Fas-Mediated Apoptosis in Cultured Human Eosinophils

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Previous studies have shown that cytokine-deprived eosinophils undergo apoptosis, yet the mechanisms governing this phenomenon remain obscure. Fas antigen is a transmembrane glycoprotein belonging to the tumor necrosis factor receptor family. Cross-linking of Fas antigen in numerous cell types leads to apoptosis. In the present study, we examined the potential role of Fas antigen in the apoptosis of purified blood eosinophils from healthy donors. Cytokine-deprived eosinophils exhibited a time-dependent loss in viability, accompanied by an increase in the number of apoptotic nuclei and in the expression of Fas antigen and its mRNA, as shown by flow cytometry and reverse transcriptase–polymerase chain reaction, respectively. Cross-linking of Fas antigen with an agonistic anti-Fas mono-
clonal antibody (MoAb) induced a dose- and time-dependent increase in the number of apoptotic nuclei. Furthermore, using an in vitro coculture system, we showed engulfment of anti-Fas MoAb–treated eosinophils by monocyte-derived macrophages. Finally, incubation of eosinophils with the corticosteroid, dexamethasone, induced apoptosis and augmented that triggered by anti-Fas MoAb. Together, these observations suggest that Fas antigen expression and activation is involved in the apoptosis of human eosinophils and may contribute to the resolution of inflammatory allergic reactions in which eosinophil accumulation is a prominent feature.

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Tissue and Blood eosinophilia are associated with a wide variety of disease states, including infection with helminthic parasites, bronchial asthma, atopic allergy, and a number of malignant disorders. Increased numbers of eosinophils are found in blood, bronchial tissue, and bronchoalveolar lavage fluid from asthmatic patients. Release of lipid mediators and cytotoxic basic proteins by activated eosinophils reportedly contributes to the development of bronchial hyperreactivity, one of the characteristic manifestations of bronchial asthma, and correlates with the degree of severity of this disease.

The infiltration and maintenance of eosinophils at the site of inflammatory reactions result mostly from the generation of cytokines, particularly from activated T-lymphocytes, such as interleukin-5 (IL-5), IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF). These cytokines induce the proliferation and differentiation of the eosinophil lineage from their precursors and prime mature eosinophils for enhancing in vitro responsiveness to various stimuli.

In vitro liquid culture systems, eosinophils do not proliferate and survive exclusively in the presence of IL-5, GM-CSF, or interferon gamma. Deprivation in these cytokines results in cell death, characterized by morphologic changes characteristic of apoptosis, including the formation of DNA fragments of approximately 185 bp because of specific cleavage between nucleosomes by intracellular nucleases.

Fas (APO-1/CD95) is a membrane glycoprotein belonging to the tumor necrosis factor receptor family. Fas antigen is expressed on the surface of a variety of cell types, including thymocytes, T and B cells, monocytes, macrophages, keratinocytes, and epithelial and endothelial cells. Cross-linking of Fas antigen with specific antibodies results in cellular apoptosis, suggesting a mechanism for in vivo cell deletion, as proposed for T lymphocytes. However, at this time, the ability of human granulocytes, in particular eosinophils, to express Fas antigen and undergo apoptosis on cross-linking with agonistic antibodies remains to be elucidated.

In the present study, we examined Fas antigen expression and function in peripheral blood (PB) eosinophils from healthy donors during in vitro culture in the absence of cytokines. Fas-dependent eosinophil apoptosis was explored using flow cytometry, DNA fragmentation, and analysis of morphologic changes and ultrastructural characteristics. Furthermore, to verify the hypothesis that ligation of Fas antigen may constitute one of the mechanisms involved in the resolution of eosinophilic inflammation, experiments were designed in which anti-Fas monoclonal antibody (MoAb)–activated eosinophils were engulfed by monocyte-derived macrophages.

Finally, to test the possibility that corticosteroids may act on eosinophil apoptosis, either directly or by interfering with Fas expression and/or activation, the effect of dexamethasone on Fas-dependent and -independent apoptotic death was also examined.

Materials and Methods

Cell Purification and Culture

Eosinophil preparation. Human peripheral venous blood from healthy donors (eosinophil counts, 0.04 to 0.51 × 10^6/L) was obtained from the Centre de Transfusion Sanguine (Paris, France) and anticoagulated with 14% (vol/vol) of a solution containing 89 mmol/L trisodium citrate, 16 mmol/L sodium phosphate, and 130 mmol/L dextrose, pH 5.6. Each experiment was performed with eosinophils obtained from a single donor.

Eosinophils were isolated after removal of mononuclear cells by separation of blood over Ficoll Paque and depletion of the neutrophils using Percoll gradient (Pharmacia Biotech, Orsay, France), followed by an immunomagnetic method. Briefly, freshly drawn blood was diluted twice in phosphate-buffered saline (PBS), pH 7.4, containing 13 mmol/L trisodium citrate and 10% human plasma (Cryo 2; Centre de Transfusion Sanguine). Thirty milliliters of diluted blood was layered on 15 mL isotonic Ficoll Paque solution at 1.077 g/mL, pH 7.4. After centrifugation (20 minutes at 1,000gmax and 20°C), platelets and mononuclear cells were removed. To lyse...
the erythrocytes, cell pellets were incubated for 10 minutes at 4°C with an ice-cold isotonic ammonium chloride solution containing 155 mmol/L NH₄Cl, 10 mmol/L KHCO₃, and 0.1 mmol/L Na₂EDTA, pH 7.1. The cells were then centrifuged (4 minutes at 400g_{max} and 4°C) and washed once (4 minutes at 400g_{max} and 20°C) in PBS containing 13 mmol/L trisodium citrate and 10% human plasma. Mixed granulocytes were resuspended in 50 mL RPMI 1640 (GIBCO BRL, Eggeny, France) supplemented with 0.5% (vol/vol) human plasma albumin (Centre Régional de Transfusion Sanguine, Lille, France) and then incubated for 30 minutes at 37°C to restore the initial densities of the cells. After this incubation period, the cells were washed and resuspended at a concentration of 40 to 60 x 10^6/mL in PBS containing 13 mmol/L trisodium citrate and 10% human plasma. Then granulocyte suspension was enriched in eosinophils by layering 1 mL of the cell suspension on 4 mL of an isotonic Percoll solution at 1.085 g/mL, pH 7.4 (Pharmacia Biotech). After centrifugation (20 minutes at 1,000 g_{max} and 20°C), normodense cells were collected from the interface at 1.085 to 1.100 g/mL and washed. To remove residual neutrophils that specifically express CD16 antigen, 15 x 10^6 cells were resuspended in 50 mL PBS supplemented with 13 mmol/L trisodium citrate and 10% human plasma and incubated for 30 minutes at 4°C with 15 µL of an anti-CD16 MoAb conjugated to magnetic microbeads (CD16 MACS Microbeads; Miltenyi Biotec, Bergisch Gladbach, Germany). The cell suspensions were subsequently transferred on the top of a separation column in a magnetic field (MiniMACS Separation Column; Miltenyi Biotec). Five hundred microliters of PBS containing 13 mmol/L trisodium citrate and 10% human plasma were passed through the column and nonmagnetically labeled eosinophils were collected and washed. Eosinophils were counted on a hemocytometer, and viability (98.3% ± 0.3%, n = 60) was assessed by the trypan blue exclusion method. Purity of the final cell suspension (99.3% ± 0.1%, n = 60) was evaluated after staining of cytospin preparations with Diff-Quik dye (Merz Dade; Baxter Dade, Duedingen, Switzerland). Eosinophils were then resuspended in RPMI 1640 (GIBCO BRL) supplemented with antibiotic-antimycotic solution (GIBCO BRL), 10% fetal calf serum (FCS) and 2 mmol/L L-glutamine (both from Boehringer Mannheim, Meylan, France) and incubated at 37°C in a 5% CO₂ atmosphere for 6 to 72 hours.

**Macrophage preparation.** Mixed mononuclear cells were aspirated from the interface of the Ficoll Paque preparation, and monocyte-derived macrophages were prepared as previously described. Briefly, mixed mononuclear cells were washed once with PBS and resuspended at 4 x 10^6 cells/mL in RPMI 1640 supplemented with antibiotic-antimycotic solution, 10% FCS, and 2 mmol/L L-glutamine. Cell suspensions (1 mL/well) were incubated in four-well Lab-Tek chamber slides (Nunc Inc, Naperville, IL) for 2 hours at 37°C in a 5% CO₂ atmosphere. Nonadherent cells were then removed, and monocytes were cultured for 3 days in the same medium as above. The medium was renewed once, and the culture was continued for 4 days. These conditions allow maturation of monocytes to macrophages, as previously described.

**Fas Expression During Spontaneous Eosinophil Apoptosis**

**Flow cytometry.** Freshly purified eosinophils were suspended at 1.5 to 3 x 10^6 cells/mL, and 200-µL suspensions were cultured in flat-bottomed 96-well culture plates (TPP, Trasadingen, Switzerland). After 24, 48, or 72 hours of culture, expression of Fas antigen, cell viability, and apoptosis were evaluated by flow cytometry. Briefly, after two washes (400g_{max} for 4 minutes at 4°C) in PBS containing 1% bovine serum albumin (BSA) (Sigma, St Louis, MO), cell pellets were incubated at 4°C for 45 minutes with 100 µL mouse antihuman Fas antigen MoAb (clone CH-11; Immugenex, Los Angeles, CA) at 20 µg/mL, or with an isotype-matched mouse IgM (Dako, Trappes, France). Cells were then washed twice, and 50 µL FITC-conjugated antimouse IgM at 17 µg/mL (Sigma) was added to each sample. After incubation at 4°C for 45 minutes and two washes, cells were resuspended in 500 µL PBS containing 0.25 µg/mL propidium iodide (Sigma) and immediately applied to a FACScan flow cytometer (Becton Dickinson, Le Pont de Claix, France) equipped with a 480-nm argon laser. A total of 5,000 viable propidium iodide–unstained cells were analyzed with a LYSIS II software (Becton Dickinson). Results are expressed as the difference between the mean fluorescence intensity (MFI) of antihuman Fas antigen MoAb–labeled cells and mouse IgM isotype–labeled cells. The percentage of dead propidium iodide–stained eosinophils was directly calculated from the gated histograms.

To evaluate eosinophil apoptosis, DNA condensation was analyzed by flow cytometry after permeabilization with Triton X-100 and staining with propidium iodide, as previously described. A total of 5,000 nuclei were analyzed.

**mRNA isolation and reverse transcriptase-polymerase chain reaction analysis.** Two milliliters of eosinophil suspensions containing 1.5 x 10^6 cells/mL were cultured in flat-bottomed 24-well culture plates (TPP) for 12, 24, 36, or 48 hours. Total cellular RNA was isolated by the acid phenol-isopropanol-phenol-chloroform procedure. RNA was then reverse-transcribed using SuperScript reverse transcriptase and oligo(dT) primers, according to the manufacturer’s instructions (GIBCO BRL). The generated complementary DNA was amplified by reverse transcriptase–polymerase chain reaction (RT-PCR) using 2.5 U Taq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT) and 1 µmol/L human Fas antigen primers (5’ primer, 5’-GTAAGATATATGATTTGTGCAGCAG-3’; 3’ primer, 5’-CATTTTCCGTTGG-3’; Eurogentec, Sealing, Belgium) or human β-actin primers (Clontech Laboratories Inc, Palo Alto, CA). The size of the PCR product (∼500 bp) amplified by Fas primers was confirmed by Southern blot using a specific Fas cDNA probe kindly provided by Dr S. Nagata (Osaka Bioscience Institute, Osaka, Japan; data not shown). RT-PCR was performed with one cycle at 94°C for 5 minutes, followed by 30 cycles (94°C for 45 seconds, 60°C for 50 seconds, and 72°C for 1.5 minutes) and was achieved by one cycle at 72°C for 5 minutes in a final volume of 100 µL. The products were separated by electrophoresis on 1% agarose gel, containing ethidium bromide in Tris borate-EDTA buffer (×90 mmol/L Tris, 90 mmol/L boric acid, and 2 mmol/L EDTA, pH 8.0).

**Induction of Eosinophil Apoptosis by Anti-Fas MoAb Stimulation**

**Evaluation of chromatin condensation by flow cytometry.** Purified eosinophils (200 µL at 1.5 x 10^6 cells/mL) were incubated in flat-bottomed 96-well culture plates with 3 to 3,000 ng/mL mouse antihuman Fas antigen MoAb or 3,000 ng/mL control mouse IgM. After 6, 12, or 24 hours, apoptosis was assessed by flow cytometry as described earlier. To verify specificity of the anti-Fas MoAb, eosinophils were incubated with 3,000 ng/mL mouse antihuman IgM MoAb directed against the granulocyte-associated antigen, CD15 (clone CS3-1; Dako), which is constitutively expressed by human normodense eosinophils. Apoptosis was evaluated at 24 hours by flow cytometry as described earlier.

**Assessment of DNA fragmentation by electrophoresis.** Freshly purified eosinophils (200 µL at 1.5 x 10^6 cells/mL) were incubated in flat-bottomed 96-well culture plates with 3,000 ng/mL mouse antihuman Fas antigen MoAb or mouse IgM. After 6, 12, or 24 hours of culture, cells were pelleted and then washed in PBS, and electrophoresis was performed according to a modification of a previously described method. Briefly, cell pellets were incubated in 40 µL lysis buffer consisting of 10 mmol/L Tris-HCl (pH 8.0), 1 mmol/L EDTA, and 0.5% (wt/vol) sodium lauryl sarcosinate. Two microliters of a solution containing 10 ng/mL RNase A (Boehringer
mannheim) were added and samples were incubated at 50°C for 1 hour. Then, 2 µL of a solution containing 200 µg/mL proteinase K (Boehringer Mannheim) were added to each sample, and incubation continued for another hour at 50°C. Then, 10 µL of a solution containing 40% (wt/vol) sucrose, 0.25% (wt/vol) bromophenol blue, and 1% (wt/vol) low–melting-point agarose (Sigma) at 50°C were added to each sample or to 5 µL 0.25–µg/µL molecular size standards (DNA-MWM VI; Boehringer Mannheim) before loading into wells of a 1.8% low–melting-point agarose gel containing ethidium bromide. Electrophoresis was performed in Tris borate–EDTA 1X.

Electron microscopy. Two milliliters of eosinophil suspensions at 2 x 10^6 cells/mL were incubated in flat-bottomed 24-well culture plates with 3,000 ng/mL mouse antihuman Fas antigen MoAb or mouse IgM. After a 12-hour culture period, eosinophils were fixed for 1 hour at 4°C in Sorensen buffer (72 mmol/L Na₂HPO₄, 2 H₂O, 28 mmol/L NaH₂PO₄, H₂O; pH 7.3) containing 1.6% (vol/vol) glutaraldehyde (Sigma). Cells were then incubated three times for 15 minutes in Sorensen buffer, followed by centrifugation for 4 minutes at 400 x g, at 20°C. One hundred microliters of Sorensen buffer supplemented with 15% (wt/vol) BSA was added to each cell pellet. After centrifugation (1 minute at 9,000 x g, and 20°C), the supernatants were removed and 100 µL of an aqueous solution containing 25% glutaraldehyde was added to the pellets. Cell pellets were detached from the tube and incubated three times for 15 minutes in Sorensen buffer, followed by buffer removal. Pellets were then post-fixed for 1 hour by adding 1 mL of Sorensen buffer containing 2% OsO₄. After acetone dehydration, pellets were embedded into epoxy resin. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined with a JEM-I00 CX microscope (Jeol, Tokyo, Japan).

**Effect of Dexamethasone on Apoptosis, Fas Antigen Expression, and Activation**

Eosinophils (1.5 x 10⁶/mL) were incubated for 24 hours with 0.05, 0.5, or 5 µmol/L dexamethasone phosphate (Sigma), and Fas antigen expression was analyzed by flow cytometry. In separate experiments, eosinophils cultured in the presence of dexamethasone, as above, were stimulated with 3 ng/mL anti-Fas MoAb or isotype-matched murine IgM, and apoptosis was evaluated by flow cytometry at 24 hours.

**Data Analysis**

Results are expressed as the mean ± SEM of the indicated number of experiments. One- or two-way analysis of variance (ANOVA) was used to determine significance among groups. If a significant variance was found, Student’s t test for unpaired values was used to assess comparability between means. A P value not greater than .05 was considered significant.

**RESULTS**

**Kinetics of changes in eosinophil viability, apoptosis, and Fas antigen expression.** Culture of PB eosinophils from healthy donors for 0 to 72 hours was followed by a loss in viability, which was accompanied by an increase in the number of apoptotic nuclei as measured by flow cytometry (Fig 1). These phenomena were time-dependent and reached a plateau between 48 and 72 hours (Fig 1). Concomitantly, an increment in Fas antigen expression was observed after 48 and 72 hours of culture (Fig 1). Freshly isolated eosinophils constitutively express Fas antigen, because MFI values for control isotype IgM- and anti-Fas MoAb–labeled eosinophils were 7.7 ± 0.4 and 24.5 ± 1.1, respectively (n = 8, P < .001).

RNA extracted from purified eosinophils cultured for 12 to 48 hours was evaluated for Fas antigen mRNA expression by RT-PCR. Cultured eosinophils exhibit Fas mRNA expression at all time points during incubation, with only trace quantities detected in freshly isolated eosinophils (Fig 2).

**Fas antigen activation.** Cross-linking of purified eosinophils with murine antihuman Fas MoAb was followed by a time-dependent increase in the number of apoptotic nuclei as measured by flow cytometry (Fig 3A). Marked apoptosis was observed 12 hours after the beginning of culture and further increased at 24 hours. At 48 hours, the proportion of apoptotic nuclei approached 90% (data not shown).

Eosinophils incubated with the isotype-matched murine IgM underwent a slight apoptosis, particularly at 24 hours (Fig 3A). This phenomenon is not likely related to IgM addition, since untreated and IgM-treated eosinophils cultured for 24 hours showed a similar degree of apoptosis (Figs 1B and 3A).

To confirm the specificity of the effect of the anti-Fas MoAb, eosinophils were incubated with 3,000 ng/mL of a mouse antihuman CD15 IgM MoAb, and apoptosis was evaluated at 24 hours by flow cytometry. Similar proportions of apoptotic nuclei were found in IgM- and anti-CD15 MoAb–treated eosinophils (20.9% ± 3.8% and 14.0% ± 0.9%, respectively, n = 5 to 12; not statistically significant).

The dose-dependence of Fas-mediated eosinophil killing was then examined. Concentrations of anti-Fas MoAb ranging between 3 and 3,000 ng/mL induced a dose-dependent increase in the number of apoptotic nuclei at 24 hours (Fig 3B).

To confirm that Fas-mediated death of cultured eosinophils was an apoptotic process, the chromosomal DNA was extracted and analyzed by electrophoresis in agarose gel. DNA fragmentation was observed 12 and 24 hours after incubation of eosinophils with 3,000 ng/mL anti-Fas MoAb, but not with the same concentration of control murine IgM (Fig 4).

Changes in ultrastructural characteristics of anti-Fas (3,000 ng/mL)–treated eosinophils were then examined by electron microscopy. Whereas IgM-treated cells showed a normal morphology, anti-Fas–stimulated eosinophils showed several of the ultrastructural features of apoptosis, including condensation of nuclear chromatin and smoothing of the cell membrane (Fig 5).
To verify whether Fas-mediated eosinophil apoptosis would participate in the clearance of these cells at sites of inflammatory and allergic reactions, experiments were designed in which apoptotic eosinophils were engulfed by macrophages. Figure 6 illustrates that a 3-hour incubation of monocyte-derived macrophages with eosinophils that had been cultured for 12 hours with 3,000 ng/mL anti-Fas MoAb led to the phagocytosis of intact cells or their apoptotic bodies. Higher proportions of macrophages ingesting anti-Fas–as opposed to IgM– and anti-CD15–treated eosinophils were observed. Indeed, 11.0% ± 1.7%, 11.7% ± 1.9%, and 18.6% ± 2.2% macrophages-containing eosinophils were enumerated after incubation of the eosinophils with isotype control IgM, anti-CD15 MoAb, or anti-Fas MoAb, respectively (n = 5 to 8, P < .05).

**Effect of dexamethasone on spontaneous apoptosis and Fas antigen expression and activation.** Incubation of eosinophils with 5 μmol/L dexamethasone increased by 71.3% ± 32.0% (n = 7, P < .01) the number of apoptotic nuclei at 24 hours, as shown by flow cytometry (Figs 7 and 8). Low concentrations of dexamethasone (0.05 and 0.5 μmol/L) were ineffective (Fig 8A).

In separate experiments, dexamethasone at 5 μmol/L significantly augmented anti-Fas (3 ng/mL)–induced apoptosis at 24 hours (Figs 7 and 8). A 29.7% and 36.1% increase of anti-Fas–induced eosinophil killing was also observed on treatment of the cells with 0.05 and 0.5 μmol/L dexamethasone, respectively (differences not statistically significant; Fig 8B).

At early time points (6 and 12 hours), the highest concentration of dexamethasone (5 μmol/L) failed to modify Fas-dependent and -independent apoptosis (data not shown).

No changes in Fas antigen expression were detected in eosinophils (5 μmol/L)-treated and -untreated eosinophils after 24 hours of culture. Indeed, MFI values for anti-Fas–labeled dexamethasone-untreated and -treated eosinophils were 26.0 ± 4.3 and 29.0 ± 5.4, respectively, (n = 5, difference not statistically significant).

**DISCUSSION**

Eosinophil accumulation and activation in blood and tissues characterize a number of inflammatory diseases, includ-
Fig 4. Fas-mediated DNA fragmentation of purified cultured human eosinophils. Eosinophils were incubated with 3,000 ng/mL mouse antihuman Fas MoAb. At 6, 12, and 24 hours, the cells were lysed and total cellular DNA was loaded on an agarose electrophoresis gel containing ethidium bromide. Chromosomal DNA from eosinophils incubated for 12 hours with 3,000 ng/mL isotype-matched mouse IgM was also analyzed (lane IgM). M represents DNA size markers (0.15 to 2.1 kb).

Fig 5. Electron transmission micrographs of purified human eosinophils cultured for 12 hours in the presence of 3,000 ng/mL (a) isotype-matched mouse IgM or (b) mouse antihuman Fas MoAb. (a) Practically all the eosinophils exhibit a normal morphology, in contrast to (b), where most of the cells showed morphologic features of apoptosis, ie, nuclear chromatin condensation, smoothing of the cell surface, and integrity (absence of swelling) of the organelles. The eosinophil indicated by the arrow shows less advanced nuclear changes than the adjacent cells. Bar, 5 μm.
We then investigated the expression of Fas antigen and its mRNA in freshly purified and cultured eosinophils and verified whether this phenomenon paralleled apoptosis. Only trace quantities of Fas mRNA were present in freshly purified eosinophils, whereas an easily detectable expression was shown at all time points during culture. Consistent with this finding, a constitutive expression of Fas antigen on the cell surface, which further increased upon culture of the cells for 48 and 72 hours, was observed. These results indicate that in the absence of triggering, eosinophils are capable of expressing Fas mRNA and the corresponding protein at their surface for a few days.

Although the expression of Fas antigen was not increased 24 hours after the beginning of culture, more than 20% of the eosinophils were already apoptotic. A number of hypotheses to explain this discrepancy may be invoked, including (1) production of Fas ligand, which, upon interaction with Fas antigen expressed constitutively on the surface of eosinophils, would trigger apoptosis during the first 24 hours of culture; or (2) decreased availability of bcl-2, an oncogene capable of blocking apoptotic death in various cell types and in cytokine-deprived hematopoietic cell lines. However, it was beyond the aim of the present study to elucidate the functional role of these factors in the regulation of eosinophil apoptosis, and accordingly, these hypotheses are areas for further investigation.

When the sensitivity of eosinophils toward Fas-mediated apoptosis was tested using an agonistic anti-Fas MoAb, a marked and dose-dependent increase in the number of apoptotic nuclei was detected by flow cytometry. Kinetics study showed that apoptosis developed at 12 and 24 but not at 6 hours after anti-Fas stimulation, a result consistent with the analysis of DNA fragmentation. Interestingly, the amounts of anti-Fas MoAb required to elicit an effect were in the same range as those used on other human, but also on murine, cell types, indicating a similar susceptibility of cells from different origins to undergo apoptotic death following anti-Fas MoAb triggering.

When the kinetics of Fas expression and anti-Fas sensitivity were compared, it emerged that eosinophils exhibited an increase in Fas antigen expression 48 hours after the beginning of culture, although they were sensitive to anti-Fas-induced apoptosis at early time points (between 6 and 12 hours). As mentioned earlier, these results may be consistent with the presence of a functional Fas antigen on the surface of freshly purified eosinophils, which would render them
prone to respond rapidly to cross-linking with agonistic anti-Fas MoAbs. Importantly, constitutive Fas expression distinguishes eosinophils from T lymphocytes, which neither express Fas antigen nor undergo Fas-mediated apoptosis unless their activation is triggered through the CD3/T-cell receptor complex or by using nonphysiologic stimuli. In this respect, human eosinophils also differ from human keratinocytes, endothelial cells, or some myeloma cells, which exhibit a constitutive, although nonfunctional, expression of Fas antigen, as demonstrated by the failure of cytotoxic MoAbs to induce apoptotic death.

Electron microscopic examination of anti-Fas MoAb–stimulated eosinophils revealed a number of morphologic features of apoptosis, particularly nuclear chromatin condensation, smoothing of the cell surface, and integrity of the organelles. These experiments were conducted 12 hours after anti-Fas triggering, because long-term stimulations led to a complete loss of eosinophil structure and to a massive formation of debris, which prevented enough numbers of entire cells to be examined.

Engulfment of apoptotic cells, including neutrophils and eosinophils, by macrophages is one of the mechanisms involved in the resolution of tissue inflammation. Here, we suggest that Fas-mediated apoptosis of human eosinophils may play an important role in the removal of these cells from inflammatory sites, because increased proportions of macrophages that had ingested intact eosinophils or their apoptotic bodies were found after stimulation with anti-Fas MoAb, as opposed to treatment of cells with the isotype control IgM or with an anti-CD15 MoAb. This latter comparison ruled out the possibility that enhanced uptake of anti-Fas–treated eosinophils by macrophages was linked to the mere binding of the antibody to the cell surface.

In rare circumstances, we also noted an engulfment of intact eosinophils with no apparent signs of apoptosis, such as chromatin condensation (Fig 6B). This contradiction may be explained by the emergence on the eosinophil surface of modifications undetectable morphologically and that characterize the early stages of apoptotic processes. These may include appearance of vitronectin receptor, which is expressed in apoptotic cells and is responsible for their recognition by and further adhesion to macrophages.

Corticosteroids have a prominent place in the therapy for several eosinophil-related disorders, including bronchial asthma, since they reduce tissue inflammation via different mechanisms, including inhibition of the transcription of cytokine genes in lymphocytes and other immune and inflammatory cells. Thus, treatment of asthmatic patients with oral corticosteroids downregulates local bronchial eosinophilia and the associated increase in gene expression for IL-4, IL-5, and GM-CSF. However, many aspects of how corticosteroids control eosinophilic inflammation remain to be characterized. Although the capacity of T lymphocytes to undergo apoptosis when cultured in the presence of corticosteroids has been widely documented, little is known about the precise effect of these drugs on eosinophil survival. In one report, Wallen et al showed that IL-3, IL-5, and GM-CSF–mediated increased survival of human eosinophils is prevented by corticosteroids, suggesting that these drugs competitively antagonize the effect of eosinophil-active cytokines. Here, we went further, because we showed that...
dexamethasone, although unable to modify Fas antigen expression, promoted eosinophil apoptosis and augmented that induced by anti-Fas MoAb. These findings suggest that corticosteroids may modulate eosinophil death by interfering with the regulation of a variety of apoptotic signals, including Fas antigen activation.

In conclusion, and as proposed recently by Simon and Blaser,4 we hypothesize that a defective regulation of apoptosis may constitute one of the mechanisms for the induction and maintenance of tissue and blood eosinophilia observed in patients with eosinophilic disorders. Our results showing that Fas antigen is involved in human eosinophil apoptosis suggest that interventions aimed to selectively favor this process may have a potential therapeutic role in the resolution of inflammatory and allergic reactions.

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