studies have shown a clear synergism for IL-4 production between IL-3 and IgE activation. As shown in Fig 6, the effects of IL-3 and αIgER MoAb on IL-13 expression were largely additive, particularly after 18 hours. These experiments also show that in the presence of IL-3, IL-13 is further released continuously between 4 and 18 hours in amounts that are not influenced by prior IgE activation. By contrast, IL-4 expression in response to IgE crosslinking is more transient and maximal after 4 hours regardless of whether IL-3 is present.

Induction of IL-13 mRNA expression in response to IgE activation or IL-3 and C5a. Because human basophils are found in only low numbers in the peripheral blood and thus cannot be purified in large quantities, semi-quantitative competitive RT-PCR was used to estimate mRNA levels of IL-13 and IL-4. We found that IL-13 mRNA was strongly induced by IgER crosslinking or IL-3 plus C5a, consistent with the data on product formation (Fig 7). IL-13 mRNA was also induced after culturing basophils during 3 hours with IL-3 alone (data not shown), in marked contrast to basophils stimulated with C5a in absence of IL-3 (Fig 7). The mononuclear cell preparations depleted of basophils purified from the same leukocyte preparations and cultured with medium alone, αIgER, IL-3, C5a, or IL-3 plus C5a did not express detectable levels of IL-13 mRNA (data not shown). Even on polyclonal stimulation with PMA and Ionophore A23187 IL-13 mRNA expression was comparably very weak and inconsistent in MNC (Fig 7, right panels) and hardly approached the levels constitutively expressed in basophils (Fig 7, left panels). Time course studies showed that the induction of IL-13 mRNA in response to IgE crosslinking was rapid and significantly above the control already 30 minutes after activation (Fig 8). It should be noted that for the different conditions the RNA was reverse transcribed.
with the same fixed amount of internal standard in the same reaction, before splitting into different aliquots and performing PCR with the appropriate specific primers using the same numbers of cycles. Thus, the ratio of the amplification products of the internal standard and that of the mRNA of the housekeeping gene \( \beta_2 \)-microglobulin, IL-4 and IL-13 can be directly compared with each other and for each condition. The data shown in Figs 7 and 8 therefore demonstrate that the messages of IL-4 and IL-13 are induced in a very important manner, although not quite to the level of the mRNA of \( \beta_2 \)-microglobulin. Figure 8 also confirms that in human basophils IL-4 mRNA is constitutively expressed\(^{13} \) and IL-4 mRNA is induced in a manner similar to IL-13 mRNA on activation. IL-13 mRNA was found to be constitutively expressed in several basophil preparations examined (see also Figs 7 and 8), a finding which was better visualized when using a lower competitive internal standard cellular mRNA ratio. However, it was a very consistent finding that IL-13 mRNA was expressed at clearly lower levels than IL-4 mRNA in unstimulated freshly purified human basophils (data not shown). It thus appears that IL-13 expression is primary regulated at the transcriptional level.

**DISCUSSION**

This study demonstrates that mature human blood basophils are a prominent source of IL-13. IL-13 is expressed in a qualitatively identical manner as the related cytokine IL-4. IL-13 shares a certain degree of sequence homology with IL-4,\(^1 \) and both are encoded in close association on the cytokine gene cluster on human chromosome 5 in a ‘tail to head’ fashion.\(^{25} \) It is therefore not surprising that IL-4 and IL-13 are coordinately expressed. On the other hand, the expression of IL-4 and IL-13 may also be separately regulated as has been shown in T cells in which IL-13 is expressed by both, Th1 and Th2 cells, in contrast to IL-4 whose expression is restricted to Th2 cells.\(^{19,22,26} \) Furthermore other cytokines on the gene cluster on chromosome 5, such as IL-3, GM-CSF, and in particular IL-5 which is also in close association with the IL-4 gene, are not expressed at all neither constitutively nor after activation by human basophils, as assessed by measuring the protein product with ELISA\(^{20} \) or the corresponding mRNA by RT-PCR (our unpublished observations). Thus, the cytokine profile of human basophils clearly differs from other cell types, including Th2 cells.

In contrast to our rapidly expanding knowledge about the bioactivity profile of IL-13, very little is yet known about the cellular sources of IL-13 and the regulation of its expression. In the human system, IL-13 mRNA expression has been demonstrated in T cells,\(^{26} \) in transformed B cells, and in B-cell tumors.\(^{27} \) It should be noted that IL-13 mRNA in B-cell tumors was demonstrated by RT-PCR only using large numbers of cycles, and the significance of this observation...
for normal B-cell physiology remains unknown. No information about IL-13 protein secretion by different leukocyte types is yet available in humans. Of interest is the recent demonstration of IL-13 expression by murine mast cell lines and immature murine bone-marrow-derived mast cells in response to IgE receptor activation. To our knowledge, this is also the only study demonstrating IL-13 protein secretion. Murine mast cell lines express, however, a large number of cytokines, including other cytokines encoded on the cytokine gene cluster on the murine chromosome 11 (the syntheic region of human chromosome 5), such as IL-3, IL-5, and GM-CSF, and thus cytokine expression by murine mast cell lines and normal human basophils appears to be regulated differently. It is also unknown whether murine mast cells are able to express IL-13 in response to IgE-independent stimuli.

Despite the qualitatively identical and co-ordinate expression of IL-4 and IL-13, some differences in the amounts of the two products formed are clearly evident. Generally higher amounts of IL-4, as compared with IL-13, are detected when measured at early time points after basophil activation, or in response to stimuli, which only leads to relatively transient induction of cytokine expression, such as IgE-receptor crosslinking. One may speculate that the higher levels of constitutively expressed IL-4 mRNA may endow the basophil to respond more rapidly and vigorously to an appropriate stimulus. When the basophils are stimulated by IgE-independent stimuli, such as IL-3 or IL-3 plus C5a, which induce a more sustained cytokine production, IL-13 is clearly the prevalent product. Using semi-quantitative RT-PCR, we found that IL-13 mRNA is induced by all the stimuli capable of promoting IL-13 protein formation and secretion. These experiments indicate that IL-13 expression is regulated primarily at the transcriptional level. This conclusion is further supported by the fact that IL-13 release is totally suppressed by a short preincubation with either actinomycin D or cyclo-
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Fig 8. Induction of IL-13 and IL-4 mRNA in basophils in response to IgE activation. Basophils were cultured in medium with (lower panel) or without (upper panel) αIgE MoAb (100 ng/mL), and RNA was extracted at times 0 minutes, 30 minutes, and 120 minutes after addition of the stimulus or buffer control. Competitive RT-PCR was performed using primers for β2-microglobulin (β2 mic.), IL-4 and IL-13. The size of the amplification product of the reverse transcribed IL-4 mRNA was 224 bp, others are as indicated in Fig 7 legend. A representative experiment (out of four) is shown.

Basophils-derived IL-4 and IL-13 in response to antigen and in particular the large amounts of IL-13 formed after IgE-independent activation by IL-3 and C5a may provide a mechanism for the marked increase in polyclonal IgE concentrations found in allergic diseases and helminth infections.

In parallel with IL-4, IL-13 also regulates the function of monocytes, macrophages, and certain tissue cells. IL-13 downregulates the production of proinflammatory cytokines, induces the secretion of cytokine antagonists and antagonistic receptors, and upregulates the expression of the low affinity IgE receptor (CD23). CD23 may also be involved in IgE regulation, and may facilitate presentation of antigens at low concentrations, a phenomenon that may be of particular importance in immediate-type hypersensitivity reactions, which occur in response to limited amounts of antigen. Like IL-4, IL-13 also upregulates the expression of VCAM on endothelial cells, providing a means by which effector leukocytes expressing αIβ2 (basophils, eosinophils, and activated T cells) are attracted to inflammatory sites in a more selective manner. Thus, basophil-derived IL-13 may play a role in regulating the cellular composition of leukocytes at inflammatory sites, eg, in allergic inflammation and other pathologies involving basophils.

IL-3 appears to be a particularly important cytokine in regulating the expression of IL-13 and IL-4. Considerable amounts of IL-13 are produced by culturing basophils in medium containing IL-3 even without further activation. There is also an important synergism for IL-13 expression between IL-3 and C5a, the latter being incapable of inducing IL-13 secretion by itself. In contrast to IL-4, which is synergistically induced by IL-3 and IgE activation, the effects of IL-3 and αIgE MoAb on IL-13 expression are largely

heximide. On the other hand, IL-13 (and in particular IL-4) production may also be regulated in part at the translational level, since: (1) some constitutive IL-13 and IL-4 mRNA can be consistently detected in purified basophil, but not in the mononuclear cells, and it is unknown at present whether these mRNAs are transcribed at a level below the detection limit of the ELISAs, or whether IL-4 and IL-13 genes are transcribed without translation. (2) We did not find an obvious difference in IL-13 mRNA levels between basophils stimulated with IL-3 versus IL-3 plus C5a, despite the marked differences in IL-13 protein secretion. Therefore, C5a may also have an effect on IL-13 mRNA translation, although one must take into account the fact that RT-PCR is only a semi-quantitative method even when using an internal competitive standard.

The simultaneous and very restricted expression of IL-4 and IL-13 in human basophils further supports the hypothesis of a key immunoregulatory role of this cell type not shared by other immune cells. In the human system, IL-13 shares most of the biological activities with IL-4, with the exception of a lack of activity on T cells, and the two cytokines also share receptor components. In particular, IL-13 is, together with IL-4, an obligatory cytokine for isotype switching to IgE in B cells. It has been shown in IL-4 knockout mice that the IL-4 gene is indispensable for IgE production, but the relative role of IL-4 and IL-13, respectively, for the induction of IgE production in humans is unclear. It is conceivable that in the human system IL-13 is at least as important for IgE production, and thus for the pathogenesis of IgE-dependent diseases, as is IL-4. We have previously shown that human basophils provide all the necessary signals to human B cells for isotype switching and IgE production.

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additive. Interestingly, important amounts of IL-13, but not of IL-4, are further produced between 4 and at least 18 hours after activation at a rate that is not influenced by prior IgER activation. One may postulate that the more transient induction of cytokine expression induced by IgER crosslinking may be due to a shut off signal occurring later after IgER activation. Our data show that even if such a mechanism should exist, it does not influence the long-lasting induction of IL-13 expression promoted by IL-3. IL-3 appears to be rather unique in regulating IL-13 expression. Although IL-3 and GM-CSF significantly enhanced IgER-dependent IL-13 secretion, and although in the presence of these cytokines some IL-13 was generated even in response to C5a, their effect was rather weak compared with that of IL-3. A large number of other cytokines tested were ineffective. These observations further support our recently proposed hypothesis of an indirect immunoregulatory role of IL-3 by strongly regulating IL-4 and IL-13 expression in human basophils and by expanding the pool of IgER positive cells secreting these cytokines.

In conclusion, we find that mature human blood basophils are capable of expressing and secreting IL-13 in response to IgER-dependent and IgE-independent activation. IL-13 is produced together with IL-4 in a very restricted manner and this expression is strongly regulated by IL-3. These data further support an immunoregulatory role of basophils, apart from their inflammatory effector functions.

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