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Functional Expression of IL-12 Receptor by Human Eosinophils: IL-12 Promotes Eosinophil Apoptosis

Esra Nutku,‡ Qianli Zhuang,‡ Abdelillah Soussi-Gounni,‡ Fadi Aris,‡ Bruce D. Mazer,‡† and Qutayba Hamid‡‡

In murine models of allergic inflammation, IL-12 has been shown to decrease tissue eosinophilia, but the underlying mechanisms are not known. We evaluated the expression of IL-12R and the effect of IL-12 on eosinophil survival. In situ hybridization demonstrated the presence of mRNA and immunoreactivity for IL-12Rβ1 and -β2 subunits in human peripheral blood eosinophils. Surface expression of IL-12Rβ1 and -β2 subunits on freshly isolated human eosinophils was optimally expressed after incubation with PMA. To determine the functional significance of IL-12R studies, we studied cell viability and apoptosis. Morphological analysis and propidium iodide staining for cell cycle demonstrated that recombinant human IL-12 increased in vitro human eosinophil apoptosis in a dose-dependent manner. Addition of IL-5 together with IL-12 abrogated eosinophil apoptosis, suggesting that IL-12 and IL-5 have antagonistic effects. Our findings provide evidence for a novel role for IL-12 in regulating eosinophil function by increasing eosinophil apoptosis. The Journal of Immunology, 2001, 167: 1039–1046.

Eosinophils are the hallmark of allergic inflammation. In allergic diseases, eosinophil numbers correlate with disease severity (1–3). Eosinophils may be critical in allergic inflammation due to the release of cytotoxic basic proteins, de novo synthesized lipid mediators, and oxygen radicals. Moreover, eosinophil-derived mediators, including cytokines, can perpetuate the inflammatory reaction and lead to structural changes (4, 5).

Tissue eosinophilia within the lungs and nose of individuals with allergic asthma and rhinitis has primarily been attributed to the influx of mature cells from the blood. However, increased eosinophil survival and/or decreased eosinophil apoptosis (programmed cell death) has also been recently proposed as a mechanism underlying tissue eosinophilia (6, 7). It is evident that Th2-type cytokines such as IL-4, IL-5, and GM-CSF are important mediators for eosinophil chemotraction and survival in allergic inflammation (8–10). Recent studies have focused on the mechanisms involved in Th2-type cytokine-eosinophil interaction.

To mitigate against the effect of Th2 cytokines, strategies have been developed to suppressed their production, thus decreasing allergic inflammation. Glucocorticoids are the most useful class of drugs for treating many eosinophil-related disorders including allergic inflammation. Glucocorticoids can cause a striking reduction in eosinophil numbers in vivo (11). They exert inhibitory effects on eosinophil survival directly through specific receptors on eosinophils, as well as indirectly through inhibition of cytokines that promote eosinophil survival, such as IL-5 and GM-CSF (12).

A second strategy focuses on cytokines that have immunoregulatory effects, such as IL-12. Produced by APC (13), IL-12 induces Th1 cell proliferation and Th1-type cytokine expression (13–16). IL-12 has also been proposed as an antiallergic cytokine by virtue of its inhibitory effects on Th2-type cytokine expression as well as eosinophilic inflammation (17–21). We have recently shown that the level of IL-12 mRNA expression in the airways of asthmatic subjects is significantly lower than that in nonasthmatic controls and was significantly increased in response to steroid treatment (22). It is unknown whether IL-12 can have a direct effect on eosinophil function.

There are two subunits of the IL-12R, IL-12Rβ1 and IL-12Rβ2 (23, 24), which belong to the gp130 group of the cytokine receptor superfamily. Both subunits can bind IL-12 independently of one another; however, interaction between them is required for IL-12 signaling. Furthermore, the IL-12Rβ2 subunit acts as a high affinity converter. Functional IL-12R have been recently described on human and murine T cells (25–27) and on NK cells (28). To date, IL-12R expression on eosinophils has not been determined. To investigate the role of IL-12 on eosinophils and to understand the mechanism by which IL-12 may exert its influence in the allergic response, we examined the expression of IL-12R on eosinophils as well as the effects of IL-12 on eosinophil survival.

Materials and Methods

Cytokines and Abs

rIL-12, rIL-5, rIFN-γ, and rat IgG2a isotype control Ab were purchased from R&D (Minneapolis, MN). Anti-CD16 and anti-CD3 mAb micobeads were from Miltenyl Biotec (Bergisch Gladbach, Germany). Rat anti-human IL-12Rβ1 and β2 IgG2a isotype Abs were gifts from Dr. D. Presky, Hoffman LaRoche, Italy. Propidium iodide (PI) was from Sigma (St. Louis, MO). Biotinylated rabbit anti-rat IgG was obtained from Vector Laboratories (Burlingame, CA). FITC, Fast Red, and streptavidin-conjugated alkaline phosphatase were purchased from Dako (Glostrup, Denmark). FCS was purchased from HyClone Laboratories (Logan, UT). Dextran T70, LM-2 emulsion, and 35S were obtained from Pharmacia (Uppsala, Sweden). Triton X, formamide, and RNase A, were obtained from Sigma (St. Louis, MO).

Peripheral blood eosinophil purification

After informed consent, 50 ml blood were obtained from volunteers with peripheral blood eosinophilia ranging from 5 to 10% (n = 25). Human eosinophils were isolated from the peripheral blood of healthy adult volunteers using a negative selection strategy. The cells were plated in the presence of IL-5 and cultured for 24 h. After culture, eosinphils were prepared for labeling with CD15 specific mAb and were stained with PI and analyzed by flow cytometry.

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blood leukocytes were obtained from whole blood by sedimentation of RBC in 6% dextran T70. Granulocytes were enriched on a Ficoll-Hypaque gradient (1.077 g/ml; Pharmacia), and eosinophils were negatively selected from the enriched cells by immunomagnetic selection on a MACS column using anti-CD16 microbeads. Eosinophil purity was consistently >98% with neutrophils being the only contaminating cells. The viability of freshly isolated eosinophils were >99% as determined by trypan blue dye exclusion.

Cell culture
Isolated peripheral blood eosinophils (1 x 10⁶/ml) were resuspended in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured at 37°C in a humidified atmosphere with IL-12 (10 ng/ml) in the absence or presence of IL-5 (2.5 ng/ml) for 18 h. To investigate the regulation of IL-12R expression, eosinophils were cultured in the presence of IL-5 (2.5 ng/ml), IFN-γ (10 ng/ml), IL-12 (10 ng/ml), IL-18 (10 ng/ml), IL-12 plus IL-18, PMA (1–10 ng/ml), or PMA plus IL-12 plus IL-18 for 12 h.

Cytosin preparations
Cytosin slides were prepared from eosinophils freshly isolated from human peripheral blood or from cultured eosinophils. The cytosins were then fixed in 4% paraformaldehyde for 20 min at room temperature and washed with 0.05 M Tris-HCl-buffered isonic saline (TBS), pH 7.6. After drying, the slides were stored at −20°C before immunocytochemistry, in situ hybridization, or morphological analysis of apoptosis.

Preparation of riboprobes
cDNAs coding for IL-12Rβ1 and -β2 were inserted into Bluescript vectors. The vectors containing either IL-12Rβ1 or -β2 cDNAs were linearized with the appropriate enzymes and transcribed in vitro with either SP6 or T7 polymerases in the presence of [35S]UTP to generate sense and antisense riboprobes.

In situ hybridization
In situ hybridization was performed as previously described (29). Briefly, cytosin preparations were first permeabilized by immersion in 0.3% Triton X-100 in PBS for 10 min followed by exposure to protease K (1 mg/ml in 20 mM Tris-HCl and 1 mM EDTA, pH 7.2) for 30 min at 37°C. The slides were then prehybridized with 50% formamide in 2× SSC for 15 min at 37°C. Hybridization was performed with [35S]UTP-labeled riboprobes (either antisense or sense) for 16 h at 42°C. Posthybridization washings were done with SSC solution (4× SSC and 0.1× SSC) followed by RNase A treatment to remove unhybridized single-stranded RNA. The preparations were dehydrated, immersed in Amersham LM-2 emulsion, and then subjected to autoradiography for 18 days. The autoradiograms were developed in Kodak n-19 developer, fixed, and subsequently counterstained with hematoxylin. As the negative control, sections were hybridized with the sense probe or were pretreated with the RNase A before hybridization with the antisense probe.

Immunocytochemistry
IL-12R immunoreactivity was detected by the avidin-biotin complex method, as previously described (30). Briefly, the cytosin preparations of eosinophils were washed with TBS. After saturation for 20 min with TBS containing 10% normal human serum and 5% normal goat serum (Cedarlane, Hornby, Ontario, Canada), cells were incubated with rat anti-human IL-12Rβ1 (BM10) or β2 (LM5) mAbs, (each at 1/20 dilution) in Ab dilution buffer (Dako) overnight at 4°C. After washing, the cells were incubated with biotinylated anti-rat Ab for 30 min at room temperature followed by streptavidin-conjugated alkaline phosphatase (1/100 dilution) for 1 h at room temperature. The slides were then developed using Fast Red, counterstained with Mayer’s hematoxylin, and examined by microscopy.

Flow cytometric analysis of IL-12R expression on eosinophils
Cells (2 x 10⁵) were incubated with saturating concentrations of primary Abs, either rat anti-human IL-12Rβ1, β2 mAbs or IgG2a isotype control Ab (1/100 dilution) in PBS for 30 min at 4°C. The cells were washed twice with PBS and incubated at room temperature for 30 min with biotinylated rabbit anti-rat Ab (1/100 dilution). After washing, the cells were incubated with PE-conjugated streptavidin (1/100 dilution; Sigma, St. Louis, MO) at room temperature for 30 min. Cells were washed and resuspended in 200 μl PBS and analyzed on FACScanLibur flow cytometer (BD Biosciences, Oxnard, CA). IL-12R expression was read using FL-2 channel and compared with the control.

Determination of eosinophil apoptosis by morphological assessment of nuclei alteration
Apoptosis was evaluated by morphological assessment (31). Briefly, cytosin preparations were stained with Leukostat (Fisher Diagnostics, Pittsburgh, PA) to reveal nuclear morphology. Apoptotic cells were discriminated from healthy cells by the condensed and rounded appearance of their nuclei under light microscopy. Cells exhibiting apoptotic nuclei were enumerated in different fields in a blinded manner using a random coded order. A minimum of 500 total cells per slide was counted. Cells were then photographed under an Axioscope microscope (Zeiss, Oberkochen, Germany) at ×400 magnification.

Cell cycle analysis
Apoptosis was assessed by cell cycle analysis using PI staining as described previously (32). Briefly, harvested eosinophils (0.2 x 10⁶ cells) were washed twice with PBS by centrifugation at 1200 rpm for 10 min. The cells were then resuspended in 250 μl FCS and 250 μl RPMI 1640 and fixed in 1.5 ml 70% ethanol at 4°C for a minimum of 30 min. The cells were washed twice with PBS by centrifugation at 4°C and 2400 rpm for 5 min, resuspended in 1 ml of PBS with 0.05 mg/ml RNase A (50 U/mg), and incubated for 30 min at 37°C. After this, 50 μg/ml PI was added. Using flow cytometry (Cell Fit Software; BD Biosciences), cell cycle analysis was performed on eosinophils gated on the basis of their cell volume (right angle scatter) and cellular DNA content with any cell debris excluded from analysis. The percentage of apoptotic cells was determined in the hypodiploid peak.

Determination of eosinophil viability
The percentage of necrotic eosinophils was determined by using trypan blue exclusion. Spontaneous uptake of trypan blue as a vital dye by eosinophils was then assessed by light microscopy.

Quantification and statistics
In situ hybridization and immunocytochemistry slides were analyzed for positive signals in a blinded fashion by two independent examiners. Data are represented within the text and figures as the mean percent ± SEM. Comparisons between groups were performed using a one-way ANOVA and Fisher post hoc test. A value of p < 0.05 was considered statistically significant (SyStat version 7.1; SyStat, Evanston, IL).

Results
Detection of mRNA encoding IL-12R in purified human peripheral blood eosinophils
In situ hybridization was performed to examine the potential of eosinophils to produce and express IL-12R. Hybridization of the [35S]UTP-labeled antisense riboprobes and mRNA encoding IL-12Rβ1 and -β2 (Fig. 1, A and B) were demonstrated by specific deposits of silver grains in the photographic emulsion overlaying the cytosins. IL-12Rβ1 and -β2 mRNA were expressed in eosinophils obtained from all donors. The mean percentage of eosinophils expressing IL-12Rβ1 mRNA was 56.4 ± 6.6%, and that for IL-12Rβ2 mRNA was 49.1 ± 3.9% (Fig. 1C, mean ± SEM, n = 12). The percentage of eosinophils expressing the IL-12Rβ1 and -β2 were significantly higher in healthy controls than in asthmatics (mean ± SEM, IL-12Rβ1: asthmatics, 36.1 ± 2.4; controls; 69.2% ± 7.01, p = 0.001. IL-12Rβ2: asthmatics, 38.6 ± 4.4; controls; 59.5 ± 1.3, p = 0.001). No positive hybridization signals were observed when the sense probe was used or when the cell preparations were pretreated with RNase before hybridization with the antisense probe.

Detection of IL-12R protein in human peripheral blood eosinophils by immunocytochemistry
Eosinophils exhibited positive immunoreactivity for IL-12Rβ1 and -β2 as detected by the presence of discrete red cytoplasmic or membranous staining (Fig. 2, A and B). Eosinophils obtained from all donors were immunoreactive for IL-12Rβ1 and -β2 subunits.
The mean percentages of eosinophil immunoreactivity for IL-12Rβ1 and -β2 were 39.9 ± 4.8 and 65.9 ± 3.4, respectively (Fig. 2D). There was no difference between the percentages of the IL-12Rβ1 and -β2 immunoreactivity between asthmatics and the control subjects (data not shown).

Surface expression of IL-12R on freshly isolated peripheral blood eosinophils

To differentiate surface from cytoplasmic expression of IL-12R, freshly isolated peripheral blood eosinophils were stained for
surface expression of IL-12Rβ1 and -β2 subunits and analyzed by indirect immunofluorescence and flow cytometry. Eosinophils were obtained from a total of eight donors. In four of the eight eosinophil preparations, IL-12Rβ1 and -β2 were detected on eosinophil cell surface (Fig. 3, A and B). Of these four positive preparations, three were from asthmatic patients and one was from a nonasthmatic healthy control. The percentages of eosinophils expressing IL-12Rβ1 and IL-12Rβ2 were 27.3 ± 3.6 and 14.3 ± 1.1, respectively, in the four subjects studied (Fig. 4). Baseline surface staining for IL-12R was not detectable in four subjects (two asthmatics and two nonasthmatic healthy controls) of eight donors (Fig. 3, C and D). Although we were unable to detect IL-12R expression by FACS analysis, using immunocytochemistry technique and the same Abs, we demonstrated the IL-12Rβ1 and -β2 expression in eosinophils from these four donors (data not shown).

Regulation of IL-12R surface expression on eosinophils

PMA (1–10 ng/ml) stimulation increased IL-12Rβ1 and -β2 surface expression on eosinophils (IL-12Rβ1, 48.4 ± 4.5; IL-12Rβ2, 49.8 ± 12.8) compared with unstimulated eosinophils (Figs. 3 and 4). In addition, PMA stimulation induced IL-12R surface expression by FACS analysis on eosinophils with undetectable baseline IL-12R expression (Fig. 3, C and D). Eosinophils cultured with rIL-5 (2.5 ng/ml), rIL-12 (10 ng/ml), rIL-18 (10 ng/ml), or rIFN-γ (10 ng/ml), did not exhibit an increase in IL-12R expression.

Effect of IL-12 on eosinophil apoptosis

In the light of previous data that demonstrate decreased eosinophilia on exogenous administration of IL-12 and the presence of IL-12R on eosinophils, we examined the effect of IL-12 on the maintenance of eosinophil survival. Eosinophils were cultured with rIL-12, rIL-5, or medium alone for 18 h, and the extent of apoptosis was evaluated by morphological analysis (27) (Fig. 5A). Fig. 5B demonstrates that IL-12 significantly increased the percentage of eosinophils undergoing apoptosis (24.3 ± 3.8, mean percent of apoptotic cells ± SEM) compared with IL-5 (8.8 ± 2.0%, p < 0.005) and medium control (13.2 ± 2.7%, p < 0.05, n = 5). The effect of IL-12 on eosinophil apoptosis was dose dependent, with apoptosis increasing at IL-12 concentrations ranging from 1 to 100 ng/ml (Fig. 6).

To confirm the effect of IL-12 on eosinophil apoptosis determined by morphological analysis, eosinophil apoptosis was assessed by cell cycle analysis using PI staining (Fig. 7, A–D). After 18 h of cell culture with IL-12, eosinophil apoptosis was 2-fold higher than that of IL-5 (Fig. 7D, IL-12 vs IL-5; 24.3 ± 2.9% vs 12.7 ± 3.5%, mean percent of apoptotic cells ± SEM, p < 0.05,
IL-5 rescues eosinophils from programmed cell death induced by IL-12 (Fig. 8). IL-5 rescued eosinophils from programmed cell death induced by IL-12, IL-5 was no longer able to reverse the effect on eosinophils (8–10). The effect of IL-12 on eosinophil apoptosis was further investigated in the presence of IL-5. Eosinophils were cultured with IL-12 (10 ng/ml) for 18 h, and cell morphology was examined. In the absence of IL-5, IL-12 significantly increased the number of eosinophils undergoing apoptosis, compared with IL-5 or medium alone (Fig. 8). The percentage of apoptotic eosinophils in the presence of IL-12 was 30.8 ± 6 (%, p < 0.005) and 21.4 ± 6 (%, p < 0.005) in medium alone (IL-12 vs medium; p < 0.05, group Ia compared with group IIa. Table I demonstrates that after a 3-h incubation, incubation with IL-5 for 3 h or more rendered the eosinophils insensitive to the action of IL-12.

**Discussion**

We report for the first time detection of functional IL-12R on eosinophils, including both IL-12Rβ1 and -β2 subunits (Figs. 1–4). This is based on direct demonstration of mRNA and protein, as well as evidence that IL-12 accelerates eosinophil apoptosis (Figs. 5–7), which may be reversed by addition of IL-5 (Fig. 8).

IL-12 receptors consist of two subunits, β1 and β2. The IL-12Rβ1 is ubiquitously expressed, yet its role in IL-12 signaling is still unknown. IL-12Rβ2 is the signal-transducing unit and has a more limited expression, to date being detected in T and B cells and NK cells (25, 28, 33). Our data demonstrating the presence of both receptor subtypes suggest that the IL-12R in the eosinophils can be functional.

IL-12 primarily regulates the balance between Th1 and Th2 cells (16, 25–27). IL-12 induces Th1 cell differentiation and proliferation, and contributes to optimal production of IFN-γ (13, 14). Besides this activity of IL-12 in inducing the Th1-type responses, IL-12 was also reported to inhibit Th2-type responses. IL-12 inhibited allergen-induced hyperresponsiveness and eosinophil accumulation in murine (18, 19, 21) models of asthma, and Ag challenged human airways (B. J. Connor, unpublished observation). In a recent study, Naseer et al. (22) demonstrated that IL-12 mRNA expression was decreased within the airways of asthmatic subjects compared with those of nonasthmatic control subjects. These findings demonstrated the relative deficiency of IL-12 in asthmatic airways, which could be due to the overexpression of IL-4 (27, 34). The inhibitory effects of IL-12 on eosinophil numbers and airway

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<th>Cytokine Conditions at Each Time Point of the Cell Culture</th>
<th>% of Apoptosis (mean ± SEM)</th>
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<td>Group</td>
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<td>Ia</td>
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<sup>a</sup> Peripheral blood eosinophils were cultured as described in Materials and Methods.

<sup>b</sup> p < 0.05, group Ia compared with group Ib.

<sup>c</sup> p < 0.005, group Ia compared with group IIa.

<sup>d</sup> p < 0.05, group Ib compared with group IIa.
hyperresponsiveness can also be attributed to IL-12-mediated inhibition of Th2-promoting cytokines such as IL-4 (16, 18, 20). These previous reports do not, however, preclude other functions for this cytokine. Taken together with our current data, the lower levels of IL-12 in asthmatics suggest that there may be less eosinophil apoptosis and thus less turnover of the receptor compared with nonasthmatic subjects. This may explain the discrepancy between the IL-12R mRNA levels detected in normal control subjects as compared with asthmatics.

To date, studies demonstrate the capacity of eosinophils to express predominantly Th2-type cytokine receptors both constitutively and after appropriate stimulation. IL-5 is the primary growth factor in eosinophils (35), and they also respond to IL-3, GM-CSF (36), and IL-9 (37). In the present study, IL-12R, known as a modulator of Th1 responses, was detected constitutively on the majority of eosinophils from both asthmatic and normal control subjects. We were unable to detect any difference in protein expression or in response to IL-12 in eosinophils derived from asthmatic compared with nonasthmatic patients.

Using flow cytometry, baseline IL-12R surface expression was detected in four of eight eosinophil preparations obtained. In all other subjects, cytoplasmic IL-12R expression determined by immunocytochemistry (data not shown). One explanation for the discrepancy between the results using these two methods is the sensitivity of detection of low surface expression in some individuals (38). This is supported by the fact that when eosinophils with low surface receptor expression were stimulated with PMA (data not shown), the expression of IL-12R was significantly induced and thus detectable by FACS analysis. This is further supported by acceleration of programmed cell death in response to IL-12 administration seen in all subjects.

The mechanism by which PMA up-regulated the surface expression of IL-12R is unknown. A possible mechanism is through induction of cytokines, including IL-12 or IFN-γ. However, none of these cytokines or IL-5 and IL-18 augmented surface expression of IL-12R. It is unclear whether the induction of surface IL-12R by PMA changes the functional response to IL-12. These studies are ongoing in our laboratory.

**FIGURE 7.** Detection of apoptosis by cell cycle analysis. Eosinophils were incubated with medium only (A), IL-5 (B), or IL-12 (C) for 18 h and assessed by cell cycle analysis after incubation with PI as in Materials and Methods. M1 indicates the eosinophil population with increased DNA fragmentation. D, PI staining of eosinophils obtained from five donors. Eosinophils demonstrated a significant increase in the percentage of apoptotic cells after culture with IL-12 compared with IL-5, *, p < 0.05; n = 4. E, trypan blue exclusion determining eosinophil viability. There was no difference in the percentage of necrotic eosinophils in cells treated with either IL-12 or IL-5 compared with medium alone.

**FIGURE 8.** IL-5 abrogates IL-12-induced eosinophil apoptosis. A and B, Morphological analysis of the percentage of apoptotic eosinophils after 18 h of cell culture. A, IL-5 overcame apoptotic effects of IL-12 on eosinophils from normal donors (subjects 1 and 3) as well as asthmatics (subject 2). B, Samples grouped and presented with measures of statistical significance. The percentage of eosinophil apoptosis induced by IL-12 was significantly decreased in the presence of IL-5, compared with cells with IL-12 alone (n = 3). *, p < 0.05; **, p ≤ 0.001.
In culture, in the absence of growth-promoting cytokines, eosinophils gradually undergo programmed cell death. We propose that the well-documented decrease in eosinophil numbers in response to IL-12 is due to the acceleration of this process. Morphological analysis of apoptotic nuclei and DNA fragmentation determined by PI staining were consistent with this hypothesis (Figs. 5–7). This effect is dose dependent, with effects between 1 and 100 ng/ml (Fig. 6). Although previous studies have demonstrated a role for IL-12 in defined Fas-mediated apoptosis in T cells (39, 40), our data provide the first report demonstrating direct effects of IL-12 on eosinophil apoptosis.

The pathway by which IL-12 induces in vitro eosinophil apoptosis is not clear. Our preliminary results suggest that neither IL-12 nor any other factors on FACS expression on eosinophils (data not shown). Similarly, IL-12 does not increase TNF-α receptor expression on eosinophils, determined by FACS analysis (E. Nutku and Q. Hamid, unpublished observation), nor does it decrease the expression of IL-5 mRNA (R. Schleimer, unpublished observation). IL-5 serves as a survival factor for eosinophils (8–10). When mature peripheral blood eosinophils are cultured in the absence of IL-5, they undergo apoptosis. In the present study, IL-5 (2.5 ng/ml) rescued eosinophils from the apoptotic effect of IL-12 (Fig. 8). The mechanism of IL-5 and IL-12 interaction is not clear. IL-5 may act by decreasing inhibition of IL-12R expression, rendering the eosinophils unresponsive to IL-12. However, IL-5 had no regulatory effects on eosinophil-IL-12R expression.

Our time course experiments (Table I) provided new perspectives to explain IL-5 and IL-12 interaction in association with eosinophil apoptosis. The presence of either of the cytokines in initial eosinophil cultures reversed the effect of the other on eosinophil apoptosis. However, addition of either IL-5 or IL-12 at later time points of the cell culture did not affect the outcome. These data may imply that IL-5 and IL-12 use different pathways to inhibit or to accelerate the eosinophil apoptosis, respectively. Stimulation of eosinophils for at least 3 h appeared to commit the cells to programmed cell death. This is consistent with the up-regulation of new proteins including caspases and Bcl family proteins involved in apoptotic processes (41, 42). Whether these proteins are associated with IL-12 effects on eosinophil apoptosis is still to be determined.

IL-12 has been shown to have important effects on other cells including T cells and NK cells. This includes induction of IFN-γ and other proinflammatory cytokines (14, 43). In addition, it has been demonstrated to decrease the synthesis of IgE (17, 20). Although eosinophils are known to produce cytokines including IL-12 (44), we do not believe that exogenous IL-12 augments production of cytokines such as IL-12 or IFN-γ, as the signal from IL-12 appears to rapidly commit eosinophils to programmed cell death. In addition we cannot exclude that IL-12 may have some effect on eosinophil progenitors, either to enhance or retard cell differentiation. This hypothesis is currently being tested in our laboratory.

Our findings suggest a mechanism by which IL-12 exerts an inhibitory effect on eosinophils in allergic and parasitic inflammation (16–18). Our experiments also demonstrate that the critical balance between dendritic cell derived cytokines such as IL-12 and Th2-derived cytokines including IL-5 in the microenvironment of an allergic reaction play a crucial role in determining the survival and effect of eosinophils in inflamed tissues.

Acknowledgments

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