Eosinophils, which are prominent cells in asthmatic inflammation, undergo apoptosis and are recognized and engulfed by phagocytic macrophages in vitro. We have examined the ability of human small airway epithelial cells (SAEC) to recognize and ingest apoptotic human eosinophils. Cultured SAEC ingested apoptotic eosinophils but not freshly isolated eosinophils or opsonized erythrocytes. The ability of SAEC to ingest apoptotic eosinophils was enhanced by interleukin-1\(\alpha\) (IL-1\(\alpha\)) or tumor necrosis factor \(\alpha\) (TNF\(\alpha\)) in a time- and concentration-dependent fashion. IL-1\(\alpha\) was found to be more potent than TNF\(\alpha\) and each was optimal at 10\(^{-10}\) mol/L, with a significant \((P < .05)\) effect observed at 1 hour postcytokine incubation that was maximal at 5 hours. IL-1\(\alpha\) stimulation not only increased the number of SAEC engulfing apoptotic eosinophils, but also enhanced their capacity for ingestion. The amino sugars glucosamine, N-acetyl glucosamine, and galactosamine significantly inhibited uptake of apoptotic eosinophils by both resting and IL-1\(\alpha\)-stimulated SAEC, in contrast to the parent sugars glucose, galactose, mannose, and fucose. Incubation of apoptotic eosinophils with the tetrapeptide RGDS, but not RGES, significantly inhibited their uptake by both resting and IL-1\(\alpha\)-stimulated SAEC, as did monoclonal antibody against \(\alpha\)\(\beta\)3 and CD36. Thus, SAEC recognize apoptotic eosinophils via lectin- and integrin-dependent mechanisms. These data demonstrate a novel function for human bronchial epithelial cells that might represent an important mechanism in the resolution of eosinophil-induced asthmatic inflammation.

© 1999 by The American Society of Hematology.

---

From the Department of Medicine & Therapeutics, University of Aberdeen Medical School, Aberdeen, UK.

Submitted October 15, 1998; accepted June 16, 1999.

Supported by the Wellcome Trust (044988/2/95/2).

Address reprint requests to Garry M. Walsh, PhD, Department of Medicine & Therapeutics, IMS Building, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK; e-mail: g.m.walsh@abdn.ac.uk.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1999 by The American Society of Hematology.

0006-4971/99/9408-0031$3.00/0

MATERIALS AND METHODS

Reagents and MoAbs. Human recombinant human IL-1α (rhIL-1α) and recombinant human tumor necrosis factor α (rhTNFα) were purchased from R&D Systems (Oxon, UK). The tetrapeptides RGDS and RGEs, monosaccharides, OPD, and bovine serum albumin (BSA) were supplied by Sigma Ltd (Poole, Dorset, UK). The specific αvβ3 MoAb 23C6,24 the CD36 MoAb SMO,25 anticytokeratin peptide 19, anti–intracellular adhesion molecule-1 (ICAM-1) and isotype-matched negative controls were all purchased from Serotec (Oxon, UK). The anti-MBP MoAb BMK-1326 was a kind gift from Prof Redwan Moqbel (University of Alberta, Edmonton, Alberta, Canada). The polyclonal antihuman endothelial membrane, anti-CD9-phycoerythrin (PE), anti-CD44, and the anti-CD11b MoAb were from Dako Ltd (High Wycombe, Bucks, UK). The latter MoAb has been previously shown to block CD11b-dependent eosinophil adhesion to cultured microvascular endothelial cells.27

Small airway epithelial cells (SAEC) culture. Small airway human bronchial epithelial cells were purchased as frozen primary cultures from Clonetics Ltd (Walkersville, MD). They were cultured in the supplier’s SAEC basal medium supplemented with gentamicin, amphotericin-B, bovine pituitary extract, hydrocortisone, human epithelial cell supplier’s serum-free growth factor, epinephrine, transferrin, insulin, retinoic acid, triiodothyronine, and bovine serum albumin. Although human SAEC can be maintained in culture for 5 to 6 passages using the supplier’s serum-free medium,28 all experiments presented here were performed with SAEC that had been passaged a maximum of 3 times to ensure no loss of phenotype. These studies used SAEC from 5 different donors and their identity as epithelial cells was guaranteed by the supplier. However, we further confirmed the epithelial identity of SAEC that had undergone 2 or 3 passages by their cobblestone morphology and uniform positive immunostaining with MoAb to the epithelial marker cytokeratin peptide 19, CD9, CD44, and ICAM-1. Furthermore, the supplemented basal epithelial medium used for SAEC culture also inhibits fibroblast growth and fibroblasts do not express CD9. Moreover, we observed no positive immunostaining with MoAb to von Willebrand factor or smooth muscle α actin (both from Novocastra Laboratories Ltd, Newcastle-upon-Tyne, UK), ruling out any contamination of our SAEC cultures by these cell types (data not shown). Cultures were split at 80% to 90% confluence by trypsin digestion and subcultured in the same SAEC medium.

Eosinophil isolation and apoptosis induction. Blood (100 mL) was obtained from normal donors or individuals with a history of mild allergic disease with an eosinophilia not greater than 0.5 × 106 eosinophils/mL who were not taking any medication at the time of venesection and who gave informed consent. Eosinophils were purified under sterile conditions using our modification of the immunomagnetic-de-Iy method of Hansel et al,29 which has been described in detail elsewhere.30 Briefly, after removal of red blood cells using dextran sedimentation, the leukocytes were centrifuged on Percoll gradients (Pharmacia Ltd, Beds, UK) at 700g for 20 minutes at 4°C. Contaminating red blood cells in the granulocyte pellet were removed by hypotonic shock with ice-cold, sterile, endotoxin-free, distilled water. Granulocytes were incubated on ice with micromagnetic beads coated with anti-CD16 for 40 minutes before passage through the magnetic-activated separation column (Miltenyi Ltd, Bisley, UK). Using this method, eosinophils with a purity of at least 99% were obtained with greater than 98% viability as assessed by trypan blue exclusion. No more than 3% of the freshly isolated eosinophil preparations displayed any morphological evidence of apoptosis. Flow cytometric analysis performed as previously described11 demonstrated that freshly isolated eosinophils did not bind annexin V-fluorescein isothiocyanate (FITC) and excluded propidium iodide.

Purified eosinophils were washed in RPMI 1640 supplemented with 10% fetal calf serum, antibiotics, and L-glutamine and resuspended in the same medium at a concentration of 1 × 106 cells/mL. Cells were cultured in 1-mL aliquots for 48 hours in a humidified atmosphere with 5% CO2 in flat-bottom 12-well plates (Life Technologies, Paisley, UK) that had been previously coated with BSA (1 mg/mL). After aging, eosinophils were assessed for apoptosis by morphological assessment and binding of annexin V-FITC.30 All apoptotic cells used in the interaction/uptake experiments were greater than 70% apoptotic and more than 90% viable as judged by trypan blue exclusion.

Interaction assay. This was a modification of a previously established assay.32 Before use, secondary passage SAEC were trypsinized and seeded into 24-well plates (Life Technologies) at a concentration of 5 × 103 cells/well and rested for 1 to 2 days before use. At this stage, cells were 80% to 90% confluent in each well as a whole, but were 95% to 100% confluent in the center of the well. After a change of medium, SAEC were stimulated with various concentrations of IL-1α or TNFα for 48, 24, 5, 2, or 1 hour. Apoptotic eosinophils were washed and added to resting or stimulated SAEC at a final concentration of 1 × 106/well and allowed to interact for 60 minutes at 37°C, which was found to be the optimum time for interaction and phagocytosis (Table 1), although we did observe evidence that by 60 minutes some ingested eosinophils had started to undergo digestion within the SAEC. Noningested eosinophils were removed by 3 vigorous washes of the SAEC monolayer with phosphate-buffered saline (PBS) supplemented with 0.02 mol/L EDTA using a 1-mL Gilson pipette, and the cells were fixed with 2% glutaraldehyde in PBS. Ingested eosinophils were stained for peroxidase by incubation with o-phenylenediamine dihydrochloride (OPD) dissolved in PBS with hydrogen peroxide. Aged eosinophils could be clearly seen as brown cells within the SAEC. SAEC alone treated with OPD showed no positive peroxidase staining. All experiments were performed in duplicate and at least 200 SAEC were counted using an inverted microscope, and the proportion that had ingested 1 or more eosinophils was expressed as a percentage. In some experiments, the number of ingested apoptotic eosinophils within each resting or cytokine-stimulated SAEC were counted. To confirm that aged eosinophils were indeed ingested by SAEC and not merely adherent to them as a result of nonspecific stickiness, trypsin was added to washed monolayers after the interaction assay, but before the cells were fixed or stained. Cytospins were prepared from the epithelial cell suspensions and the cells fixed as before in glutaraldehyde/PBS and stained with OPD.

In addition, SAEC were also grown in chamber slides and the following experiments were performed. SAEC that had ingested apoptotic eosinophils were permeabilized and stained with MoAb BMK-13, which is specific for the eosinophil granule protein MBP.26 Ingested aged eosinophils were visualized by the addition of antiamOUSE IgG-FITC before viewing with a fluorescent microscope. Apoptotic eosinophils were also stained with a CD9 MoAb conjugated to PE (CD9-PE) before incubation with SAEC that had been stained with anti-CD44 MoAb and antimouse IgG-FITC before the interaction assay. The 2 cell types were then visualized with a fluorescent microscope. Interaction experiments were also performed with human erythrocytes oposenoned with an antilymphocyte membrane polyclonal IgG antibody as previously described.13

Inhibition assays. For these experiments, resting or cytokine-stimulated SAEC were preincubated for 30 minutes at 37°C with MoAb to αvβ3, CD36, isotype-matched controls at a final concentration of 10

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>% Engulfment by SAEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>4 ± 0.9</td>
</tr>
<tr>
<td>30</td>
<td>17 ± 2.1</td>
</tr>
<tr>
<td>45</td>
<td>21 ± 5.6</td>
</tr>
<tr>
<td>60</td>
<td>25 ± 2.6</td>
</tr>
</tbody>
</table>

Table 1. Determination of Optimal Timepoint for Uptake of Apoptotic Eosinophils by Unstimulated Monolayers of SAEC (n = 4, ±SEM)
μg/mL. Both untreated and treated SAEC were washed before use. We also assessed the tetrapeptides RGDS or RGES (final concentration, 2 mmol/L) and monosaccharides (final concentration, 25 mmol/L) for their effects on the uptake of apoptotic eosinophils by resting or IL-1α–stimulated SAEC. Aged eosinophils were preincubated for 30 minutes at 37°C with the tetrapeptides or sugars before their use in the interaction assay. We did not observe any increase in the uptake of trypan blue by treated aged eosinophils compared with untreated cells (data not shown). Aged eosinophils were also treated with a known adhesion blocking MoAb to CD11b to control for the possibility that adhesion via this receptor was responsible for apoptotic eosinophil interaction with SAEC.

Measurement of expression of αvβ3 and CD36 by SAEC. The expression of the integrins αvβ3 and CD36 by resting and IL-1α–stimulated or TNFα-stimulated SAEC were measured using a specific enzyme-linked immunosorbent assay (ELISA) performed as previously described.29 Briefly, SAEC were seeded into 96-well flat-bottomed plates and grown to 90% confluence before stimulation with IL-1α or TNFα (final concentration, 10−10 mol/L, for 5 or 20 hours). The resting or cytokine-stimulated SAEC were washed, fixed in 2% glacial acetic acid, and blocked with 0.5% BSA. Specific and control MoAb (10 µg/mL) were added in triplicate and incubated overnight at 4°C. After several washes in PBS, the SAEC were treated with rabbit antimouse IgG conjugated to horseradish peroxidase (HRP) for 30 minutes at room temperature (RT), washed in PBS, and treated with an HRP-conjugated goat antimouse IgG. After development with OPD, the plate was read on an automated ELISA plate reader at an absorbance of 490 nm.

Statistical analysis. All data are presented as the mean ± SEM and where n is given this represents the number of experiments each performed in duplicate. Statistical analysis was by the unpaired 2-tailed Student’s t-test, where a P value of less than .05 was considered significant.

RESULTS

Resting and cytokine-stimulated SAEC ingest apoptotic eosinophils. Figure 1A is a photomicrograph of a representative experiment that clearly demonstrates uptake of apoptotic eosinophils by unstimulated SAEC. Several SAEC can be seen to have ingested apoptotic eosinophils visualized as dark staining peroxidase-positive cells. Noningested or adherent aged eosinophils were removed by vigorous washing with PBS/EDTA, as described above. We confirmed that eosinophils had been ingested by addition of trypsin to washed SAEC after their interaction with aged eosinophils. Cytosins of the cell suspension were prepared, fixed, and stained with OPD. Figure 1B clearly shows apoptotic peroxidase-positive eosinophils inside SAEC; note the absence of eosinophils on other areas of the slide. We further confirmed our observation by permeabilizing SAEC that had ingested apoptotic eosinophils and adding a MoAb specific for MBP. Stained eosinophils were visualized by the addition of antimouse IgG-FITC before viewing the cells with a fluorescent microscope. Figure 1C clearly demonstrates the presence of bright green-staining apoptotic eosinophils inside SAEC. Figure 1D shows an SAEC stained with CD44 MoAb followed by antimouse IgG-FITC before the addition of apoptotic eosinophils stained with CD9-PE before the interaction assay. In this example, 2 apoptotic eosinophils can be seen. The PE-positive eosinophil is interacting with the SAEC membrane but is not yet completely engulfed and, therefore, shows red fluorescence. The other is within a phagosome within the SAEC and is thus surrounded by a CD44+ membrane and, therefore, exhibits green fluorescence. We observed no evidence of either interaction with or the ingestion of freshly prepared nonapoptotic eosinophils or of human erythrocytes opsonized with IgG by either resting or cytokine-stimulated SAEC (data not shown). Any adhesive interactions between the freshly prepared eosinophils and the resting or cytokine-stimulated SAEC would have been disrupted by a combination of the 3 stringent washes together with the use of 0.02 mol/L EDTA in the washing buffer. Finally, our preliminary experiments determined that the optimal time-course for the engulfment of apoptotic eosinophils by SAEC was 60 minutes (Table 1).

Having made the initial observation that SAEC are indeed capable of ingesting apoptotic eosinophils, the effect on this process of stimulation of SAEC with the proinflammatory cytokine IL-1α was examined. Stimulation with IL-1α resulted in a marked enhancement of the ability of SAEC to ingest apoptotic eosinophils (Fig 2A). The proportion of SAEC that ingested eosinophils was enhanced by IL-1α stimulation, as was the ability of individual SAEC to ingest multiples of aged cells (Fig 2B).

Dose-response and kinetics of stimulation of SAEC with IL-1α or TNFα. We next assessed the effects on uptake of aged eosinophils by SAEC after stimulation with a dose-range of IL-1α or TNFα. Figure 3A shows the effect on the uptake of apoptotic eosinophils by resting SAEC after prestimulation with increasing concentrations of IL-1α or TNFα. The maximal increase in the number of SAEC ingesting apoptotic eosinophils was seen with IL-1α at a concentration of 10−10 mol/L, and this represented an approximate doubling of the numbers of stimulated SAEC capable of ingesting apoptotic eosinophils. Kinetic studies showed that a significant (P < .05) increase in uptake of apoptotic eosinophils by SAEC was observed at 1 hour post–IL-1α incubation, an effect that essentially had plateaued at 5 hours (Fig 3B). TNFα stimulation of SAEC resulted in a more modest, but statistically significant (P < .05), increase in the ability of SAEC to ingest apoptotic eosinophils (Fig 3A). Again, the optimal cytokine concentration was 10−10 mol/L, with the effect plateauing at 2 to 5 hours poststimulation (Fig 3B). In all subsequent interaction experiments that used cytokine-stimulated SAEC, IL-1α was used at a concentration of 10−10 mol/L for 24 hours.

The receptors involved in apoptotic eosinophil recognition and engulfment by SAEC. We assessed the effect of amino sugars to confirm that phagocytosis of aged eosinophils by resting and cytokine-stimulated SAEC was a specific receptor-mediated process as described in other phagocytic cells, including macrophages.18 Preincubation of apoptotic eosinophils with the amino sugars glucosamine, N-acetyl glucosamine, and galactosamine significantly inhibited their uptake by both resting and IL-1α–stimulated SAEC. In contrast, the parent sugars glucose, galactose, mannose, and fucose had no measurable inhibitory effect (Fig 4). Incubation of apoptotic eosinophils with the tetrapeptide RGDS significantly inhibited their uptake by both resting and IL-1α–stimulated SAEC, whereas the control tetrapeptide RGES had no measurable effect on ingestion of apoptotic eosinophils (Fig 5). These data provided evidence that ingestion of apoptotic eosinophils by resting or IL-1α–stimulated SAEC is dependent on an RGD-dependent signal and that a membrane receptor molecule of the integrin
family is involved. The effects of MoAb against αvβ3 and CD36 were therefore assessed, because these have been shown to be important in macrophage recognition of apoptotic eosinophils or neutrophils. Both MoAbs, but not an isotype-matched control or a known adhesion blocking MoAb against CD11b, significantly inhibited ingestion of apoptotic eosinophils by both resting and IL-1α–stimulated SAEC (Fig 6). No additive effect was observed either when both MoAbs were used in combination or when either αvβ3 or CD36 MoAb was used in combination with the amino sugars glucosamine, N-acetyl glucosamine, and galactosamine by either resting or IL-1α–treated SAEC (data not shown).

Expression of αvβ3 and CD36 by resting or cytokine-stimulated SAEC. To assess whether the observed increase in the capacity of cytokine-stimulated SAEC to ingest aged eosinophils was due to increased expression of αvβ3 or CD36, we performed specific ELISAs on resting and IL-1α–stimulated or TNFα-stimulated SAEC derived from 3 different donors. We observed modest but consistent expression of αvβ3 by resting SAEC comparable to that of ICAM-1, whereas expression of CD36 was somewhat higher. Incubation of the SAEC with either IL-1α or TNFα for 5 hours did not result in a significant increase in the expression of αvβ3 or CD36, although expression of ICAM-1 was enhanced to a modest degree (Table 2).
Near identical results were obtained when the incubation time with either IL-1α or TNFα was extended to 24 hours (data not shown).

**DISCUSSION**

In the present study, we demonstrate for the first time that human SAEC recognize and ingest apoptotic eosinophils via specific recognition mechanisms and that this process is upregulated by the proinflammatory cytokine IL-1α and, to a more modest degree, TNFα. We have used several different approaches to confirm that apoptotic eosinophils were engulfed by SAEC and that internalized eosinophils are within phagosomes (Fig 1D). This might represent an additional pathway by which intact apoptotic tissue eosinophils are safely removed from the tissues surrounding the airways, thus preventing in situ leakage of their ubiquitous, granule-derived, highly cytotoxic proteins. It can be envisaged that failure to remove apoptotic eosinophils might result in secondary necrosis and subsequent leakage of their potent proinflammatory mediators, thereby making a major contribution to asthma pathogenesis. Thus, induction of
apoptosis in eosinophils and their subsequent removal by macrophages and resident cells such as epithelial cells represents a potentially important therapeutic strategy in asthma treatment. Moreover, our observation that stimulation of SAEC with IL-1α or TNFα enhances their phagocytic capacity for apoptotic eosinophils is interesting in the light of the fact that these same cytokines are thought to promote eosinophil accumulation through upregulation of endothelial adhesion receptors, most notably vascular cell adhesion molecule-1 (VCAM-1), which interacts with eosinophil VLA-4.36 However, because inflammation is normally a beneficial and self-limiting response, it would make sense that the cytokines involved in eosinophil accumulation would also prepare resident cells to remove apoptotic eosinophils, a process analogous to that described for clearance of apoptotic neutrophils by cytokine-stimulated monocyte-derived macrophages.37

The mechanisms by which apoptotic cells are recognized appears to vary according to the cell type responsible for their engulfment. To date, at least 4 recognition mechanisms for

**Table 2. ELISA Demonstrating Expression of αvβ3, CD36, or ICAM-1 by Resting and IL-1α-Stimulated or TNFα-Stimulated SAEC (10⁻¹⁰ mol/L Final Concentration for 5 Hours)**

<table>
<thead>
<tr>
<th>Isotype</th>
<th>αvβ3 (Mean ± SEM)</th>
<th>CD36 (Mean ± SEM)</th>
<th>ICAM-1 (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.07 ± 0.02</td>
<td>0.16 ± 0.005</td>
<td>0.26 ± 0.06</td>
</tr>
<tr>
<td>IL-1α</td>
<td>0.07 ± 0.02</td>
<td>0.16 ± 0.02</td>
<td>0.29 ± 0.06</td>
</tr>
<tr>
<td>TNFα</td>
<td>0.08 ± 0.02</td>
<td>0.15 ± 0.005</td>
<td>0.24 ± 0.04</td>
</tr>
</tbody>
</table>

Data (± SEM) are from 3 separate experiments that used SAEC from different donors, each performed in triplicate.

*P < .01.
†P < .005.
apoptotic cells have been described: (1) an uncharacterized lec- 
tin-dependent interaction; (2) a complicated charge- 
sensitive process involving the CD36/vitronectin receptor com- 
plex (αvβ3) on the macrophage surface interacting with un- 
known moieties on the apoptotic neutrophil surface via a 
thrombospondin bridge; (3) a stereo-specific recognition of 
phosphatidylserine that is expressed on the surface of the 
apoptotic cell after the loss of membrane asymmetry, and 
(macrophage scavenger receptors. In the present study, 
we observed that, in some respects, SAEC recognition of 
apoptotic eosinophils is similar to that of the human monocyte- 
derived macrophage reported by Stern et al. We found that the 
amino sugars glucosamine, N-acetyl glucosamine, and galacto- 
amine significantly inhibited uptake of aged human eosinophils 
by both resting and IL-1α-stimulated SAEC, as did RGD- 
containing tetrapeptides. MoAbs to both αvβ3 and CD36 
hindered the phagocytic process. However, data from a sensitive 
ELISA indicated that the increased capacity of SAEC to 
ingest aged eosinophils after stimulation with either IL-1α or 
TNFα did not appear to be a consequence of increased 
expression of either αvβ3 or CD36, both of which had low but 
consistent constitutive expression by SAEC. These data suggest 
that factors other than a simple increase in the expression of 
αvβ3 or CD36 are involved, such as a conformational change in 
these receptors leading to increased affinity for their ligand. 
Expression of αvβ3 or CD36 has not been reported in studies 
that have examined the expression of integrins by the bronchial 
epithelium. The possibility that expression of αvβ3 or CD36 
by SAEC is an artefact of their culture in vitro cannot therefore 
be excluded, although uniform positive immunostaining with 
MoAb to cytokernetin peptide CD9, CD44, and ICAM-1 
confirmed the epithelial identity of the SAEC. Moreover, 
bronchial airway epithelial cell expression of integrins is 
complex and, in particular, appears dependent on a number of 
different factors, including exposure to pollutants such as ozone, 
malignancy, and their stage of development or repair. 
Thus, other receptors, including αvβ3 or CD36, might also be 
expressed by the bronchial epithelium under certain circum-
stances. Our data suggest that both resting and cytokine- 
activated SAEC recognize apoptotic eosinophils via VnR- 
dependent and sugar–lecitin–dependent mechanisms. However, 
it remains to be determined whether other receptors shown to be 
involved in both professional and nonprofessional phagocyte 
recognition and engulfment of apoptotic cells, including 
CD14, CD68, or the ABC1 transporter system, might 
also be involved in the recognition of apoptotic eosinophils by 
SAEC.

Macrophages are thought to be one of the most important and 
efficient cells in the recognition and engulfment of apoptotic 
cells. These professional phagocytes have the ability to ingest 
multiples of apoptotic cells that, combined with rapid digestion 
of their apoptotic meal, probably explains much of their 
efficiency. A number of studies have demonstrated that several 
nonprofessional phagocyte cell types are also capable of 
recognizing and ingesting apoptotic cells. Moreover, a 
recent report has confirmed that dendritic cells recognize and 
ingest apoptotic cells via the scavenger receptor CD36 and also 
use a receptor not expressed by macrophages, namely αvβ5. 
whereas human liver Kupffer cells phagocytose apoptotic 
lymphocytes via lectin-dependent recognition of increased 
expression of n-acetylglucosamine, D-galactose, and manno-
se residues. Hall et al. have shown that human fibroblasts, 
including those derived from the lung, recognize and ingest 
apoptotic neutrophils and that this involves the participation of 
the vitronectin receptor and a mannos-fucose-specific lectin. 
However, although SAEC also use the vitronectin receptor in 
recognition of apoptotic eosinophils, we did not observe a role 
for the mannos-fucose-specific lectin, because neither sugar had 
any inhibitory effect on recognition or engulfment. More-
over, these workers did not observe any uptake of apoptotic 
neutrophils by human epidermal keratinocytes or mammary 
epithelial cells. These and the observations presented here 
suggest that epithelial cells at different organ sites vary in their 
ability to recognize and engulf apoptotic cells. The reasons for 
such differences are unclear. We also observed variation in the 
ability of SAEC to ingest more than 1 apoptotic eosinophil. This 
was particularly marked after IL-1α stimulation, with the 
majority of SAEC ingesting 2 or 3 aged eosinophils, whereas 
most unstimulated SAEC had only 1 or 2 ingested cells. The 
most likely explanation for this observation is the nature of the 
conditions required for the culture of SAEC. All of the 
experiments presented in this study were performed with SAEC 
that were 80% to 90% confluent. Culturing SAEC to 100% 
confluence is not possible, because this results in contact- 
dependent cell morbidity and loss. Thus, a minority of the 
SAEC in the monolayers used in these studies were at different 
stages of cell division and/or maturity that might explain in part 
the variability in their ability to ingest apoptotic eosinophils.

The present study has focused on clearance of apoptotic 
eosinophils by human small airway epithelial cells given the 
potential relevance of this process to asthma pathology. We 
therefore did not examine the ability of SAEC to ingest 
apoptotic neutrophils and neither did we examine the engulf-
ment ability of large airway epithelial cells. Apoptosis is 
associated with the swift recognition of intact cells by macro-
phages or resident cells followed by their engulfment and 
degradation with the result that detection in vivo can be 
difficult. This fact is the most likely explanation for the reason 
that, to date, there have been no reports of the presence of 
apoptotic eosinophils inside epithelial cells in, for example, 
bronchial biopsies from patients with mild asthma.

Apoptotic cells are recognized and cleared by macrophages 
or nonprofessional phagocytes by a specific recognition pro-
cess. Crucially, not only does the rapid phagocytosis of 
apoptotic cells prevent local tissue injury or inflammation, but it 
also suppresses activation of the macrophages’ usual proinflam-
matory secretory response. Similarly, engulfment of apoptotic 
eosinophils exerts profound effects on the functional ability of 
the ingesting macrophage, inducing an anti-inflammatory cyto-
kine and mediator secretory profile, ie, TGFβ and prostaglandin 
E2. In contrast, ingestion of necrotic eosinophils induces a 
proinflammatory cytokine and mediator profile, ie, release of 
thromboxane B2 and granulocyte-macrophage colony-stimulat-
ing factor (GM-CSF). A number of studies have demonstrated 
that airway epithelial cells are also potent sources of proinflam-
matory substances, including cytokines and chemokines. The 
question as to whether ingestion of apoptotic eosinophils by
SAEC modulates their ability to release chemokines and/or cytokines will be the subject of a future report.

In summary, we have demonstrated that human SAEC are capable of recognizing and ingesting apoptotic eosinophils and that this process can be enhanced by stimulation of SAEC with the proinflammatory cytokines IL-1α and, to a lesser extent, TNFα. The membrane receptors involved in SAEC recognition of apoptotic eosinophils appear similar to those reported for human monocyte-derived macrophages. These observations demonstrate that, together with macrophages and other resident cells, human SAEC might be active participants in the removal of apoptotic eosinophils and therefore play an important role in the resolution of eosinophilic inflammation in the asthmatic lung.

ACKNOWLEDGMENT

The authors thank Prof Andy Rees for his valuable comments on the manuscript and Sharon Gordon for providing some of the eosinophil preparations.

REFERENCES

32. Erwig LP, Gordon S, Walsh GM, Rees AJ: Previous uptake of apoptotic neutrophils or ligation of integrin receptors downmodulates the ability of macrophages to ingest apoptotic neutrophils. Blood 93:1406, 1999
35. Savill JS, Dransfield I, Hogg N, Haslett C: Vitronectin receptor-


49. Ramprasad MP, Fischer W, Witzum JL, Sambrano GR, Quehenberger O, Steinberg D: The 94- to 97-kDa mouse macrophage membrane protein that recognizes oxidized low density lipoprotein and phosphatidylserine-rich liposomes is identical to macrosin, the mouse homologue of human CD68. Proc Natl Acad Sci USA 92:9580, 1995.


Resting and Cytokine-Stimulated Human Small Airway Epithelial Cells Recognize and Engulf Apoptotic Eosinophils

Garry M. Walsh, Darren W. Sexton, Morgan G. Blaylock and Catherine M. Convery