

Human alveolar epithelial cells engulf apoptotic eosinophils by means of integrin- and phosphatidylserine receptor-dependent mechanisms: A process upregulated by dexamethasone

Darren W. Sexton, MSc, Morgan G. Blaylock, PhD, and Garry M. Walsh, PhD

Aberdeen, United Kingdom

Background: We previously demonstrated receptor-mediated apoptotic-eosinophil engulfment by human small airway epithelial cells, which may represent a potentially important mechanism in the resolution of allergic and asthmatic inflammation.

Objective: A549 cells were selected as being representative of alveolar epithelial cells, and their ability to ingest human apoptotic eosinophils was examined in terms of the effects of dexamethasone treatment and the receptor-mediated recognition mechanisms important in this process.

Methods: A549 epithelial-cell expression of $\alpha\text{v}\beta 3$, $\alpha\text{v}\beta 5$, CD36, and the phosphatidylserine receptor was established by using immunostaining and flow cytometry, and inhibition assays were examined by using the role of these receptors in apoptotic-eosinophil recognition by resting and dexamethasone-treated A549 epithelial cells. Electron microscopy confirmed engulfment of apoptotic eosinophils, and receptor clustering around apoptotic eosinophils was examined by using immunofluorescent labeling.

Results: A549 epithelial cells recognized and engulfed apoptotic eosinophils but not freshly isolated cells. Dexamethasone enhanced the number of A549 cells ingesting apoptotic eosinophils and dose dependently increased their capacity for ingestion. The tetrapeptide RGDS significantly inhibited apoptotic-eosinophil uptake by A549 cells, indicating a role for integrins in the recognition process. A549 cells expressed $\alpha\text{v}\beta 3$, $\alpha\text{v}\beta 5$, $\beta 5$, CD36, and the phosphatidylserine receptor, and expression of these receptors was not significantly increased after dexamethasone treatment. Engulfment of apoptotic eosinophils by A549 cells involved $\alpha\text{v}\beta 3$ -, CD36-, $\alpha\text{v}\beta 5$ -, and phosphatidylserine receptor-mediated recognition mechanisms and was associated with the formation of integrin focal adhesion complexes around apoptotic eosinophils.

Conclusions: These data further suggest a nonpassive role for airway epithelium in the resolution of eosinophilic inflammation in asthma. (*J Allergy Clin Immunol* 2001;108:962-9.)

Key words: Epithelial cells, eosinophils, apoptosis, asthma, phagocytic recognition, corticosteroids

Eosinophils play a critical role in diverse human conditions, most notably in allergic diseases, such as asthma.¹ Asthma is a complex disorder characterized by reversible airway obstruction, bronchial hyperresponsiveness, and airway inflammation, and the increased numbers of eosinophils found in the blood, sputum, and bronchoalveolar lavage fluid of asthmatic patients correlate with disease severity. Inappropriate accumulation of eosinophils and the subsequent release of their potent armory of mediators, including cytotoxic granule proteins, lipid mediators, cytokines, and chemokines, are thought to contribute significantly to the airway inflammation underlying asthma pathogenesis.^{2,3}

Apoptosis or programmed cell death is fundamental to a viable immune system⁴ and vital for the resolution of inflammation; cells are rapidly phagocytosed whole or as fragments bound by an intact membrane, preventing exposure of adjacent tissues to their intracellular contents. A number of studies provide evidence that eosinophil clearance by means of apoptosis induction has clinical relevance in asthma and that this may represent a plausible target for novel therapeutic intervention.⁵⁻⁷ Several signals that induce or accelerate eosinophil apoptosis have been described,⁸ and macrophages swiftly recognize and ingest apoptotic eosinophils by means of integrin- and sugar/lectin-dependent mechanisms.⁹ However, the mechanisms responsible for the recognition and clearance of apoptotic eosinophils from the asthmatic lung remain poorly understood. It is relevant to note that a number of non-professional phagocytes,¹⁰ including smooth muscle cells,¹¹ lung fibroblasts,¹² human liver Kupffer cells,¹³ and dendritic cells,^{14,15} also have the capacity to recognize and ingest apoptotic cells. Our own studies¹⁶ demonstrated that human small airway bronchial epithelial cells (SAECs) ingest apoptotic eosinophils, but not freshly isolated viable eosinophils, in a specific lectin or $\alpha\text{v}\beta 3$, CD36 membrane receptor-mediated fashion, a process enhanced by stimulation with IL-1 α or TNF- α . However, the data from that study indicated the existence of other membrane receptor-mediated pathways by which apoptotic eosinophils are recognized by bronchial epithelial cells.

From the Department of Medicine and Therapeutics, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen.

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Reprint requests: Garry M. Walsh, PhD, Department of Medicine and Therapeutics, IMS Building, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK.

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Abbreviations used

PE: Phycoerythrin
PS: Phosphatidylserine
PSR: Phosphatidylserine receptor
SAEC: Small airway epithelial cell

In the present study we have shown that the well-characterized type II alveolar adenocarcinoma epithelial cell line A549 recognizes and ingests apoptotic eosinophils in an $\alpha\beta 3$, CD36-dependent manner and also demonstrates a role for both integrin $\alpha\beta 5$ and the phosphatidylserine receptor (PSR). Integrin-dependent recognition involved the formation of focal adhesion complexes around apoptotic eosinophils. Glucocorticoids can cause a striking reduction in eosinophil numbers *in vivo*,¹⁷ and in their inhaled form, they remain the mainstays of anti-inflammatory therapy in asthma.¹⁸ Although their precise mechanism of action remains to be determined, glucocorticoids accelerate apoptosis in blood¹⁹ and tissue eosinophils.²⁰ We therefore investigated the effect of the corticosteroid dexamethasone on the apoptotic-eosinophil engulfment ability of the A549 epithelial cells.

METHODS

Reagents and mAbs

The tetrapeptides RGDS and RGEs, *o*-dianisidine, dexamethasone, monosaccharides, and BSA were supplied by Sigma Ltd (Poole, Dorset, United Kingdom). The specific $\alpha\beta 3$ mAb 23C6,²¹ the CD36 mAb SMO,²² the anti-CD44 mAb F-10-44-Z, and isotype-matched negative controls were all purchased from Serotec (Oxon, United Kingdom). The $\alpha\beta 5$ mAb MAB1961Z and anti-CD9-phycoerythrin (PE) were purchased from Chemicon International Inc (Temecula, Calif) and Pharmingen (San Diego, Calif), respectively. The $\beta 5$ polyclonal Ab E-19 was obtained from Santa Cruz Biotechnology, and anti-CD11b mAb was from Dako Ltd (High Wycombe, Bucks, United Kingdom). The PSR mAb²³ was a kind gift from Dr Valerie Fadok (National Jewish Medical and Research Center, Denver, Colo).

Cell culture and eosinophil purification

Blood (80 mL) was obtained from normal donors or individuals with a history of mild allergic disease and an eosinophilia not greater than 0.5×10^6 eosinophils/mL, who were not taking any medication at the time of venesection and who gave informed consent. Eosinophils were purified by using dextran sedimentation and centrifugation on Percoll gradients, followed by CD16-dependent negative immunomagnetic selection, as previously described.²⁴ Apoptotic eosinophils were generated by aging in culture, as previously described,¹⁶ which provided eosinophils that were greater than 70% apoptotic and greater than 90% viable, quantified as previously described.²⁵ A549 cells were purchased from the American Tissue Culture Collection and cultured in RPMI-1640 with glutamax (both from Life Technologies Ltd, Paisley, United Kingdom) and 10% fetal clone III (Perbio Science, Cheshire, United Kingdom). SAECs were cultured in the supplier's epithelial cell medium, as previously described.¹⁶ All cultures were split at 95% confluence by means of trypsin digestion. Dexamethasone treatment involved 24-hour incubations of confluent monolayers of A549 cells with growth medium supplemented with the glucocorticoid at the requisite concentration.

Uptake of apoptotic eosinophils by A549 cells

Interaction assays were performed as previously described.¹⁶ Briefly, A549 epithelial cells or SAECs were trypsinized, seeded onto 24-well plates (1×10^5 cells/well), and allowed to divide to confluence. Confluent SAECs were washed with supplemented RPMI-1640 and rested in the same medium overnight before use. Confluent monolayers of A549 cells or SAECs were washed with supplemented RPMI-1640 immediately before use. Washed apoptotic eosinophils (3×10^5 /well) were added to resting or stimulated epithelial monolayers for 60 minutes at 37°C, which was established as the optimum time point in preliminary experiments (data not shown). After stringent washing with EDTA-PBS buffer to remove noningested eosinophils and fixation, internalized eosinophils were visualized by staining for peroxidase with *o*-dianisidine. At least 200 epithelial cells in duplicate wells in randomly selected fields were counted by a blinded investigator, and the proportion that had ingested one or more eosinophils was expressed as a percentage.

Apoptotic eosinophils were interacted with confluent A549 cells to confirm ingestion, as described above. After removal of non-ingested apoptotic eosinophils, A549 cells were removed from the tissue culture plastic with a nonenzymatic adherent cell-disassociation solution (Sigma Ltd, Poole, Dorset, United Kingdom), pelleted, fixed in 2.5% glutaraldehyde, and prepared for examination by means of electron microscopy. In addition, A549 cells were also grown to confluence in chamber slides and, before the interaction assay, were stained with mAb against CD44, CD36, $\alpha\beta 3$, $\alpha\beta 5$, PSR, or isotype-matched controls and visualized with an anti-mouse IgG-FITC. Before interaction with the A549 monolayer, apoptotic eosinophils were stained with a CD9 mAb conjugated to phycoerythrin. The 2 cell types were then visualized with a fluorescent microscope. We also examined whether the A549 cells remained viable and were capable of further division after apoptotic-eosinophil ingestion. For these experiments, apoptotic eosinophils were interacted with resting or dexamethasone-treated A549 cells, which were approximately 50% confluent. After removal of noningested apoptotic eosinophils, as detailed above, sample wells were examined for A549 viability by using trypan blue exclusion. The remaining A549 monolayers were then returned to the CO₂ incubator before assessment for their ability to continue to divide until near confluence by means of microscope inspection.

Expression of receptors involved in apoptotic-eosinophil recognition by bronchial epithelial cells

Resting or dexamethasone (10^{-6} mol/L)-stimulated confluent A549 epithelial cells were removed from the tissue culture plastic by using the nonenzymatic disassociation solution, thus preserving membrane receptor expression. A549 cells (2×10^5) were immunostained with the relevant antibodies or isotype-matched controls at saturating concentrations for 40 minutes at 4°C. After a wash and staining with an appropriate FITC-conjugated secondary mAb, the cells were analyzed on a flow cytometer (FACScan, Becton Dickinson, Oxon, United Kingdom), with A549 cells gated on the basis of their forward- and side-scatter profile and any cell debris excluded from analysis.

Inhibition assays

For these experiments, resting or dexamethasone-stimulated A549 cells were preincubated for 30 minutes at 37°C with mAb/pAb to $\alpha\beta 5$, $\beta 5$, PSR, $\alpha\beta 3$, CD36, a known adhesion-blocking mAb to CD11b, or isotype-matched controls at a final concentration of 10 μ g/mL. Both untreated and treated A549 cells were washed before

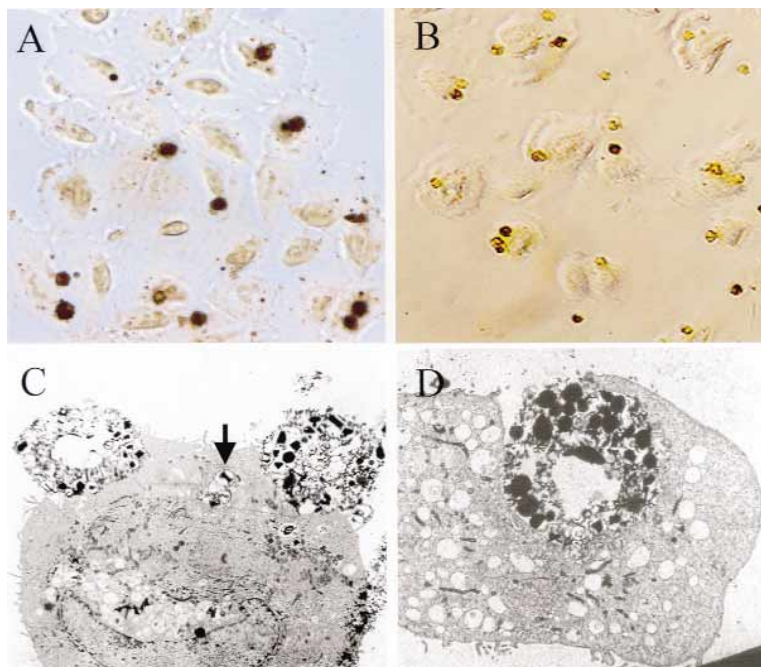


FIG 1. Representative light (original magnification 400 $\times$) and electron (original magnification 5000 $\times$) photomicrographs of epithelial cells ingesting apoptotic eosinophils at 60 minutes' interaction. A549 epithelial-cell engulfment of apoptotic eosinophils (**A**), which are visualized as dark-staining peroxidase-positive cells, compared with ingestion by SAECs (**B**). Electron micrographs of partial apoptotic-eosinophil ingestion (**C**) and near-total engulfment of an apoptotic eosinophil (**D**) by A549 cells. The arrow in (**C**) demonstrates evidence of partial digestion of an apoptotic eosinophil (ie, a phagosome containing a degenerated eosinophil, including an eosinophil granule).

use. We also assessed the tetrapeptides RGDS and RGEs (final concentration, 2 mmol/L) or monosaccharides (final concentration, 25 mmol/L) for their effects on the uptake of apoptotic eosinophils by resting or dexamethasone-stimulated A549 cells. Aged eosinophils were preincubated for 30 minutes at 37°C with the tetrapeptides or monosaccharides before their use in the interaction assay. The apoptotic eosinophils were added to the A549 monolayers, together with the appropriate tetrapeptides or monosaccharides. We did not observe any increase in the uptake of trypan blue by treated aged eosinophils compared with untreated cells (data not shown).

Statistical analysis

All data are presented as means $\pm$ SEM, and where *n* is given, this represents the number of experiments, each performed in duplicate. Statistical analysis was performed with the unpaired 2-tailed Student *t* test, and a *P* value of $<.05$ was considered significant.

RESULTS

A549 epithelial cells ingest apoptotic eosinophils

A549 epithelial cells recognized and ingested apoptotic eosinophils that were visualized as dark-staining peroxidase-positive cells (Fig 1, *A*), and this was comparable with apoptotic-eosinophil engulfment by SAECs (Fig 1, *B*). We observed no ingestion of freshly prepared non-apoptotic eosinophils or of human erythrocytes opsonized with IgG by either resting or dexamethasone-stimulated A549 cells (data not shown). Electron microscopy confirmed engulfment of apoptotic eosinophils by A549 cells

and also provided evidence of digestion of ingested eosinophils by the epithelial cells (Fig 1, *C* and *D*). The apoptotic eosinophils seen in Fig 1, *A*, were not merely adherent to the A549 cells because the stringent washing procedure with buffer containing 0.02 mol/L EDTA removed noningested apoptotic eosinophils and resulted in there being no adhesive interactions with A549 cells or SAECs. This step also prevented any adhesion molecule-dependent interactions between freshly prepared viable eosinophils and the resting or dexamethasone-stimulated epithelial cells. Furthermore, it has recently been reported that resting or platelet-activating factor- or IL-5-activated eosinophils did not adhere to A549 epithelial cells, even when the latter were stimulated with TNF- α , IFN- γ , or a mix of TNF- α , IFN- γ , and IL-1 β .²⁶

Effect of dexamethasone stimulation of A549 cells

Dexamethasone treatment for 24 hours increased the percentage of A549 cells or SAECs that recognized and ingested apoptotic eosinophils (Fig 2, *A*) to a level comparable with that produced by IL-1 β stimulation (data not shown). The optimal dexamethasone concentration was 10⁻⁶ mol/L for both epithelial cell types, although a significant dose-dependent effect at the lower concentrations was only observed for SAECs. However, dexamethasone treatment enhanced the capacity of A549 cells to ingest multiples of apoptotic eosinophils (Fig 2, *B*) compared

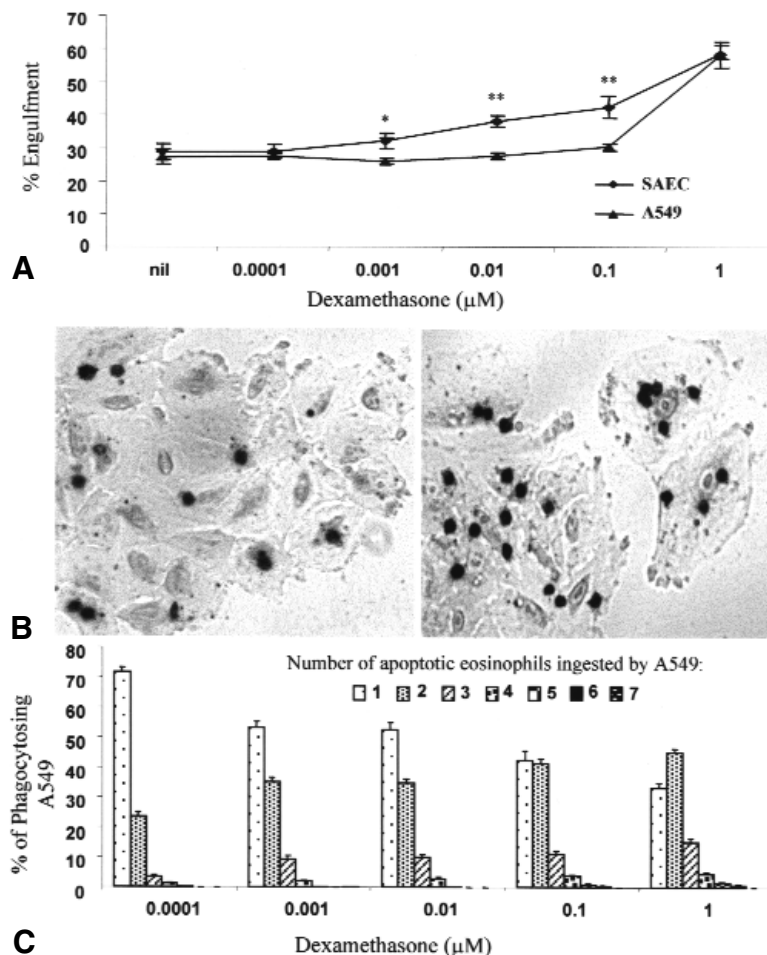


FIG 2. A, Effect of increasing concentrations of dexamethasone on apoptotic-eosinophil uptake by A549 cells compared with SAECs (percentage engulfment with each point representing the mean $\pm$ SEM of 5 experiments: * $P < .05$, ** $P < .005$). B, Representative photomicrographs of basal ingestion of apoptotic eosinophils by resting A549 cells (*left*) and engulfment after treatment of the A549 monolayers with the glucocorticoid dexamethasone (*right*, 10^{-6} mol/L). C, Stimulation of A549 epithelial cells for 24 hours with a concentration range of dexamethasone cells increased their ingestion capacity for phagocytosis of apoptotic eosinophils. Each *bar* represents the mean $\pm$ SEM of 5 experiments.

with untreated epithelial cells in a dose-dependent manner (Fig 2, C). At the optimal concentration of dexamethasone, greater than 60% of A549 cells ingested 2 or more apoptotic eosinophils, whereas the lowest dose yielded less than 30% such cells (Fig 2, C). Engulfment of apoptotic eosinophils by resting or dexamethasone-treated A549 cells did not lead to a loss of viability, and they retained their ability to divide (data not shown).

Receptors involved in A549 recognition of apoptotic eosinophils

We next investigated which receptors were responsible for the recognition of apoptotic eosinophils by A549 epithelial cells. The tetrapeptide RGDS significantly inhibited apoptotic-eosinophil engulfment by resting A549 cells ($24\% \pm 1.7\%$ reduced to $14\% \pm 1.9\%$ [$P < .05$] of A549 cells engulfing apoptotic eosinophils in the presence of RGDS). IL-1 stimulation (10^{-10} mol/L for 24 hours) of A549 cells enhanced uptake of apoptotic eosinophils to

$41\% \pm 1.4\%$, which was reduced to $23\% \pm 3.1\%$ ($P < .05$) in the presence of RGDS. RGES had no measurable effect on ingestion by either resting or IL-1-stimulated A549 cells. In contrast, the amino sugars glucosamine, n-acetyl glucosamine, and galactosamine only partially and non-significantly inhibited apoptotic-eosinophil uptake by A549 cells, and the parent sugars glucose, galactose, mannose, and fucose had no measurable inhibitory effect (data not shown). These findings provided evidence that ingestion of apoptotic eosinophils by resting or dexamethasone-stimulated A549 cells is dependent on an RGD-dependent signal and the involvement of integrins. We therefore established expression of the integrins $\alpha v \beta 5$, $\beta 5$, $\alpha v \beta 3$, CD36, and PSR by resting and dexamethasone-stimulated confluent A549 cells. Fig 3 is a representative flow cytometry experiment demonstrating that resting A549 cells express $\alpha v \beta 5$, $\beta 5$, $\alpha v \beta 3$, CD36, and the recently defined PSR.²³ Expression of $\beta 5$ and CD36 by dexamethasone-treated A549 epithelial cells (10^{-6} mol/L for 24 hours) was

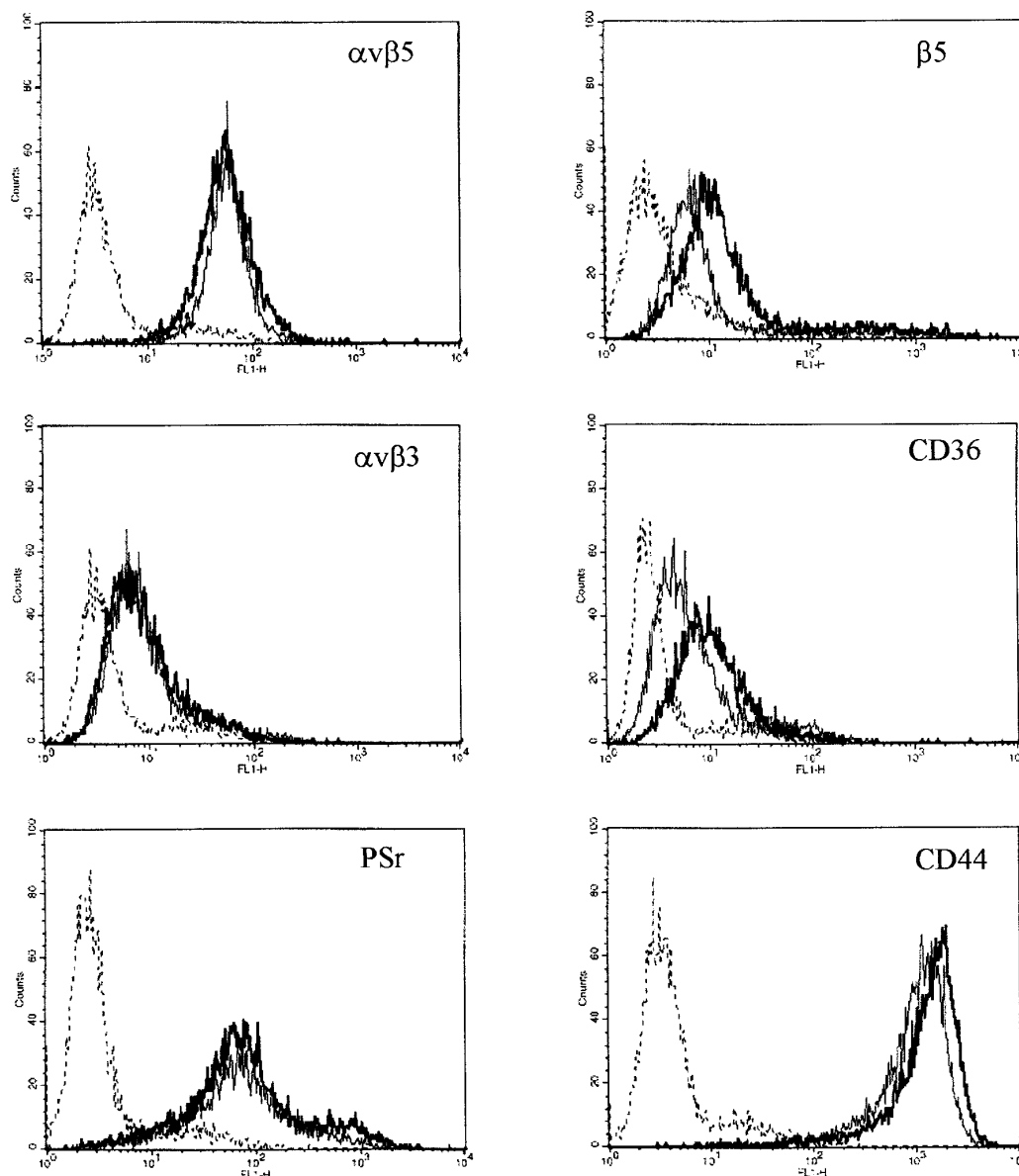


FIG 3. Representative expression data from flow cytometric analysis of A549 cell-surface receptors involved in phagocytosis of apoptotic eosinophils ($\beta 5$, $\alpha v\beta 5$, $\alpha v\beta 3$, CD36, or PSR). In each histogram the *dotted line* represents the isotype-matched negative control, the *solid line* represents immunostaining with the indicated primary antibody, and the *heavy solid line* denotes immunostaining with the indicated primary antibody after incubation of the A549 monolayer with dexamethasone (10^{-6} mol/L for 24 hours). CD44 represents the positive control.

increased to a small degree, but in 5 independent experiments the enhanced expression of these 2 receptors failed to reach statistical significance (data not shown).

Inhibition studies

The role of $\alpha v\beta 5$, $\beta 5$, $\alpha v\beta 3$, CD36, and PSR in recognition of apoptotic eosinophils by A549 cells was examined with inhibition assays. All mAbs, but not isotype-matched controls or a known adhesion-blocking mAb against CD11b, significantly inhibited ingestion

of apoptotic eosinophils by both resting and dexamethasone-stimulated A549 epithelial cells, with the greatest inhibitory effect given by mAbs against $\alpha v\beta 5$. Moreover, the anti-PSR mAb significantly decreased apoptotic-eosinophil uptake by resting and steroid-treated A549 epithelial cells (Fig 4). No additive effect was observed when the mAbs to either $\alpha v\beta 5$, $\alpha v\beta 3$, CD36, or PSR were used in combination with either resting or dexamethasone-treated A549 epithelial cells (data not shown).

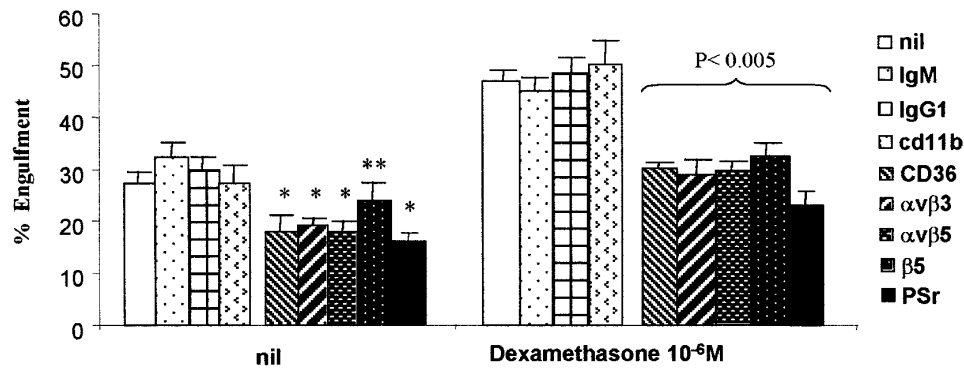


FIG 4. Inhibition of apoptotic-eosinophil phagocytosis by A549 cells (percentage engulfment) through integrin, CD36, or PSR blockade. The contribution of these receptors to apoptotic-eosinophil engulfment by both resting and dexamethasone (10^{-6} mol/L)-treated A549 cells was assessed by using mAbs against these receptors and the appropriate isotype controls. Significant, comparable, and incomplete inhibition was observed with mAbs specific for $\alpha\text{v}\beta 5$, $\alpha\text{v}\beta 3$, CD36, and PSR, with no significant inhibition observed for the anti-CD11b mAb. Each bar represents the mean $\pm$ SEM of 5 experiments (* P < .05, ** P < .005 compared with the relevant isotype-matched control in each case).

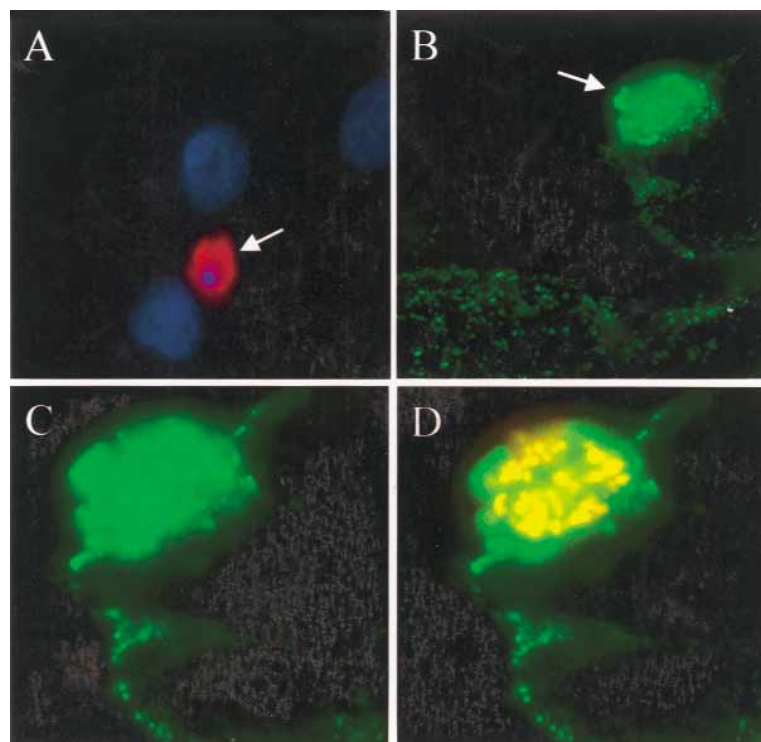


FIG 5. Representative experiments showing immunostaining of A549 monolayers with isotype negative control, CD44, or $\alpha\text{v}\beta 5$ mAbs. **A**, Negative staining with an isotype control mAb together with DAPI staining (blue) of epithelial nuclei. Note the presence of a red-immunostained CD9-PE-positive apoptotic eosinophil (arrow). **B**, Positive immunostaining of A549 monolayer with an $\alpha\text{v}\beta 5$ mAb visualized with anti-mouse antibody-FITC, demonstrating a phagosome containing a CD9-PE-labeled apoptotic eosinophil (arrow). **C**, Demonstration of $\alpha\text{v}\beta 5$ integrin receptor clustering around an apoptotic eosinophil inside a newly formed phagocytic vessel within an A549 epithelial cell. Note the diffuse membrane immunostaining for integrin $\alpha\text{v}\beta 5$ on A549 cells, while the apoptotic eosinophil is surrounded by a $\alpha\text{v}\beta 5$ -positive phagosome, which has resulted in a high level of FITC-positive immunostaining in this area. All representative photomicrographs are at 400 $\times$ magnification. The area of interest in **B** has been enhanced with Adobe PhotoShop.

Receptor clustering

Fig 5 demonstrates FITC-labeled $\alpha\text{v}\beta 5$ receptor clustering around an apoptotic eosinophil that is inside a newly formed phagocytic vessel. Apoptotic eosinophils were prelabeled with a CD9-PE-conjugated mAb. After inter-

action with the A549 monolayer, DAPI staining revealed both epithelial and apoptotic-eosinophil nuclei (Fig 5, A). A549 monolayers that had ingested apoptotic eosinophils were stained with an anti- $\alpha\text{v}\beta 5$ mAb and visualized with an FITC-conjugated anti-mouse antibody. Fig 5, B,

demonstrates diffuse membrane immunostaining of integrin $\alpha\text{v}\beta 5$ by A549 cells, whereas the apoptotic eosinophil is almost completely surrounded by an $\alpha\text{v}\beta 5$ -positive phagosome, resulting in a high level of FITC staining in the area occupied by the apoptotic eosinophil (Fig 5, C and D). Comparable results were obtained with the CD36, $\alpha\text{v}\beta 3$, and PSR mAbs. These observations are in accordance with the proposed clustering model of phagocytosis and further reinforce our observation of phagocytic ingestion of apoptotic eosinophils by epithelial cells.

DISCUSSION

The type II adenocarcinoma-derived cell line A549 has been well characterized as being representative of alveolar epithelial cells, and these exhibited comparable levels of apoptotic-eosinophil engulfment, as we previously reported for SAECs.¹⁶ Electron microscopy confirmed apoptotic-eosinophil engulfment by A549 cells and was also suggestive that A549 cells were capable of at least partial digestion of the engulfed apoptotic eosinophils. Integrin involvement in the recognition of apoptotic eosinophils was evident in A549 cells that expressed and used $\alpha\text{v}\beta 3$ and CD36 in apoptotic-eosinophil clearance, with results comparable with those of SAECs.¹⁶ The integrin $\alpha\text{v}\beta 5$ and its subunit $\beta 5$ were expressed at moderate to high levels in A549 cells, although inhibition levels were not significantly different from those obtained with mAbs to $\alpha\text{v}\beta 3$ and CD36. Having previously demonstrated that SAECs also recognize apoptotic eosinophils through lectin interactions,¹⁶ we were somewhat surprised that preincubation of apoptotic eosinophils with the amino sugars glucosamine, *n*-acetyl glucosamine, or galactosamine only partially and nonsignificantly inhibited their uptake by A549 cells (data not shown). These observations might be indicative of bronchial epithelial cells from different areas of the lung using different recognition receptors for the engulfment of apoptotic eosinophils. However, the most likely explanation is that A549 cells are a transformed cell line and may therefore be deficient in their ability to recognize apoptotic eosinophils through cell-surface lectin interactions.

The $\alpha\text{v}\beta 5$ integrin is used in the recognition and engulfment of apoptotic cells by immature dendritic cells¹⁵ and is not expressed on the surface of macrophages.²⁷ Moreover, $\alpha\text{v}\beta 5$ -dependent internalization of apoptotic targets by dendritic cells was dependent on signaling through the $\beta 5$ cytoplasmic tail. Engagement of the $\alpha\text{v}\beta 5$ heterodimer resulted in recruitment of the Crkl-Dock180-Rac1 molecular complex and subsequent rearrangement of the actin cytoskeleton and phagosome formation.²⁸ It has been suggested that $\alpha\text{v}\beta 5$ may cooperate with CD36 and thrombospondin in a similar way to $\alpha\text{v}\beta 3$ in the phagocytosis of outer rod segments by retinal pigmented epithelium,²⁷ although an independent role for $\alpha\text{v}\beta 5$ could not be discounted. This probable association of $\alpha\text{v}\beta 5$ and CD36 in a fashion similar to $\alpha\text{v}\beta 3$ cooperation with CD36 could explain our observation of a lack of increased inhibition when combinations

of blocking mAbs were used. We did not observe more than 50% to 60% inhibition of phagocytosis of apoptotic eosinophils by A549 epithelial cells by any anti-integrin, anti-CD36, or anti-PSR antibody or any combination of these, suggesting that recognition is not dependent on any single receptor. It is possible that one receptor can substitute for the others or that other, as-yet unidentified receptors could be involved in apoptotic-eosinophil recognition by bronchial epithelial cells.

We observed the greatest inhibition of uptake of apoptotic eosinophils after mAb-dependent blockade of the PSR. This receptor recognizes one of the earliest markers of apoptosis, the exposure of phosphatidylserine (PS) from the inner to the outer leaflet of the cell membrane caused by inhibition of PS translocase activity and activation of PS scramblase.^{29,30} We have extended previous findings for PSR in phagocyte-apoptotic cell interactions^{23,29,31} to show a role for this receptor in A549 epithelial-cell recognition of apoptotic eosinophils. This receptor is critical in mediating the uptake of apoptotic cells to produce an anti-inflammatory effect, a hallmark of apoptotic-cell phagocytosis. Indeed, phagocytosis of apoptotic cells by macrophages not only fails to release phlogistic agents, such as eicosanoids or proinflammatory cytokines, but can actually suppress the inflammatory response.^{32,33} Engulfment of apoptotic eosinophils also induces an anti-inflammatory cytokine and mediator secretory profile in the macrophage.⁹ Whether bronchial epithelial cells can switch to an anti-inflammatory cytokine profile after ingestion is currently under investigation.

The ability of glucocorticoids to resolve eosinophilic inflammation may involve acceleration of eosinophil apoptosis^{19,20} and their subsequent recognition and engulfment by alveolar macrophages.⁷ However, increased induction of apoptosis in eosinophils is only beneficial if there is a concomitant increase in the phagocytic capacity of the cells in their environment. Eosinophils that have become apoptotic but have not been phagocytosed will exhibit secondary necrosis that is associated with loss of membrane integrity and subsequent release of toxic granule contents, resulting in a propagated inflammatory response. Dexamethasone increased both the percentage of cells engulfing apoptotic eosinophils and in particular the number of apoptotic eosinophils ingested by each A549 epithelial cell. Dexamethasone treatment of A549 cells resulted in a small and nonsignificant degree of enhanced expression of CD36 and $\beta 5$, with no change in expression of the other receptors. Thus increased receptor expression did not appear to be responsible for increased A549 phagocytic capacity. There are a number of potential reasons for this observation. These include the participation of as-yet unidentified recognition receptors or a conformational change in the receptors under investigation, which increased their affinity for ligand in a manner analogous to that described for activated leukocyte integrin-dependent interaction with endothelial cells.³⁴

In summary, we have established that A549 alveolar epithelial cells are capable of recognizing and engulfing

apoptotic eosinophils by using receptors known to be involved in macrophage and small airway epithelial cell recognition of apoptotic eosinophils, namely CD36 and $\alpha\text{v}\beta 3$. We also established A549 expression of $\alpha\text{v}\beta 5$ and PSR and demonstrated a role for them in the recognition of apoptotic eosinophils. Dexamethasone treatment enhanced phagocytic capacity without significant increased receptor expression in A549 cells. These observations reinforce our conjecture that rather than being a passive victim of eosinophilic inflammation, the lung epithelium plays an active role in the fate of eosinophil infiltrates into the lung.

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