

Ligation of intercellular adhesion molecule 3 induces apoptosis of human blood eosinophils and neutrophils

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Background: Intercellular adhesion molecule 3 (ICAM-3) is highly expressed on human granulocytes, including eosinophils and neutrophils, but the functions of ICAM-3 in these cells are not well understood.

Objective: Our studies test the hypothesis that ICAM-3 regulates granulocyte apoptosis.

Methods: Intercellular adhesion molecule 3 was activated by a mAb that recognizes an ICAM-3 epitope that binds its ligand, CD11a/CD18. In some experiments with eosinophils, recombinant human IL-5 or GM-CSF was added to mimic *in vivo* antiapoptotic conditions. Staining with annexin V–fluorescein isothiocyanate and propidium iodide identified apoptotic cells.

Results: Binding of ICAM-3 increased apoptosis of both eosinophils (18 and 48 hours) and neutrophils (18 hours). At 18 hours, eosinophil apoptosis increased from 31.4% $\pm$ 3.5 SE (IgG control) to 45.2% $\pm$ 3.8 SE (anti-ICAM-3), and neutrophil apoptosis increased from 48% $\pm$ 4.1 SE (IgG control) to 55.3% $\pm$ 4.5 SE (anti-ICAM-3). At 48 hours, eosinophil apoptosis increased 2-fold under baseline conditions and also in the presence of recombinant human IL-5 or GM-CSF. In both eosinophils and neutrophils, incubation with a blocking antibody against CD18 integrins blunted ICAM-3-induced apoptosis. In eosinophils, blocking peptides for caspases 8 and 9, proteases critical to apoptosis, also decreased ICAM-3-induced apoptosis to control levels.

Conclusion: Through its effect on eosinophil and neutrophil apoptosis, ICAM-3 may be an important anti-inflammatory molecule that can oppose the proinflammatory effects of IL-5 and GM-CSF.

Clinical implications: These findings provide a mechanism for apoptotic clearance of eosinophils and neutrophils involved in allergic inflammation that, unlike necrosis, does not cause nonspecific tissue injury. (*J Allergy Clin Immunol* 2006;118:831-6.)

Key words: ICAM-3, adhesion molecules, neutrophils, eosinophils, apoptosis

Granulocytes, specifically eosinophils and neutrophils, infiltrate tissues in many inflammatory diseases, including allergy and asthma. Granulocyte activation and clearance from tissues is regulated in part by apoptosis, or programmed cell death.¹⁻³ The mechanisms that control eosinophil and neutrophil apoptosis may thereby be critical in the pathophysiology of inflammatory diseases like allergy and asthma.^{4,5}

Intercellular adhesion molecule (ICAM)–3 (CD50) is one of many molecules that can modulate leukocyte apoptosis. ICAM-3 is unique in the ICAM adhesion protein family in that ICAM-3 is expressed only on leukocytes.^{6,7} ICAM-3 regulates leukocyte-leukocyte adhesion by binding to 3 ligands: two CD18 (β_2) integrins (CD11a/CD18 and CD11d/CD18) and dendritic cell-specific C-type lectin.⁷⁻¹⁰ In addition, studies that use activating mAbs to ligate ICAM-3 and mimic ligand (CD18 integrin) binding show that ICAM-3 induces leukocyte functions, including apoptosis. For example, ICAM-3 ligation decreases lymphocyte proliferation and induces thymocyte and bone marrow cell apoptosis.¹¹⁻¹³

Although ICAM-3 is highly expressed on eosinophils and neutrophils, how it modulates apoptosis in these cells is unknown. As in lymphocytes, ICAM-3 on eosinophils and neutrophils functions in both adhesion and intracellular signaling. Two adhesive roles of ICAM-3 include neutrophil-neutrophil adhesion during aggregation¹⁴ and neutrophil-macrophage adhesion during phagocytosis.¹⁵ Two signaling roles of ICAM-3 include CD18 integrin activation and cytokine secretion. In neutrophils, ICAM-3 ligation activates surface CD18 integrins to a proadhesive state¹⁴ and regulates IL-8 production.¹⁶ In eosinophils, ICAM-3 ligation decreases the production of GM-CSF, a cytokine that opposes granulocyte apoptosis.⁶ On the basis of this observation in eosinophils and the role of ICAM-3 in lymphocyte apoptosis, we hypothesized that ICAM-3 could induce eosinophil and neutrophil apoptosis.

METHODS

Subjects

Cells were isolated from the peripheral blood of subjects with mild allergic asthma and/or rhinitis. Immediate hypersensitivity was confirmed in each subject by at least 1 positive skin reaction by the

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Abbreviations used

FITC: Fluorescein isothiocyanate
 FMK: Fluoromethyl ketone
 ICAM: Intercellular adhesion molecule
 PI: Propidium iodide

prick-puncture technique to common allergens. Asthma was diagnosed by medical history and a physician diagnosis. No subjects received corticosteroids within 6 months of the study. The University of Wisconsin Institutional Review Board approved this study, and all participants gave written consent.

Isolation of eosinophils and neutrophils

Eosinophils and neutrophils were isolated from heparinized blood as previously described.¹⁷ Blood was centrifuged (700g for 20 minutes) over a 1.090 g/mL Percoll gradient (Amersham Pharmacia, Uppsala, Switzerland), and the mononuclear cell band was discarded. After hypotonic lysis of red blood cells, anti-CD16 beads and an AutoMacs magnetic system (Miltenyi Biotec, Auburn, Calif) were used to isolate eosinophils (negative selection) and neutrophils (positive selection) that were greater than 95% pure by direct microscopy and viable by trypan blue exclusion.

Eosinophil and neutrophil apoptosis assay

Isolated granulocytes were resuspended to 1×10^6 cells/mL in RPMI media (Invitrogen, Carlsbad, Calif) supplemented with L-glutamine (Invitrogen), penicillin-streptomycin (Invitrogen), and FBS (Invitrogen). Eosinophils were incubated with 1% FBS, and neutrophils were incubated with 10% FBS. Twenty-four-well plates were blocked with FBS for 1 hour. Then 500 μ L of the eosinophil or neutrophil suspension was added to each well. Mouse antihuman ICAM-3 mAb CAL 3.10 (R&D Systems, Minneapolis, Minn) or control IgG (irrelevant antikeyhole limpet hemocyanin mAb; R&D Systems) was added to 10 μ g/mL. In some experiments, cells were preincubated 15 minutes with antihuman CD18 (clone L130; Sigma, St Louis, Mo) at 10 μ g/mL before adding anti-ICAM-3 mAb. Select wells were treated with rhIL-5 or rhGM-CSF (0.1 ng/mL; R&D Systems).¹⁸ Autocrine GM-CSF production was triggered by incubating eosinophils in fibronectin-coated 96-well tissue culture plates (BD Biosciences, San Jose, Calif) with TNF- α (10 ng/mL) and cellular fibronectin (20 μ g/mL; Sigma).¹⁹

After incubation at 37°C/5% CO₂ for 18 to 48 hours, cells were harvested, centrifuged, and resuspended in binding buffer (Annexin/PI Apoptosis Detection Kit I; Pharmingen/BD, San Diego, Calif). Apoptosis was determined by staining with annexin V-fluorescein isothiocyanate (FITC; 5 μ L/test) and propidium iodide (PI; 5 μ L of 50 μ g/mL) according to kit instructions. Cells were analyzed by flow cytometry using a 15-mW argon ion laser at 488 nm. PI-negative and annexin V-positive cells were defined as apoptotic.

To determine whether tyrosine phosphorylation or oxygen radical production was important in ICAM-3-induced apoptosis, experiments were performed after pretreatment for 15 minutes with tyrosine phosphorylation inhibitors (genistein, 1 μ M; herbimycin A, 1 μ M) or antioxidants (catalase or superoxide dismutase, 500 U/mL; Sigma).

Caspase inhibition assay

The caspase inhibition assay used synthetic peptides (BioVision, Inc, Mountain View, Calif) that irreversibly inhibit caspase-3 (Z-DEVD-fluoromethyl ketone [FMK]), caspase-8 (Z-IETD-FMK), or caspase-9 (Z-LEHD-FMK). An inhibitor of all caspases

(Z-VAD-FMK), and a negative control peptide (Z-FA-FMK) served as controls. Eosinophils were pretreated for 1 hour with caspase inhibitors (2 μ M final concentration) before adding anti-ICAM-3 mAb or controls. After an 18-hour incubation, apoptosis levels were determined by annexin V/PI as previously described.

Activated caspase-3 assay

In an alternative approach, the role of caspase-3 was investigated by using a FITC-labeled mAb that detects the activated form of caspase-3 (Apo-Active 3 kit from Cell Technology, Mountainview, Calif). Eosinophils were incubated 18 hours with anti-ICAM-3 mAb or control IgG, resuspended to 1×10^6 cells/mL in fixative solution for 20 minutes, washed in PBS (Invitrogen), and resuspended in PBS with 1% Saponin (Sigma) to permeabilize the cell membrane. Samples were incubated 60 minutes with rabbit antiactive-caspase-3 (1 μ L per 10 μ L cell sample), washed, incubated 60 minutes with FITC-labeled goat-antirabbit antibody (10 μ L 1X), washed, and then analyzed by flow cytometry.

Data analysis

Results are expressed as the mean $\pm$ SEM. A 2-tailed *t* test (paired or unpaired) or ANOVA was used to determine significance between groups.

RESULTS**ICAM-3 ligation for 18 hours increased eosinophil and neutrophil apoptosis**

Ligation of ICAM-3 for 18 hours by a mAb (clone Cal 3.10) that mimics CD11a/CD18 binding^{20,21} increased eosinophil and neutrophil apoptosis (Figs 1 and 2). Apoptotic eosinophils (Fig 2, A) increased from 31.4% $\pm$ 3.5 (IgG control) to 45.2% $\pm$ 3.8 (anti-ICAM-3; *P* = .0045; *n* = 13). Live eosinophils (Fig 2, B) decreased from 51.5% $\pm$ 4.4 (IgG control) to 41.4% $\pm$ 3.7 (anti-ICAM-3; *P* = .034; *n* = 13). Although ICAM-3 binding also decreased dead eosinophils in some experiments, data did not reach statistical significance (16.4% $\pm$ 2.8 [IgG] to 11.9 $\pm$ 2.5 [anti-ICAM-3], *P* = .11).

Similarly, apoptotic neutrophils increased from 48% $\pm$ 4.1 (IgG control) to 55.3% $\pm$ 4.5 (anti-ICAM-3; *P* = .0077; *n* = 11). Live neutrophils decreased from 9.7% $\pm$ 1.6 (IgG control) to 3.4% $\pm$ 0.7 (anti-ICAM-3; *P* = .04; *n* = 11). ICAM-3 ligation did not affect dead neutrophils (data not shown). Treatment with IgG control did not change eosinophil or neutrophil apoptosis compared with media control (*P* > .05).

ICAM-3 ligation for 48 hours increased eosinophil apoptosis

We next examined the effect of ICAM-3 ligation on eosinophils incubated 48 hours. These experiments focused on eosinophils, because most neutrophils are apoptotic or dead beyond 24 hours. Experiments included eosinophils incubated in the presence of rhIL-5 or rhGM-CSF,²² antiapoptotic cytokines that simulate the *in vivo* prosurvival conditions that occur during inflammation.

Intercellular adhesion molecule 3 (ICAM-3) increased eosinophil apoptosis during long-term incubations, just as it did at 18 hours. At 48 hours, eosinophil apoptosis increased from 12.2% $\pm$ 1.2 (IgG control) to 21% $\pm$ 2

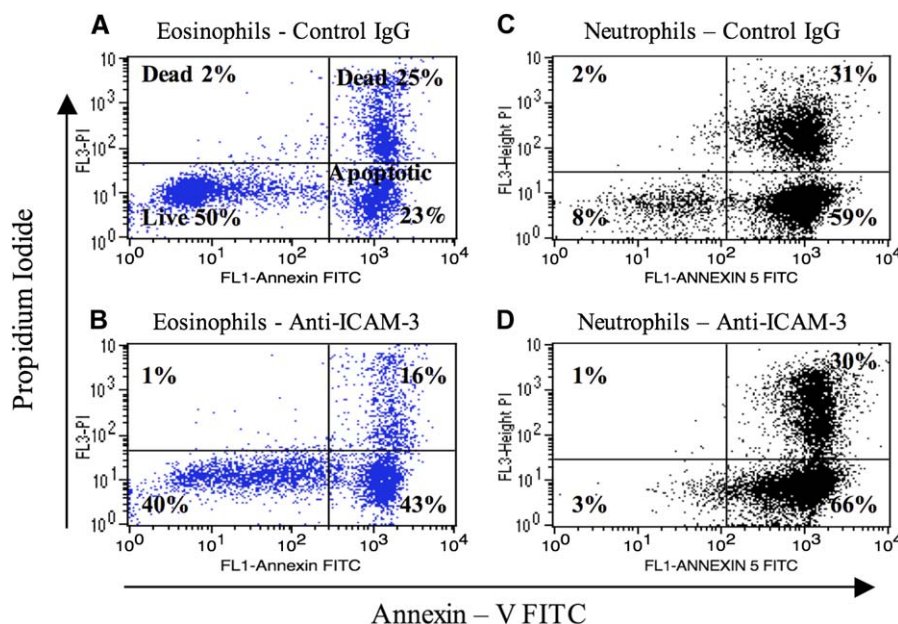


FIG 1. Distribution of live, apoptotic, and dead eosinophils (A, B) and neutrophils (C, D) after ICAM-3 ligation. Flow cytometry was used to detect live (PI⁻, annexin V⁻), apoptotic (PI⁺, annexin V⁻) and dead (PI⁺) cells after incubation for 18 hours with 10 μ g/mL anti-ICAM-3 mAb or IgG control. Representative dot plots of $n = 11$.

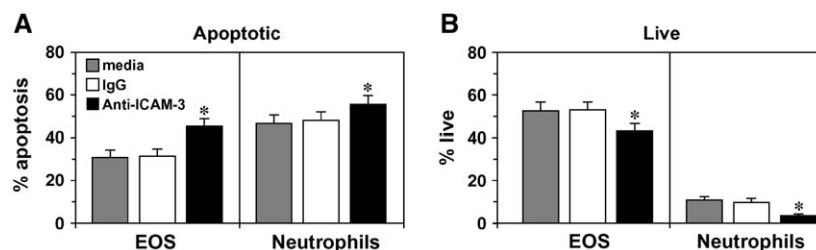


FIG 2. Effect of ICAM-3 ligation on baseline eosinophil (EOS) and neutrophil apoptosis (18 hours). EOS or neutrophils were incubated 18 hours with anti-ICAM-3 mAb or IgG control. The percent apoptotic (A) and live (B) cells was determined by flow cytometry. ICAM-3 ligation increased apoptotic and decreased live populations of EOS ($n = 13$) and neutrophils ($n = 11$). * $P < .05$.

(anti-ICAM-3; $P = .001$; $n = 11$; Fig 3). ICAM-3 increased eosinophil apoptosis even in the presence of rhIL-5 and rhGM-CSF, cytokines that promote eosinophil survival (Fig 3). ICAM-3 ligation doubled eosinophil apoptosis in the presence of rhIL-5 ($8.8\% \pm 1.1$ vs $15.6\% \pm 1.8$; $P = .002$; $n = 11$) or rhGM-CSF ($7.3\% \pm 1.8$ vs $15.8\% \pm 3.1$; $P = .03$; $n = 6$). As expected, GM-CSF and IL-5 decreased apoptosis compared with media control ($P < .05$). Under conditions (exposure to TNF- α and fibronectin) that stimulate eosinophil autocrine GM-CSF production, ICAM-3 ligation tripled apoptosis ($9.8\% \pm 2.2$ vs $28.2\% \pm 5$; $P = .001$; $n = 6$).

Blockade of CD18 abrogated ICAM-3-induced eosinophil and neutrophil apoptosis

A blocking mAb against CD18 integrins was used to investigate the role of CD18 integrins in ICAM-3-induced eosinophil and neutrophil apoptosis (Fig 4). Eosinophil apoptosis was lower after coincubation with anti-ICAM-3 and a blocking anti-CD18 mAb ($26.2\% \pm 3.4$)

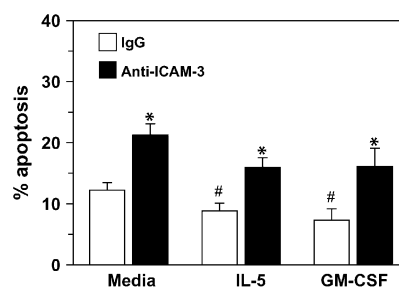


FIG 3. Effect of ICAM-3 ligation on eosinophil apoptosis in the presence of antiapoptotic cytokines (48 hours). Eosinophils were incubated 48 hours with media or antiapoptotic cytokines (0.1 ng/mL). ICAM-3 ligation increased apoptosis in eosinophils cultured in media ($n = 11$; $P = .001$), GM-CSF ($n = 6$), or IL-5 ($n = 11$) * $P < .05$, anti-ICAM-3 compared with IgG control. # $P < .05$, IL-5 and GM-CSF compared with media.

compared with anti-ICAM-3 alone ($41.3\% \pm 3.3$; $P = .0021$; $n = 11$). Similarly, neutrophil apoptosis was lower after coincubation with anti-ICAM-3 and a blocking

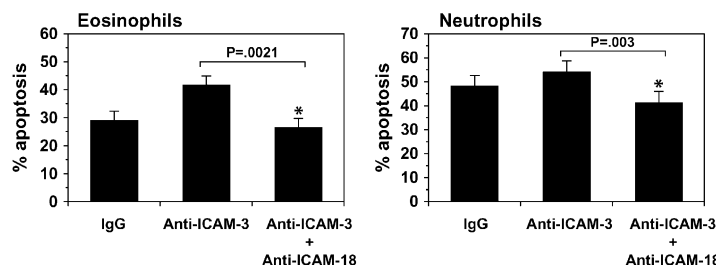


FIG 4. Role of CD18 binding on ICAM-3-induced apoptosis. Eosinophils or neutrophils were pretreated with a blocking antibody against CD18 integrins and incubated 18 hours in the presence of anti-ICAM-3 mAb or control IgG. Anti-CD18 mAb blocked ICAM-3-induced apoptosis ($n = 6$). * $P < .05$.

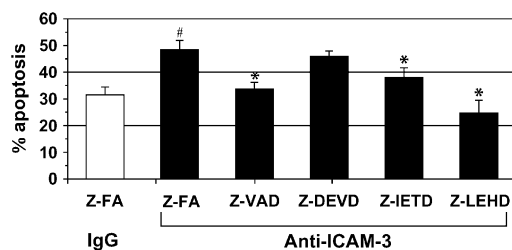


FIG 5. Role of caspase activation in ICAM-3-induced apoptosis. Eosinophils were incubated 18 hours with anti-ICAM-3 mAb and caspase inhibitors: Z-FA (negative control), Z-VAD (caspase family), Z-DEVD (caspase-3), Z-IETD (caspase-8), and Z-LEHD (caspase-9). Inhibitors of the caspase family ($n = 10$), caspase-8 ($n = 10$) or caspase-9 ($n = 4$), decreased apoptosis. * $P < .05$ for anti-ICAM-3/Z-FA compared with anti-ICAM-3/caspase inhibitors. # $P < .05$ for IgG/Z-FA compared with anti-ICAM-3/Z-FA.

anti-CD 18 mAb ($41.1\% \pm 4.8$) compared with anti-ICAM-3 alone ($53.8\% \pm 4.7$; $P = .003$; $n = 10$). In both eosinophils ($n = 6$) and neutrophils ($n = 4$), apoptosis in cells treated with anti-CD18 mAb alone was not different from IgG control (data not shown).

To determine whether ICAM-3 could induce eosinophil apoptosis through tyrosine phosphorylation or reactive oxygen species generation, we incubated eosinophils with pharmacologic inhibitors of tyrosine phosphorylation (herbimycin, genistein) or superoxide generation (superoxide dismutase [SOD], catalase). We found no difference in ICAM-3-induced apoptosis in the presence of media (48.3 ± 9.6 ; $n = 4$) compared with herbimycin (37.2 ± 6.8 ; $n = 4$), genistein (43.7 ± 10.7 ; $n = 4$), SOD (56.1 ± 19.4 ; $n = 3$), or catalase (58.4 ± 24.7 ; $n = 3$).

Blockade of caspase activation blunted ICAM-3-induced eosinophil apoptosis

Multiple signaling pathways are involved in eosinophil apoptosis, including activation of proteases in the caspase family. We measured ICAM-3-induced eosinophil apoptosis at 18 hours (Fig 5) in the presence or absence of synthetic peptides bound to FMK that irreversibly inhibit caspase activity: caspase family (Z-VAD), caspase-3 (Z-DEVD), caspase-8 (Z-IETD), and caspase-9 (Z-LEHD). In the presence of the peptide Z-FA, a negative control, ICAM-3-induced apoptosis was $47.3\% \pm 3.6$. The caspase family inhibitor decreased apoptosis to $32.2\% \pm 2.5$ ($P = .002$; $n = 10$). Both the caspase-8 inhibitor

($38\% \pm 3.7$; $P = .03$; $n = 10$) and the caspase-9 inhibitor ($24.7\% \pm 4.7$; $P = .003$; $n = 4$) also decreased apoptosis. Interestingly, a caspase-3 inhibitor did not block apoptosis ($46.6\% \pm 2.1$, $P = 0.4$; $n = 10$). The control peptide (Z-FA) compared with media had no effect on apoptosis in the presence of control IgG (data not shown) and or anti-ICAM-3 ($n = 10$, $P < .05$ for IgG/Z-FA vs anti-ICAM-3/Z-FA).

To confirm that caspase-3 is not involved, we investigated caspase-3 activation using an antibody specific for activated caspase-3. After incubation with anti-ICAM-3 or control IgG, eosinophils were permeabilized and incubated with anti-activated caspase-3-mAb and then a FITC-conjugated secondary antibody. Ligation of ICAM-3 did not increase levels of activated caspase-3 in eosinophils (mean fluorescence IgG control, 32.3 ± 12.4 , vs anti-active caspase 3, 20.9 ± 4.8 ; $n = 4$).

DISCUSSION

Our results show that ICAM-3 modulates human eosinophil and neutrophil apoptosis via integrin and caspase-dependent mechanisms. In eosinophils, the apoptotic signaling induced by ICAM-3 counteracts the prosurvival effects of exogenous rhIL-5 or rhGM-CSF. In neutrophils, even though more cells are apoptotic compared with eosinophils at baseline, as expected, ICAM-3 ligation still increases apoptosis. Both eosinophils and neutrophils require CD18 signaling. ICAM-3-induced apoptosis is thereby distinct compared with other mediators, such as glucocorticoids, that have opposing effects on eosinophil versus neutrophil apoptosis.²³ Unlike CD18 integrin blockade, inhibition of tyrosine phosphorylation or generation of reactive oxygen species did not block apoptosis. Our data suggest that the mechanism of ICAM-3-induced eosinophil apoptosis involves caspases 8 and 9, two proteases also important in spontaneous and Fas-induced eosinophil apoptosis.^{24,25} Unlike Fas-induced apoptosis, ICAM-3-induced apoptosis does not require caspase-3 activation, suggesting a role for other caspases or unique pathways in ICAM-3 signaling.

Intercellular adhesion molecule 3-induced eosinophil and neutrophil apoptosis may have important implications in inflammation and host defense. During allergic inflammation, for example, GM-CSF and IL-5 help recruit eosinophils and neutrophils to the lung and increase their

survival.¹ How lung inflammation subsequently resolves is not well understood, although cell death by apoptosis appears to be critical.¹⁻⁵ Our data suggest one way ICAM-3 could promote resolution of granulocyte inflammation. We speculate that when granulocytes are recruited to the lung, ICAM-3 and CD18 on neighboring leukocytes bind and thereby induce apoptosis. Apoptosis attenuates granulocyte functions and, unlike necrosis, is not accompanied by toxic mediator release and nonspecific tissue injury.²⁶ Finally, apoptosis induces surface expression of receptors, like phosphatidyl serine, that promote macrophage phagocytosis.

Our experimental design has some limitations that may affect our data interpretation. First, ICAM-3 ligation by a mAb may not absolutely mimic binding by the natural ligand, CD11a/CD18. However, this strategy has been useful in defining the signaling potential of ICAM-3 and other adhesion molecules, such as Fas, CD30, and CD69.^{14,27-32} Second, our experimental approach cannot rule out costimulation of apoptosis by granulocyte Fc receptors. However, our IgG control confirms that the response is not solely a result of Fc ligation. Third, the definition of apoptosis varies in the literature. Our data agree with other studies showing that eosinophils and neutrophils in early apoptosis externalize phosphatidyl serine (bind annexin V) and exclude PI.²⁶ PI⁺ cells are variably defined as late apoptotic or dead, and the functional relevance of these cells is unclear.

The mechanisms that mediate ICAM-3 apoptotic signaling are not yet defined, so we looked at several possibilities known to be important in granulocyte survival: (1) intracellular signaling events (tyrosine phosphorylation), (2) secondary mediators (oxygen metabolites, cytokines), (3) adhesion molecules (CD18), and (4) apoptotic proteases (caspases). Our investigations focused primarily on eosinophils because these cells have the greatest increase in ICAM-3–induced apoptosis. The first mechanism examined was tyrosine phosphorylation, an event that occurs in granulocytes after ICAM-3 ligation^{14,33} and during apoptosis initiated by other mediators.^{34,35} Our data using the nonspecific tyrosine phosphorylation inhibitors, herbimycin A and genistein, do not support a clear role for tyrosine phosphorylation in ICAM-3–mediated apoptosis. Studies with tyrosine kinase inhibitors that target the src pathways specifically activated by ICAM-3 ligation could show different results. The second mechanism examined was secondary mediators including oxygen metabolites and cytokines. Oxygen metabolite production contributes to constitutive, glucocorticoid, and Fas-induced eosinophil apoptosis.³⁶⁻³⁸ Two antioxidants, superoxide dismutase and catalase, do not inhibit ICAM-3–induced apoptosis. We previously showed that ICAM-3 ligation decreases eosinophil GM-CSF production. This event is a possible mechanism for increased apoptosis in the current study, particularly because ICAM-3 ligation increased apoptosis under conditions of autocrine GM-CSF production.

The third mechanism examined was CD18 integrin binding. We previously showed that ICAM-3 activates the

binding avidity of CD18 integrins on human neutrophil surfaces and induces homotypic aggregation.¹⁴ In the current study, granulocyte preincubation with anti-CD18 mAb decreased ICAM-3–induced apoptosis to control levels. Other studies on the mechanisms by which integrins regulate granulocyte apoptosis show conflicting results, suggesting that CD18 integrin signaling can both promote and inhibit apoptosis.^{39,40} Although CD18 blockade did not decrease ICAM-3–induced apoptosis in bone marrow leukocytes, both CD30-induced eosinophils and TNF- α –induced or FAS-induced neutrophil apoptosis were CD18-dependent.^{13,31,40}

The signaling mechanisms of CD18 and ICAM-3 are unclear. Ligation of ICAM-3 by our activating mAb induces both neutrophil¹⁴ and eosinophil aggregation (Kessel, unpublished data, July 2000). Anti-CD18 mAb completely inhibits neutrophil aggregation, presumably by blocking CD18 binding to ligands on neighboring neutrophils.¹⁴ Similarly, ICAM-3 signaling may activate CD18 integrins and induce adhesion to ICAM-3 or ICAM-1, events that would be inhibited by our blocking anti-CD18 mAb.^{20,21} We cannot exclude the possibility that CD18 integrin binding to ICAM-3 augments the signaling generated by our activating anti-ICAM-3 mAb. However, it seems more likely that CD18 integrin signaling is a required apoptotic cosignal for ICAM-3, as it is for Fas and TNF- α . In support of ICAM-3 and CD18 cosignaling, other studies have mechanistically linked CD18 signaling and caspase activation.^{40,41} Further studies will be needed to clarify the contribution from each β_2 integrin (CD11a/CD18, CD11b/CD18, CD11c/CD18 and CD11d/CD18).

The final mechanism examined was caspase activation. Stimulus-specific cascades of initiator and effector caspases cleave cellular proteins that activate apoptosis.²⁶ In eosinophils, ICAM-3–induced apoptosis requires caspases 8 and 9, similar to spontaneous apoptosis.²⁴ Fas-induced eosinophil apoptosis, in comparison, involves caspases 8 and 3. ICAM-3–induced eosinophil apoptosis does not require caspase-3, as shown by both inhibitory peptides and anti-activated caspase-3 mAbs. Glucocorticoid-induced eosinophil apoptosis also does not require caspase-3.⁴² Other caspases (6, 7) may be involved. How ICAM-3 and caspase signaling interact is not known. The 37–amino acid cytoplasmic tail of ICAM-3 does not contain a death domain, a unique 80–amino acid sequence in the cytoplasmic tail of Fas and other death receptors that activates caspase precursors.^{26,43}

Our data demonstrate that ICAM-3 joins the ranks of other surface receptors—including Fas, CD137, CD30, CD69, and CD45—that cause eosinophil apoptosis and override the effects of antiapoptotic cytokines.^{28,30,31,32,44} These data, in combination with our previous finding that ICAM-3 inhibits GM-CSF production, argue that ICAM-3 has anti-inflammatory effects that drive granulocytes to undergo apoptosis even in the cytokine-laden environment of inflamed tissue. ICAM-3–induced apoptosis may have an important role in regulating eosinophil and neutrophil inflammation in allergy and asthma.

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