

IL-4-induced apoptosis in peripheral blood eosinophils

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Background: The regulation of eosinophil survival and apoptosis may play a major role in diseases demonstrating increased numbers of circulating and tissue eosinophils such as allergic reactions. Because few promoters of eosinophil apoptosis have been described so far, the objective of this study was to elucidate the role of endogenous factors on eosinophil survival and apoptosis. **Methods and Results:** Highly purified peripheral blood eosinophils were analyzed in the time course from 24 up to 144 hours in culture. Eosinophil survival was assessed with trypan blue dye exclusion and apoptosis was determined by DNA fragmentation gel analysis and ELISA technique with anti-histone antibodies. We confirmed previous results demonstrating prolonged eosinophil survival and inhibited apoptosis by IL-3, IL-5, and GM-CSF. In contrast, eosinophil apoptosis was significantly enhanced by corticosteroids, particularly dexamethasone and hydrocortisone. However, IL-1 β , IL-8, IL-12, platelet-activating factor, TNF- α , and eotaxin had no effect on eosinophil survival or apoptosis when compared with culture medium alone. In contrast, IL-4 at concentrations of 100 U/mL or more inhibited eosinophil survival and induced apoptosis. This effect was time dependent and abrogated by preincubation with neutralizing anti-IL-4 antibodies. However, after 96 hours in cocubation, IL-4 did overcome the survival-prolonging effect of IL-3, IL-5, and GM-CSF. IL-4 did not enhance eosinophil surface expression of APO-1/Fas antigen (CD95). In assessing IL-4-mediated effects on eosinophil function, we found no response by means of the release of eosinophil cationic protein or reactive oxygen species.

Conclusions: Taken together, our data present direct evidence for the presence of functional IL-4 receptors on human eosinophils and indicate that IL-4 may lead to resolution of chronic inflammation by induction of eosinophil apoptosis. (*J Allergy Clin Immunol* 1998;102:1013-20)

Key words: Eosinophils, apoptosis, IL-4, Fas antigen

Parasitic infections and the hypereosinophilic syndrome as well as sites of allergen challenge in the nose, lung, and

Abbreviations used

ECP:	Eosinophil cationic protein
PAF:	Platelet-activating factor
rh:	Recombinant
ROS:	Reactive oxygen species

skin are mediated in part by the recruitment of potent proinflammatory eosinophils. However, the mechanisms by which eosinophils accumulate in tissues are still unclear. In allergic diseases this process appears to occur without increased accumulation of neutrophils and therefore appears to have a selective component. The direction of the eosinophil targeting process is dependent on chemoattractants such as C3a, C5a, platelet activating factor (PAF), IL-8, RANTES, macrophage inflammatory protein-1 α , monocyte chemoattractant protein-2 and -3, and the recently described eotaxin.¹⁻³ Additionally, proinflammatory mediators such as IL-1 β , IL-2, IL-12, and TNF- α are suggested to be involved in eosinophil recruitment or accumulation at the inflammatory site.⁴⁻⁶ Because eosinophil granulocytes represent short-lived, terminally differentiated cells undergoing apoptosis,⁷ accumulation of the motile eosinophils may be mediated not only by chemokinesis or chemotaxis but also by dysregulated apoptosis.⁸ Apoptosis is a normal physiologic response to cell-specific environmental influences, resulting in engulfment of intact cells without disgorgement of their histotoxic contents, thus contributing to the control or resolution of tissue inflammation. Through the release of cytotoxic granule proteins and other inflammatory mediators, the eosinophil represents a potent proinflammatory cell; therefore a better understanding of eosinophil apoptosis may provide a useful tool to develop new therapeutic strategies in allergic inflammation and diseases associated with eosinophilia. Whereas cytokines such as IL-3, IL-5, and GM-CSF have been shown to promote eosinophil viability and to inhibit apoptosis in vitro,⁹⁻¹¹ only few promoters of eosinophil apoptosis have been described so far. Among these are tumor growth factor- β ¹² and corticosteroids.^{13,14} In contrast, in neutrophils, corticosteroids significantly delay apoptosis,¹⁵ indicating that apoptosis may be selectively regulated among different subtypes of leuko-

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Supported by research grants We-1912-3/1 and Ka-578/7-1 from the Deutsche Forschungsgemeinschaft.

Received for publication Dec 30, 1997; revised June 9, 1998; accepted for publication July 14, 1998.

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0091-6749/98 \$5.00 + 0 1/1/93060

cytes. Therefore the objective of this study was to find endogenous mediators regulating survival and apoptosis in eosinophil granulocytes.

METHODS

Materials

Human recombinant (rh) GM-CSF (6.85×10^8 U/mg), IL-1 β (1×10^8 U/mg), IL-3 (6.9×10^7 U/mg), IL-4 (4.47×10^7 U/mg), IL-5 (4×10^7 U/mg), TNF- α (4×10^7 U/mg), monocyte-derived IL-8, and neutralizing polyclonal sheep antibodies to purified human IL-4 (0.1 to 1 μ g neutralize the bioactivity of 250 pg/mL solution of IL-4) were obtained from Genzyme (Boston, Mass). IL-12 (1×10^7 U/mg) was from R&D Systems (Minneapolis, Minn). Human recombinant eotaxin was from Peprotech Inc (Rocky Hill, NJ). L- α -phosphatidylcholine, β -acetyl- γ -O-hexadecyl (PAF) and rh complement C5a were purchased from Sigma (Deisenhofen, Germany). Before testing, all stimuli were diluted in HBSS containing 1 mg/mL BSA (control medium). Dynabeads M-450 sheep anti-mouse IgG were purchased from Dynal A.S., Oslo, Norway. All monoclonal antibodies used for flow cytometry were purchased from Immunotech, Marseille, France. All antibodies were FITC-conjugated F(ab')₂ fragments. The Cell Death Detection ELISA^{PLUS} was obtained from Boehringer Mannheim, Germany. Pharmacia CAP System eosinophil cationic protein (ECP) fluoroimmunoassay and Ficoll-Paque solution were purchased from Kabi Pharmacia Diagnostics AB, Uppsala, Sweden.

Ribonuclease A and Proteinase K were purchased from Merck, Darmstadt, Germany. DNA standard was made by restricting lambda DNA with *EcoRI* and *HindIII* (Boehringer Mannheim, Germany). RPMI-1640, FCS, penicillin, and streptomycin were obtained from Life Technologies, Eggenstein, Germany. Cell culture medium was RPMI-1640 with 10% FCS, 1% penicillin/streptomycin (culture medium). All other chemicals and reagents including dexamethasone and hydrocortisone were obtained from Sigma Chemical, Deisenhofen, Germany.

Subjects

Peripheral venous blood was obtained after informed consent from nonatopic subjects. Blood donors were carefully screened by (1) negative personal history of seasonal asthma or allergic rhinitis and no history of dermatitis, (2) negative specific IgE antibodies (radioallergosorbent test for at least 7 frequent allergens = Sx1), and (3) negative skin prick test reaction to a panel of 10 common aeroallergens (atopy screening test). Subjects did not receive systemic corticosteroids or antihistamines.

In some experiments, peripheral blood eosinophils of subjects with active atopic dermatitis defined according to the criteria of Hanifin and Rajka¹⁶ with the presence of erythema, papulation, and excoriation involving more than 20% of skin surface were compared with nonatopic eosinophils.

Purification of eosinophils

Peripheral blood eosinophils were separated by Ficoll density gradient centrifugation and an improved immunomagnetic negative selection procedure with the use of anti-CD16 antibody-coated Dynabeads, as described in detail.^{17,18} Purity and viability were 99% or more after isolation procedure, as assessed with Kimura staining and trypan blue dye exclusion, respectively.

Eosinophil survival in vitro

Eosinophils were resuspended in culture medium to 1×10^6 /mL, and 1 mL aliquots were added to 24-well, flat-bottomed tissue culture plates and stimulated with an equal volume of control medium, dexamethasone, hydrocortisone (10^{-5} to 10^{-6} mol/L each), IL-1 β (100 to 1000 U/mL), IL-3 (100 U/mL), IL-4 (10 to 2000 U/mL), IL-5 (100 U/mL),

IL-8 (50 to 500 ng/mL), IL-12 (100 to 500 U/mL), GM-CSF (100 U/mL), TNF- α (100 to 1000 U/mL), PAF (10^{-5} to 10^{-6} mol/L), and eotaxin (100 to 500 ng/mL), respectively, at 37° C, 5% CO₂. Cells were removed from each well by gentle pipetting. To confirm that all the cells had been removed, the wells were examined by direct vision with the use of an inverted microscope. Viability of cultured eosinophils was assessed at different time points with trypan blue dye exclusion.

At least 100 eosinophils were counted under the light microscope. Those eosinophils that excluded trypan blue after a 5-minute incubation were considered viable cells. Thereafter, cultured eosinophils were pelleted by 5-minute centrifugation at 1000 rpm at 4° C, and the supernatants were carefully removed and immediately frozen at -80° C.

In further experiments, eosinophils were costimulated with either IL-3, IL-5, or GM-CSF (100 U/mL each) plus IL-4 (1000 U/mL) or preincubated with IL-4 (1000 U/mL) for 30 minutes and thereafter stimulated with either IL-3, IL-5, or GM-CSF (100 U/mL each).

In another assay, inhibition of IL-4-mediated apoptosis by neutralizing polyclonal sheep anti-human IL-4 antibodies was assessed.

Quantitative determination of apoptosis by ELISA technique

The Cell Death Detection ELISA^{PLUS}, a commercially available photometric enzyme immunoassay,¹⁹ was used according to the instructions of the manufacturer for the quantitative in vitro determination of cytoplasmic histone-associated DNA fragments (mononucleosomes and oligonucleosomes) in the eosinophil culture supernatants. The test is performed with mouse monoclonal antibodies directed against DNA and histone. A DNA-histone complex served as positive control. The eosinophil culture supernatants were diluted 1:50 in RPMI before testing. In previous experiments it was demonstrated that supernatants of fresh isolated eosinophils as well as culture medium alone did not give positive absorbance values (A_{450nm}) compared with background control.

DNA fragmentation gel analysis

Pelleted eosinophils were lysed overnight by the addition of 250 μ L lysis buffer (10 mmol/L EDTA, pH 8.0, 50 mmol/L Tris, pH 8.0, 0.5% N-lauroylsarcosine, 0.5 mg/mL Proteinase K) at 50° C. After RNase A 250 μ g/mL digestion for more than 1 hour, 50 μ L 5 mol/L NaCl was added, and the proteins were sedimented by centrifugation at 13,000g for 15 minutes at 4° C. The supernatants were transferred to fresh tubes and nucleosomal DNA fragments were precipitated by adding 2.5 $\times$ volume of ice-cold ethanol (100%) and incubation at -80° C overnight. After centrifugation at 13,000g for 60 minutes, nucleosomal DNA pellets were resuspended in Tris-ethylenediamine tetraacetic acid buffer, pH 8.0, and applied to the slots of a 1.8% agarose gel. Electrophoresis was carried out in 0.5 $\times$ Trisborate-ethylenediamine tetraacetic acid buffer containing 0.1 μ g/mL ethidium bromide. After electrophoresis, fragments were visualized by UV light and photographed.

Flow cytometric analysis of eosinophil surface antigen expression

To characterize the effect of IL-4 on the pattern of eosinophil Fas antigen surface expression, single-color immunofluorescence technique was performed as described previously.²⁰ Eosinophil granulocytes were stained directly with FITC-conjugated monoclonal antibodies against Fas/CD95 (clone UB2; isotype IgG1) and the isotypic control IgG1. Monoclonal antibodies against the eosinophil marker p24/CD9 (clone ALB6; isotype IgG1) and Fc γ RIII/CD16 (clone 3G8; isotype IgG1) were used as controls.

Release of eosinophil cationic protein

For the detection of ECP release, a total of 5×10^5 /mL freshly isolated nonatopic eosinophils in PBS-BSA (plus calcium/magnesium)

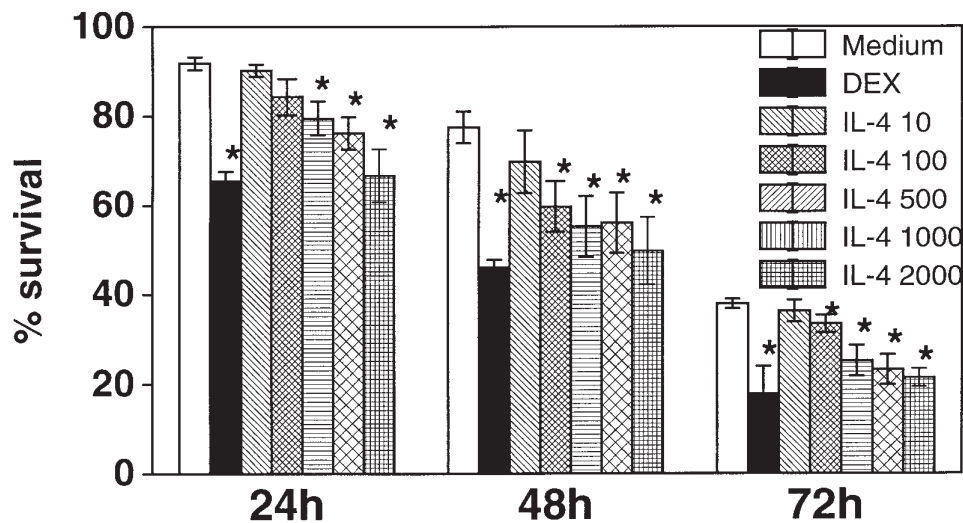


FIG 1. IL-4-inhibited eosinophil survival in vitro. Comparison of eosinophil survival stimulated by control medium, by dexamethasone (DEX, 10^{-6} mol/L), or by 10 to 2000 U/mL IL-4 after 24 to 72 hours in culture. Data are given as mean $\pm$ SEM of 6 to 8 separate experiments. * $P < .05$ vs control medium.

were incubated with or without 5 μ g/mL cytochalasin B for 5 minutes at 37° C, 5% CO₂, as described previously.¹⁷ In brief, eosinophils were stimulated for 2 hours at 37° C with control medium, GM-CSF, IL-1 β , IL-3, IL-4, IL-5 (100 U/mL each), IL-8 (50 ng/mL), C5a (10^{-8} mol/L), and PAF (10^{-6} mol/L), respectively. Total content of ECP was determined by the addition of 0.3% (f.c.) hexadecyltrimethylammoniumbromide. Thereafter, eosinophils were sedimented at 1200 rpm at 4° C for 5 minutes, and the supernatants were carefully removed and immediately frozen at -80° C. ECP concentration in the supernatants was determined by the commercially available CAP System ECP fluorescence enzyme immunoassay (measuring range 2 to 200 μ g/L) according the instructions of the manufacturer. Supernatants obtained after lysis with hexadecyltrimethylammoniumbromide were diluted 1:10 in culture medium before testing.

Lucigenin-dependent chemiluminescence

Furthermore, we investigated the effect of IL-4 on the release of reactive oxygen species (ROS) by the highly sensitive lucigenin-dependent chemiluminescence. Chemiluminescence was quantitated by a single-photon imaging system (MTP reader, Hamamatsu Photonics, Herrsching, Germany) as described previously.^{17,21} In brief, 100 μ L aliquots of eosinophils resuspended to 5×10^4 /mL in HBSS, pH 7.4, containing 200 μ mol/L lucigenin and 1 mg/mL BSA, were placed in flat-bottomed, white microtiter plates (Microfluor, Dynatech Deutschland, Denkendorf, Germany). Measurements were done in triplicate at 37° C. Integral counts were obtained from a 0- to 60-minute incubation interval immediately after addition of stimuli and were indicated as intensity integral counts $\times 10^{-3}$ in the figure.

Statistical analysis

All data are presented as mean $\pm$ SEM. Depending on the type of distribution, the statistical significance between groups was determined by Student *t* test or Mann-Whitney rank sum test, with a statistical software package (SigmaStat for Windows, Jandel Scientific, Erkrath, Germany) being used. A value of $P < .05$ was considered statistically significant.

RESULTS

Effect of stimulation on eosinophil survival in culture

With increasing time in culture, there was a progressive decrease of eosinophil survival. As has been reported previously,^{9,11} IL-3, IL-5, and GM-CSF prolonged eosinophil survival to high significance (not shown). When compared with the medium control, IL-1 β , IL-8, IL-12, PAF, eotaxin, and TNF- α were not able to modulate occurrence of eosinophil death (not shown). In contrast, dexamethasone or hydrocortisone used in concentrations known to significantly inhibit apoptosis in human neutrophils (10^{-5} to 10^{-6} mol/L) resulted in a significant induction of eosinophil apoptosis. These results are in line with published data.^{13,14} After 24 hours, 1000 to 2000 U/mL IL-4 inhibited eosinophil survival, whereas after 48 hours, IL-4 at concentrations of 100 U/mL or more markedly reduced eosinophil survival in all subjects studied (Fig 1). Compared with the effect of dexamethasone (Fig 1) and hydrocortisone (not shown), IL-4 was less potent in the reduction of eosinophil survival. To investigate whether IL-4 elicits this lethal effect even in the presence of the cytokines IL-3, IL-5, and GM-CSF, cocubation and preincubation studies were done. However, IL-4 in preincubation for 30 minutes (not shown) and in cocubation (Fig 2) did not overcome the survival-prolonging effects of IL-3, IL-5, and GM-CSF after 48 to 72 hours. Only after 96 hours was IL-4 able to significantly inhibit the survival-promoting effect of the proeosinophilic cytokines (Fig 2).

To verify that the observed effects were, in fact, mediated by IL-4, eosinophils were incubated with either IL-4 alone or in combination with polyclonal anti-IL-4 antibodies. It was shown that this polyclonal antibody neu-

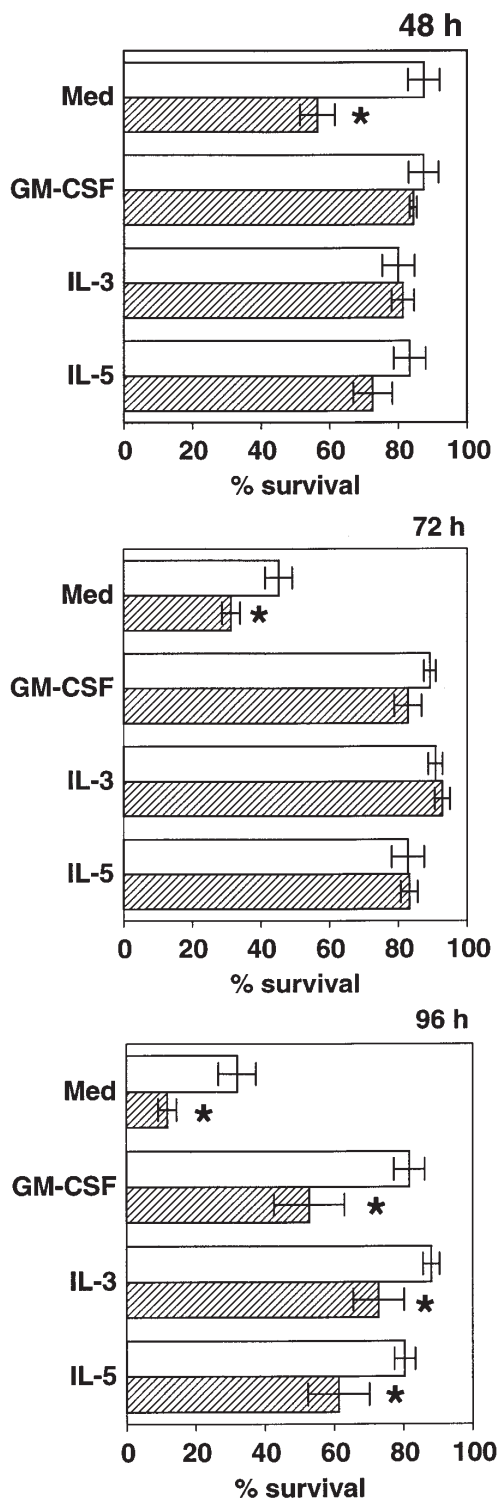


FIG 2. Effect of IL-4 on eosinophil survival in coincubation with cytokines. After 96 hours, costimulation with IL-4 antagonized survival-prolonging effect of IL-3, IL-5, and GM-CSF. Eosinophils were incubated with either IL-3, IL-5, or GM-CSF (100 U/mL each) with (filled bars) or without 1000 U/mL IL-4 (unfilled bars) for up to 96 hours. Data are presented as mean $\pm$ SEM of 5 to 11 separate experiments with eosinophils obtained from nonatopic subjects. Similar results were obtained after preincubation with IL-4 for 30 minutes followed by stimulation with IL-3, IL-5, or GM-CSF (not shown).

tralized the induction of eosinophil cell death mediated by IL-4, whereas eosinophils stimulated with the neutralizing antibody or control antibody alone showed survival rates similar to medium (Fig 3).

In further experiments, it was demonstrated that IL-4 not only decreased eosinophil survival in nonatopic eosinophils but also in subjects with active atopic dermatitis (Fig 4). In vitro, we recently demonstrated significantly delayed eosinophil apoptosis in subjects with atopic dermatitis when compared with nonatopic subjects.²² Thus in atopic dermatitis, IL-4 has a much more dramatic effect on the induction of eosinophil cell death, and this effect reached high significance even at later time points (up to 144 hours).

IL-4 induces eosinophil apoptosis

It was confirmed by the ELISA technique that the IL-4-mediated inhibition of eosinophil survival was a result of induction of apoptosis. Induction of eosinophil apoptosis by IL-4 in a concentration-dependent manner was confirmed by the detection of significant higher levels of nucleosomal DNA fragments in the eosinophil culture supernatants when compared with enriched medium alone (Fig 5).

Furthermore, eosinophils stimulated with hydrocortisone, dexamethasone, and IL-4 exhibited the classic "ladder" pattern of internucleosomal DNA cleavage indicative of endogenous endonuclease activation, the hallmark of apoptosis. IL-4 at concentrations of 100 U/mL or more induced apoptosis, as demonstrated by DNA fragmentation (Fig 6). When eosinophils were preincubated with neutralizing antibodies against IL-4 followed by stimulation with IL-4, the classic "ladder" pattern was not found (Fig 6). Whereas IL-3, IL-5, and GM-CSF significantly delayed the occurrence of the DNA laddering when compared with medium, IL-1 β , IL-8, IL-12, TNF- α , and eotaxin had no effect (not shown).

No effect of IL-4 on eosinophil Fas antigen surface expression, the release of ECP, and the release of ROS

It has been demonstrated that Fas antigen is a functional molecule on human peripheral blood eosinophils that can regulate eosinophil apoptosis in vitro.²³ To assess potential involvement of Fas antigen in IL-4-mediated induction of eosinophil apoptosis, a flow cytometric analysis was performed. Constitutive Fas antigen/CD95 expression on eosinophils did not change over time in culture, and stimulation of eosinophils with IL-4 (10 to 2000 U/mL) did not modulate eosinophil surface expression of Fas antigen in any of the subjects studied (not shown). Moreover, IL-4 had no effect on the eosinophil expression of CD9 or CD16 (not shown).

To further determine functional activities of IL-4 on freshly isolated peripheral blood eosinophils, the release of ECP was assessed. It was shown that after 2 hours, neither IL-4 nor IL-1 β , IL-3, IL-5 (100 U/mL each), IL-8 (50 ng/mL), GM-CSF (100 U/mL), or PAF (10⁻⁶ mol/L) were able to stimulate the release of ECP in the supernatant in the absence or presence of cytochalasin B. In contrast, as has been described previously,¹⁷ C5a (10⁻⁸

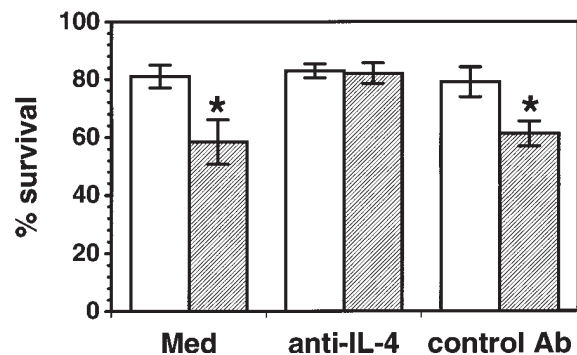


FIG 3. IL-4-inhibited eosinophil survival was abrogated in copresence of neutralizing anti-human IL-4 antibody. Eosinophils were incubated with control medium (*Med*), neutralizing polyclonal sheep anti-human IL-4 antibodies (neutralizing 100 U/mL), or with control antibody (*Ab*) and stimulated with control medium (unfilled bars) or with IL-4 (100 U/mL, filled bars) for 48 hours. Viability of eosinophils was determined with trypan blue dye exclusion. Data are presented as mean $\pm$ SEM of 7 experiments.

mol/L) in the presence of cytochalasin B (5 μ g/mL) resulted in a significant release of ECP (125 ± 32 ng/mL supernatant; $P = .002$) (not shown).

Confirming previously published results, C5a (10^{-8} mol/L), PAF (10^{-7} mol/L), IL-5, TNF- α , and GM-CSF (100 U/mL each) resulted in a significant release of ROS, whereas 10 to 2000 U/mL IL-4 did not induce the release of ROS in fresh eosinophils, as assessed by highly sensitive lucigenin-dependent chemiluminescence (not shown).

DISCUSSION

Taken together, the current data clearly demonstrate by qualitative and quantitative assay systems that IL-4 induces apoptosis in peripheral blood eosinophils *in vitro*. Our data are in line with published results demonstrating inhibition of eosinophil apoptosis by IL-3, IL-5, and GM-CSF and induction of eosinophil apoptosis by corticosteroids.^{10,11,13} However, we could not confirm previous data suggesting an inhibitory effect of PAF on eosinophil survival.²³ In contrast, IL-1 β , IL-8, and PAF, which have been shown to be involved in the accumulation of tissue eosinophils,^{24,25} had no significant effect on eosinophil survival. In addition, the recently defined eotaxin, representing a potent chemotaxin and activator of eosinophils, had no effect on eosinophil apoptosis. TNF- α , known to be a potent inducer of apoptosis in several cell types, did not induce eosinophil apoptosis. Thus the IL-4-induced apoptosis in eosinophils may be a cytokine-specific effect. As mentioned in previous experiments, IL-4 was unable to maintain the viability of eosinophils.¹¹ However, the data achieved with IL-4 were not shown in detail, and thus the authors may not have recognized the reducing effect of IL-4 on cell viability. Our findings are supported by previous results demonstrating that rhIL-4 inhibited IL-3-induced and IL-5-induced eosinophil differentiation from cord blood

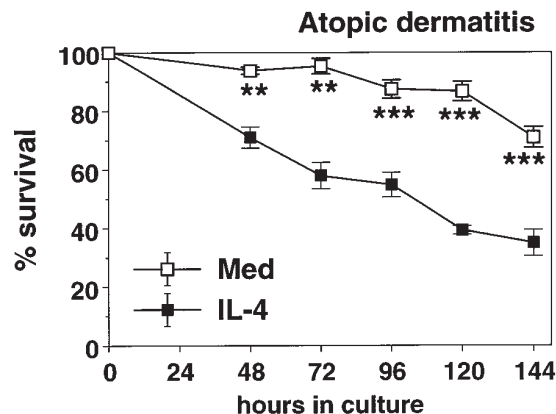


FIG 4. IL-4-inhibited survival in atopic dermatitis eosinophils. Comparison of eosinophil survival stimulated by medium (unfilled bars) or by 100 U/mL IL-4 (filled bars) after up to 144 hours in subjects with active atopic dermatitis ($n = 4$ to 6). Data are given as mean $\pm$ SEM. ** $P < .01$, *** $P < .0001$, IL-4 vs medium.

mononuclear cells and that eosinophil differentiation was significantly increased by the addition of anti-IL-4 antibody.²⁶ Moreover, data presented clearly demonstrated that IL-4-mediated induction of apoptosis was abolished by the addition of neutralizing antibody to IL-4, strongly suggesting that reduced viability and induced apoptosis are specific for IL-4.

IL-4 has been shown to be uniquely effective in the enhancement of apoptosis in human monocytes that have had prior exposure to either bacterial products or inflammatory cytokines.²⁷ In this study, IL-4 alone acted directly on resting eosinophils without the need of prior preactivation.

In previous studies, a differential effect of IL-4 on normal versus atopic individuals has been demonstrated. For example, IL-4 was shown to induce chemotaxis of blood eosinophils from patients with atopic dermatitis but not from normal individuals.²⁸ Moreover, IL-4 upregulated Fc ϵ RI α -chain messenger RNA in activated eosinophils of subjects with nasal allergy but not in normal individuals.²⁹ This differential response to IL-4 is confirmed by our findings that showed a more dramatic induction of apoptosis in atopic dermatitis eosinophils compared with eosinophils from nonatopic subjects. As published recently, it was shown that monocytes in subjects with atopic dermatitis did not undergo apoptosis in response to IL-4, whereas normal monocytes did.³⁰ Of note, in some cultures of atopic dermatitis monocytes, the opposite effect, a further reduction in apoptosis, was demonstrated with addition of IL-4 (100 and 500 U/mL).³⁰ Taken together, the findings by Mangan et al,²⁷ Bratton et al,³⁰ and the data here demonstrating IL-4-mediated induction of apoptosis in potent inflammatory cells suggest that the response to IL-4 may contribute to resolution of inflammation.

Recently, it has been shown that IL-4 delays neutrophil apoptosis.³¹ Thus not only glucocorticosteroids

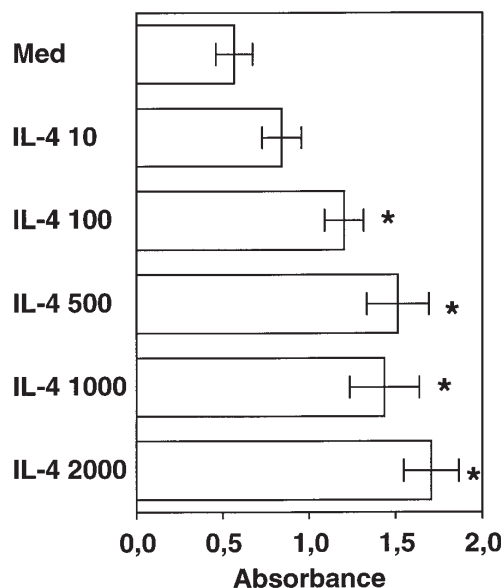


FIG 5. Stimulation with IL-4 resulted in detection of nucleosomal DNA fragments in eosinophil culture supernatants. Apoptosis as determined by amount of nucleosomal DNA fragments (*Absorbance*) in supernatants of eosinophils cultured for 48 hours with medium (*Med*) or 10 to 2000 U/mL IL-4 (for details of ELISA procedure see Methods section). * $P < .05$ indicated IL-4 concentration vs medium. Data are presented as mean $\pm$ SEM of 5 separate experiments with different donors.

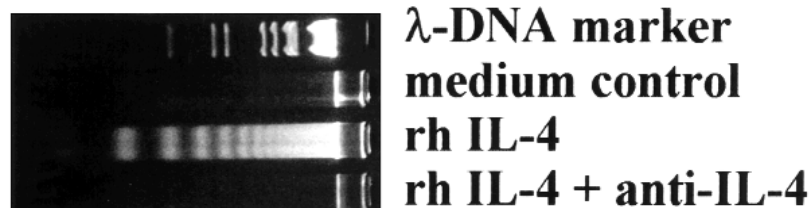


FIG 6. Anti-IL-4 mAb-abrogated eosinophil apoptosis induced by IL-4. Analysis in agarose gels of DNA fragments isolated from human peripheral blood-derived eosinophils cultured for 48 hours in medium control, rhIL-4 (100 U/mL), or rhIL-4 (100 U/mL) preincubated with polyclonal sheep anti-human IL-4 mAb (neutralizing 100 U/mL IL-4) by method described in Methods section. After electrophoresis of DNA in 1.8% agarose and staining with ethidiumbromide, DNA was viewed by UV fluorescence. Only eosinophils stimulated with IL-4 exhibited typical ladder consisting of characteristic 200 base pair DNA fragments. Eosinophils stimulated with anti-human IL-4 mAb alone did not demonstrate DNA laddering (not shown).

but also IL-4 show opposite effects on the apoptosis of neutrophils and eosinophils, respectively.¹⁵

Fas ligand/Fas interactions were shown to be involved in the regulation of eosinophil apoptosis.³² We therefore studied the expression of Fas antigen on eosinophils in the context of IL-4-induced apoptosis. It was shown that eosinophils expressed low but consistently detectable levels of Fas antigen (CD95), but induction of apoptosis by IL-4 did not show any modulating effect.

Our findings strongly provide evidence for the presence of functional IL-4 receptors on eosinophils. There have been few reported studies proposing the presence of functional IL-4 receptors on human eosinophils.³³ How-

ever, it is important to note that eosinophils express the common γ chain, which is an indispensable functional component of IL-2, IL-4, and IL-7 receptors.³⁴ With regard to functional effects, IL-4 was, for example, suggested to facilitate eosinophil recruitment from circulation into tissue³⁵ and to inhibit IgG-triggered eosinophil function,³⁶ and it was implicated to mediate antitumor activity through eosinophils.³⁷ To investigate potential effects of IL-4 on freshly isolated eosinophils, triggering of representative functions such as degranulation and release of ROS was evaluated. It was shown that IL-4 stimulation for 2 hours did not induce the release of ECP in the presence or absence of cytochalasin B. Only C5a

in the presence of cytochalasin B induced a significant release of ECP, as reported previously.¹⁷ Confirming published data, we found that IL-5, PAF, C5a, GM-CSF, and TNF- α directly induced eosinophil superoxide production, as determined by lucigenin-dependent chemiluminescence.^{17,38,39} However, IL-4 did not induce eosinophil superoxide production in vitro.

IL-4 is a cytokine present, for example, in the milieu of atopic dermatitis and allergic asthma.⁴⁰⁻⁴³ On the one hand, a role for IL-4 regulation of IgE synthesis is supported, and on the other hand, several studies showed that IL-4 is a growth factor for T cells and has proinflammatory effects on monocytes. Our observation that IL-4 mediates induction of eosinophil apoptosis seems to contradict a number of recent studies suggesting that overproduction of IL-4 may be important for induction of allergic inflammation.^{40,44} However, in contrast to these proinflammatory effects, IL-4 inhibits antibody-dependent cellular cytotoxicity, inhibits expression of Fc γ receptors, and downregulates production of nitric oxide, IL-1, IL-6, and TNF- α while stimulating production of IL-1 receptor antagonist (see Reference 45 for review).

The paradoxical finding of eosinophilia and IL-4 overproduction together with IL-4-mediated eosinophil apoptosis may be explained by the speculation that in vivo, other cytokine(s) might alter IL-4 responsiveness. Thus IL-4-mediated eosinophil apoptosis may have physiologic relevance if the eosinophil is not primed by the so-called proeosinophilic cytokines (IL-3, IL-5, and GM-CSF) and if IL-4 responsiveness is not altered by other cytokine(s) or after priming for several days when IL-4 is able to overcome the survival-prolonging effect.

Locally, there may be a balance between IL-4 and the cytokines IL-3, IL-5, and GM-CSF in regulating eosinophil apoptosis. In atopic dermatitis, for example, the cell infiltrate depends on the duration of the skin lesion. Increased numbers of eosinophils are observed in chronic but not acute atopic dermatitis skin lesions.⁴⁶ Moreover, in contrast to acute lesions of atopic dermatitis, chronic lesions of atopic dermatitis were found to be associated with relatively few cells expressing mRNA for IL-4 and significantly greater numbers of IL-5 mRNA-positive cells.⁴⁶ Taken together, acute skin inflammation in atopic dermatitis is associated with a predominance of IL-4 expression accompanied by a lymphocytic cell infiltrate, whereas maintenance of chronic inflammation is predominantly associated with decreased IL-4 expression but a significant increase in IL-5 expression and eosinophil infiltration. In asthma, this progress may be confirmed by data demonstrating an apparent decrease in the number of IL-4-positive cells in tissues from mildly asthmatic patients subjected to allergen challenge compared with diluent.⁴⁷ Moreover, "intrinsic" asthmatic subjects exhibiting normal levels of IgE and IL-4 but increased levels of IL-5 (IL-5⁺, IL-4⁻) often have eosinophilia to a greater degree than "extrinsic" asthmatic subjects who have increased levels of both interleukins (IL-5⁺, IL-4⁺).^{48,49} Thus the temporal and quantitative relation of cytokines produced during inflammation may

determine the ultimate fate of eosinophils at the site of allergic inflammation.

In conclusion, our results strongly suggest that IL-4 specifically promotes eosinophil apoptosis and that depending on the target cell, IL-4 appears to have not only proinflammatory activities but also an anti-inflammatory potential. Because IL-4 enhances apoptosis in eosinophils, it may hasten the elimination of eosinophils from a chronically inflamed lesion and thereby control eosinophil-mediated pathogenesis of inflammation.

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