Isolation technique alters eosinophil migration response to IL-8

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Abstract

Disparate reports exist on the eosinophil chemotactic capacity of interleukin-8 (IL-8). We hypothesized that the difference is due to the methods used to purify eosinophils. We therefore compared the eosinophilotactic capacity of IL-8 on human cells isolated by Percoll (positive selection) vs. magnetic cell separation system (MACS) (negative selection). Discontinuous Percoll gradients were preceded by dextran and Ficoll-Paque steps, and followed by gelatin wash and red blood cell (RBC) lysis. MACS isolation included: Percoll 1.090 g/ml layering and RBC lysis; incubation with CD16 antibody conjugated to magnetic beads (to bind neutrophils); and isolation of eluate from column positioned in magnet. Percoll isolated eosinophils migrated to IL-8 in a dose-responsive fashion. Although MACS isolation provided a greater number and higher purity of eosinophils, these eosinophils did not migrate to IL-8. Neither dextran sedimentation, Ficoll-Paque and Percoll prior to, nor Percoll discontinuous gradients subsequent to, MACS isolation reversed the negative chemotactic response. Moreover, Percoll-isolated eosinophils further purified with CD16 MicroBeads no longer chemotactically responded to IL-8. This inhibition was not due to change in eosinophil purity, a loss of eosinophil adhesion molecules or activation markers, the presence of a soluble neutrophil or eosinophil inhibitor or the effect of the magnet. Thus, the technique used to isolate eosinophils clearly affects the chemotactic responsiveness of this cell to IL-8. Since several in vivo studies suggest that IL-8 is an eosinophil chemoattractant, Percoll isolation of these cells might be more appropriate for studies involving eosinophil chemotactic responses to IL-8.

Keywords: Percoll; Magnetic cell sorting; IL-8; Chemotaxis assay

1. Introduction

Interleukin-8 has been shown to play a role in lung inflammatory responses (Baggiolini and Clark-Lewis, 1992; Carre et al., 1991). Clearly a neutrophil chemoattractant, there is considerable controversy as to IL-8’s potential as an eosinophil chemoattractant (Shute, 1994; Smith et al., 1991). Erger and Casale (1995) have shown that IL-8 does induce dose-dependent eosinophil migration through naked filters and endothelial and epithelial barriers. Others have
shown eosinophil chemotactic responsiveness to IL-8 as well (Dubois et al., 1994; Sehmi et al., 1993). In these studies, eosinophils were isolated by discontinuous gradients. Other investigators have reported the inability of IL-8 to induce a chemotactic response in eosinophils isolated by MACS (Schweizer et al., 1994; Ebisawa et al., 1994; Villar et al., 1993; Warringa et al., 1993). We hypothesized that the discrepancy in results could be due to the eosinophil isolation techniques used.

Positive and negative selection techniques are currently being used for isolating eosinophils. The well-established discontinuous Percoll gradient technique is a positive selection technique (Gartner, 1980). With this technique, varying densities of eosinophils can be separated. This is an advantage in answering the questions of activation and cell densities in regards to chemotaxis and other cellular functions. There are, however, numerous disadvantages with this technique including the requirement for donors with elevated blood eosinophils due to low yield using this procedure. Also of concern is the purity of the cell preparation. Purity of eosinophils from the same donor ranges from 70% to 99% with no obvious reason for the broad range.

Recently, the MACS isolating technique has been shown to be advantageous in improving a consistent yield and purity (Hansel et al., 1991; Bach et al., 1990; Chihara et al., 1995). This method utilizes negative selection by the removal of neutrophils which are bound to CD16 MicroBeads and magnetic column. Another advantage of this technique is the ability to isolate eosinophils from the blood of any donor.

Different isolation techniques have been shown to influence the responsiveness of isolated cells. Blum et al. (1995) have shown that eosinophils isolated by the MACS technique show stronger chemotactic responses towards C5a and PAF than those cells isolated by the fMLP method which utilizes discontinuous Percoll gradients. MACS eosinophils also have superior capacity to mount a respiratory burst than metrizamide-isolated eosinophils (Hansel et al., 1989). The effect of dextran sedimentation was determined to induce the release of specific granule compartments, resulting in changes in eosinophil density (Berends et al., 1994).

Although the MACS isolation technique appears to be a superior method for eosinophil isolation in regards to less donor prerequisites and better yield and purity, it is important to determine whether this method alters the chemotactic responsiveness of eosinophils to IL-8. In this study, we compared IL-8-induced eosinophil chemotaxis for cells isolated by the positive selection technique of Percoll versus the negative selection technique of MACS.

2. Materials and methods

2.1. Reagents

Dextran (MW 260 kDa), neonatal calf serum (NCS), bovine serum albumin (BSA) and phosphate buffered saline (PBS) were purchased from Sigma Chemical Co., St. Louis, MO. Gelatin and ethylenediaminetetraacetic acid (EDTA) were purchased from Fisher Scientific, Itasca, IL. Ficoll-Paque and Percoll (polyvinyl pyrrolidone-coated silica gel) were purchased from Pharmacia Biotech, Piscataway, NJ. Hanks’ balanced salt solution (HBSS) was purchased from Gibco, Gaithersburg, MD. CD16 MicroBeads and magnetic columns were purchased from Miltenyi Biotec, Sunnyvale, CA. Recombinant human IL-8 was purchased from R&D Systems, Minneapolis, MN. Transwell tissue culture plates were purchased from Costar, Cambridge, MA. Hansel stain was purchased from Lide Laboratories, Florissant, MO. Ham’s F12K was purchased from Irvine Biological, Irvine, CA. Primary human umbilical vein endothelial cells (HUVEC) were purchased from American Type Culture Collection, Rockville, MD. Monoclonal antibodies were purchased from Becton Dickinson, San Jose, CA.

2.2. Blood donors

15 adult volunteer blood donors were used on multiple occasions. The use of blood cells for these studies was approved by the University of Iowa Institutional Review Board for Human Studies, and all blood donors signed an informed consent form. Donors included fourteen with allergic rhinitis and/or asthma. Peripheral blood eosinophil counts ranged from 150 to 633 cells/mm³.
2.3. Isolation procedures

2.3.1. Percoll

Eosinophils were isolated from 0.1% EDTA-anticoagulated blood (180 ml) collected by venipuncture. Four parts of blood were mixed with one part of 4.5% dextran in 0.85% NaCl and RBC were allowed to sediment for 30–45 min. The dextran supernatant (3 ml) was layered over Ficoll-Paque 1.077 g/ml (3 ml) and centrifuged at 400 × g for 15 min to further fractionate leukocytes. The mononuclear cell band was aspirated, and the granulocyte pellet was washed twice and then resuspended in Ca/Mg-free HBSS with 5% NCS to a concentration of 2.0 × 10^6 cells/ml. The granulocyte suspension (2 ml) was layered over five-layer, discontinuous density gradients of Percoll (1.080, 1.085, 1.090, 1.095 and 1.100 g/ml), prepared as previously described (Little and Casale, 1991). Density gradients were centrifuged at 760 g for 20 min and cell bands which formed at each interface were collected and rinsed with 1% gelatin made in Ca/Mg-free HBSS. After lysis of residual RBC with water (4.5 ml H_2O for 30 s, then equilibrated to isotonicity with 0.5 ml 10 × Ca/Mg-free HBSS), each band was analyzed for eosinophil number using a hemocytometer and for purity with Hansel-stained cytospin preparations. Approximately 10% of peripheral blood eosinophils were recovered in band 5 (density > 1.095 g/ml) and used in subsequent assays. Cell preparations used for experiments had a range of eosinophil purity from 69% to 92% from band 5 and were normodense. Trypan Blue stain was used to determine viability of eosinophils. Eosinophils isolated by the Percoll technique were greater than 95% viable.

2.4. MACS

Eosinophils were isolated from 0.1% EDTA-anticoagulated blood (60 ml). Diluted blood (1/2 with Ca/Mg-free HBSS, 20 ml) was layered over 1.090 g/ml Percoll (10 ml) and centrifuged at 700 × g for 20 min at 20°C to fractionate leukocytes. After the mononuclear cell band was aspirated, the pellet was washed with Ca/Mg-free HBSS. Red blood cells were lysed with water (27 ml H_2O for 30 s, then equilibrated to isotonicity with 3 ml 10 × Ca/Mg-free HBSS). After washing with 2% NCS in Ca/Mg-free HBSS (NCS buffer), cell concentration was adjusted to no greater than 2.0 × 10^6 cells/tube. After centrifuging, the cell pellet was resuspended with 50 μl NCS buffer and 50 μl CD16 MicroBeads then incubated 40 min at 4°C. The magnetic column was prepared by rinsing with each of the following: 70% ETOH, Ca/Mg-free HBSS and NCS buffer. During cell-bead incubation, the column was stored at 4°C filled with NCS buffer. Ca/Mg-free HBSS (4 ml) was added to cell suspension and contents loaded onto magnetic column. The cell preparation was run through the column while in contact with the magnet and the flow-through fraction was collected. Cells collected were pelleted and analyzed for eosinophil number and purity. Approximately 65% of peripheral blood eosinophils were recovered with an eosinophil purity that ranged from 86% to 100%. Eosinophils isolated by the MACS technique were also greater than 95% viable by Trypan Blue staining.

2.5. Cell culture of HUVEC

HUVECs were grown in Ham’s F12K medium supplemented with 20% FCS, heparin (100 μg/ml), and endothelial cell growth supplement (50 μg/ml) (Erger and Casale, 1995). The HUVEC monolayers were grown to confluence on collagen coated filters in the Transwell Tissue culture plates (24-well, 6.5 mm diameter, 3 μm pore size, polycarbonate membrane) at 37°C, 5% CO_2 and 100% humidity for 3 days.

2.6. Chemotaxis and transmigration

Eosinophil chemotaxis through filters and HUVEC monolayers were performed using the Transwell plates as previously described (Erger and Casale, 1995). Prior to pipetting eosinophils onto monolayers for chemotaxis assays, monolayers/filters were briefly washed with HBSS with 2% BSA (assay buffer) to remove Ham’s F12K. Approximately 1.5 × 10^5 eosinophils in 100 μl of assay buffer were placed in the upper chamber above the filter. The chemoattractant, IL-8, in 500 μl assay buffer was pipetted into the lower chamber, below the filter. Buffer alone was used as a negative control in each experiment. Each variable was tested in duplicate or triplicate. Plates were incubated at 37°C in 5% CO_2 and 95% humidity for 3 h.
tion, filters were removed and cells in the lower chambers were resuspended with a Pipetman and were counted using a hemocytometer. The data were expressed as percent net stimulated migration (NSM) using the formula:

\[
\text{NSM} = \frac{[\text{cells in experimental well}] - [\text{cells in negative control well}]}{\text{total number of cells added to chamber}} \times 100
\]

Cells counted in the negative control wells for filters and monolayers averaged 10% and 3%, respectively, of total Percoll cells added compared to 3% and 1%, respectively, of MACS cells added.

2.7. Immunofluorescence and flow cytometry

Monoclonal antibodies used were fluorescein isothiocyanate (FITC)-conjugated anti-Leu-11a (CD16), phycoerythrin (PE)-conjugated anti-very-late antigen (VLA)-\(\alpha 4\) (CD49d), FITC-conjugated anti-Leu-23 (CD69), PE-conjugated anti-Leu 15 (CD11b) and FITC-conjugated anti-leukocyte function-associated (LFA)-1/\(\beta\) (CD18). PE-conjugated isotype-matched control antibodies were used as controls to exclude non-specific binding. Cell populations used included those isolated by the Percoll technique from bands 3, 4 and 5 and by the MACS technique pre- and post-CD16 MicroBead incubation. Cells were washed with cold PBS containing 1% BSA and 0.1% NaN\(_3\) (FACS buffer) and pelleted at 400 \(\times\) g for 10 min at 4°C. Supernatant was aspirated to 100 \(\mu\)l/tube of cells. Direct fluorochrome-conjugated antibody (10 \(\mu\)l) was added and incubated with cells for 15–30 min on ice in the dark. After this incubation period, cells were washed twice with cold FACS buffer then resuspended and immediately analyzed. Propidium iodine (40 \(\mu\)l of 5 \(\mu\)g/ml) was used to identify dead cells. Analysis was performed with a FACSscan cytofluorimeter (Becton Dickinson). Fluorescence intensity was determined on at least 10,000 cells from each sample. Dead cells were excluded from analysis by gating out propidium iodide positive cells.

2.8. Soluble inhibitor experiment

To determine if a soluble inhibitor was produced during incubation with CD16 MicroBeads, cells were first isolated by the Percoll method. Neutrophils were used from the band 3 fraction (1.090 g/ml) and eosinophils from the band 5 fraction (1.100 g/ml). One aliquot of eosinophils, the neutrophils, and buffer (50 \(\mu\)l) were each incubated with 50 \(\mu\)l CD16 MicroBeads for 40 min at 4°C. After the incubation, 1.5 ml of assay buffer were added to each group, supernatants collected, checked to make sure no cells were present, then used to resuspend three of the eosinophil aliquots. A fifth aliquot of eosinophils was resuspended with assay buffer as per standard procedure. Subsequently, the eosinophils were used in a standard chemotactic assay.

2.9. Statistics

Comparisons of eosinophil migration were performed by one-way ANOVA using the Minitab software program (State College, PA). Reported \(p\) values were then computed by Student's \(t\) test comparison of means.

3. Results

3.1. Dose response of IL-8

We first examined the NSM of eosinophils towards different concentrations of IL-8 \((10^{-10} - 10^{-8}\) M). A direct comparison of eosinophil migration with Percoll- versus MACS-isolated eosinophils was performed (Fig. 1). There was a significant difference in chemotaxis between the two populations at

![Fig. 1. Dose-response of IL-8-induced migration of Percoll- vs. MACS-isolated eosinophils. Data are from five direct comparisons. Results represent mean \pm SEM of eosinophil NSM. * \(p < 0.05\).](image-url)
all doses of IL-8. Eosinophils isolated by the MACS technique did not respond to IL-8 at the concentrations shown in Fig. 1. Moreover, expanding the concentration range from $10^{-12}$ to $10^{-7}$ M did not affect the trends shown in Fig. 1 (data not shown).

3.2. Time course

Next, we examined if this difference in chemotaxis was due to the length of the migration assay. A time course experiment with MACS-isolated eosinophils revealed no change in migration pattern at 4, 5 or 6 h as compared to the standard 3 h assay (data not shown).

3.3. Density

We next questioned whether the difference in chemotaxis was related to the density of the eosinophils isolated. Cells used in the Percoll-isolated eosinophil migration assays had a density greater than 1.095 g/ml, whereas MACS-isolated eosinophil migration assays used cells with densities greater than 1.090 g/ml. We first determined the densities of the eosinophils isolated by the two techniques as a reflection of the percent of total eosinophils. Cell densities for the MACS-isolated eosinophils were determined by the same Percoll discontinuous gradients used to isolate eosinophils. We found no differences in the proportional densities of the eosinophils isolated by the two techniques (Fig. 2), indicating that the MACS procedure likely did not alter eosinophil density. We then determined that MACS-isolated eosinophils did not migrate in response to IL-8 regardless of the density of the eosinophil (Fig. 3). For example, eosinophils isolated by the MACS technique had little to no migration response to IL-8 whereas eosinophils of the same density (> 1.095 g/ml) isolated by Percoll had significant IL-8 induced NSM. Finally, these data also show that MACS-isolated eosinophils do not migrate after being placed on a Percoll gradient.

3.4. Chemotaxis through HUVEC

Dose-response experiments were performed simultaneously with the same donors using both filter and HUVEC monolayer barriers (Fig. 4). Migration of Percoll-isolated eosinophils through HUVEC monolayers was dose-responsive and slightly greater than that measured through filters at $10^{-8}$ M IL-8. The presence of an endothelial barrier, however, did not affect IL-8 induced migration of eosinophils isolated by the MACS technique. These eosinophils were still unresponsive to IL-8.

3.5. FACS

Because of the importance of activation and adhesion prior to chemotaxis, we studied whether differences existed in cell surface markers between different populations of cells isolated by the two techniques. Cells were labeled with antibodies to: CD11b and CD18 adhesion molecules; CD49d, the
eosinophil specific marker; CD16, the neutrophil specific marker; and CD69, the granulocyte activation marker. No differences between the different cell populations were observed in regards to the percentage of cells having CD11b and CD18 (Table 1). CD16 positive cells reflected the neutrophil contamination of the preparations, whereas CD49d positive cells corresponded well with the expected percentage of eosinophils. None of the cells had a high percentage of CD69 present.

3.6. Effect of isolation steps

We next questioned whether specific steps involved in the two techniques altered cell chemotaxis. First, we examined the Percoll isolation steps. Cells exposed to dextran then subsequently isolated by the MACS procedure did not migrate to $10^{-8}$ M IL-8. Neither cells exposed to dextran and Ficoll-Paque nor those exposed to dextran, Ficoll-Paque and Percoll gradients before MACS isolation migrated to $10^{-8}$ M IL-8 (Fig. 5). In all three sets of experiments, eosinophils isolated by the standard MACS and Percoll procedures had migration patterns similar to those shown in Fig. 1 and Fig. 4. In data not shown, we also found that gelatin (used in washes) did not significantly affect IL-8-induced migration of eosinophils isolated by either the Percoll or MACS procedures.

Table 1

<table>
<thead>
<tr>
<th>Band 3 Percoll</th>
<th>Band 4 Percoll</th>
<th>Band 5 Percoll</th>
<th>Pre-MACS beads</th>
<th>Post-MACS beads</th>
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<tr>
<td>CD16</td>
<td>CD49d</td>
<td>CD16</td>
<td>CD69</td>
<td>CD11b</td>
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<td>11</td>
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<td>94</td>
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</table>

Data expressed as percent of total cells having a specific marker was obtained from two donors. Only live cells were analyzed and markers examined were: CD16 (neutrophil specific); CD49d (eosinophil specific); CD69 (activation marker); and CD11b and CD18 (adhesion molecules).

Having ruled out a facilitatory effect on eosinophil chemotaxis by the Percoll procedure, we then asked whether there was an inhibitory effect by the MACS procedure. Eosinophils isolated by the Percoll technique were incubated with the CD16 MicroBeads and run through the magnetic column. A significant inhibition of chemotaxis was seen in those eosinophils (Fig. 6).

3.7. Effects of purity

Since Percoll-isolated eosinophils further purified by CD16 MicroBeads had diminished migration to

**Fig. 5.** Effects of Percoll isolation steps on MACS-isolated eosinophil migration to $10^{-8}$ M IL-8. Peripheral blood was dextran sedimented (DEX-MACS), dextran sedimented then layered onto Ficoll-Paque (DEX-F/P-MACS) or dextran sedimented, layered onto Ficoll-Paque and then placed on a discontinuous Percoll gradient (DEX-F/P-PERCOLL-MACS) prior to MACS isolation steps. Migration of these eosinophils is compared to those isolated by the standard Percoll technique. Results are expressed as mean ± SEM from eight experiments. *, $p < 0.05$.  **Fig. 4.** Dose response of IL-8-induced eosinophil migration with Percoll isolated cells through filters (PERCOLL-FILTERS) and HUVEC monolayers (PERCOLL-HUVEC) compared to MACS isolated cells through filters (MACS-FILTERS) and HUVEC monolayers (MACS-HUVEC). Data are from simultaneous experiments using both barriers and the doses of IL-8 listed. Results represent means ± SEM of eosinophil NSM from four experiments.
IL-8, we questioned the effects of eosinophil purity on migration. We found that when Percoll-isolated eosinophil purity was as high as 92%, migration was still much higher than that with MACS-isolated eosinophils having a purity of 86% (data not shown). Therefore, purity was not a significant factor.

3.8. Presence of soluble inhibitor

The production of a soluble inhibitor due to CD16 MicroBeads incubation with neutrophils or eosinophils could alter the eosinophil migration response to IL-8. However, after resuspending Percoll-isolated eosinophils with supernatants from neutrophils, eosinophils or buffer incubated with CD16 MicroBeads, we found no differences in migration between these cells and those obtained by standard Percoll isolation (data not shown).

Furthermore, the CD16 MicroBeads are shipped by Miltenyi Biotec in medium containing sodium azide. We therefore examined whether the presence of azide could alter eosinophil migration to IL-8. We found that eosinophils isolated by CD16 MicroBeads with or without azide did not migrate to IL-8 (data not shown).

3.9. Effect of magnetic field

We next asked whether the magnetic field somehow affected eosinophil migration. We ran Percoll-isolated eosinophils through the magnetic column without any CD16 MicroBeads. Migration patterns to IL-8 were not altered due to this procedure (data not shown).

4. Discussion

The results found through these numerous experiments clearly indicate that the method of eosinophil isolation affects the chemotactic responsiveness of eosinophils to IL-8. These differences were not due to the viability of cells isolated. We found that IL-8 is capable of inducing human eosinophil migration in a dose dependent fashion when cells are isolated by discontinuous Percoll gradients (Fig. 1 and Fig. 4). However, when eosinophils were isolated by the negative selection technique of MACS using CD16 MicroBeads to remove neutrophils from the cell preparation, no IL-8-induced eosinophil NSM was observed.

The differences in migration between the eosinophils isolated by the two methods could have been due to an alteration of eosinophil density by either method. There have been many studies showing functional differences between eosinophils of different densities (Wardlaw, 1995; Winqvist et al., 1982; De Simone et al., 1982; Prin et al., 1984; Shult et al., 1988). Wardlaw et al. (1986) suggested that hypodense eosinophils have greater chemotactic activity. We found no difference in the density distribution of eosinophils isolated by the two methods (Fig. 2). Furthermore, MACS-isolated eosinophil migration was not affected by the densities of cells used in the migration assay (Fig. 3). These data are consistent with a study by Little and Casale (1991) which showed that density does not affect eosinophil chemotactic responsiveness to PAF.

Recruitment of eosinophils from the circulation into the perivascular tissue requires eosinophils to first adhere to the endothelial lining. Although the exact mechanisms that initiate this process are unknown, evidence suggests that adhesion molecules expressed on both endothelial cells and eosinophils play a crucial role (Kroegel et al., 1994). While endothelial cells express ICAM-1, ICAM-2 and VCAM (Springer, 1990), eosinophils express LFA-1 (CD11a/CD18), Mac-1 (CD11b/CD18) and VLA-4.
(Cd49d) that can bind to ICAM-1, ICAM-2 and VCAM, respectively (Dobrina et al., 1991, Bochner et al., 1991, Weller et al., 1991, Kyan-Aung et al., 1991). We evaluated if CD18 and CD11b receptors were present on the cells isolated by the two techniques. By using CD16 and CD49d, we were able to distinguish neutrophils from eosinophils. We also examined if any eosinophils expressed the CD16 receptor. We found that most cells isolated by both techniques expressed CD18 and CD11b (Table 1). Therefore, any reduction in migration in the MACS-isolated eosinophils could not be explained by the lack of an adhesion molecule needed to initiate the sequence of eosinophil recruitment. We also determined that only an average of 7% of the CD49 positive cells in band 5 (> 1.095 g/ml density) also expressed CD16 and would therefore be removed by the MACS technique employing CD16 MicroBeads. This small percentage of cells could not account for the differences in net stimulated migration between the cells isolated by the two techniques (Fig. 1 and Fig. 4).

Subsequent to adhesion, eosinophils must cross endothelial barriers. IL-8’s potential to induce eosinophil transendothelial migration is supported by studies showing that eosinophils have IL-8 receptors (Villar et al., 1993) and that IL-8 can induce the expression of endothelial adhesion molecules (Carolan et al., 1993). Furthermore, the present studies confirm previous data showing that IL-8 can induce Percoll-isolated eosinophils to cross endothelial barriers (Erger and Casale, 1995). Moreover, investigators using MACS isolated eosinophils have found that IL-8 does not mediate transendothelial migration (Ebisawa et al., 1994). Thus, the data in the present study are consistent with previous positive and negative studies and provide an explanation to account for the disparate results concerning IL-8 reported in the literature.

We also evaluated the percentage of cells which were activated subsequent to isolation by the two techniques. These studies were done since it has been suggested that activated cells migrate more (Dubois et al., 1994; Schweizer et al., 1994; Warringa et al., 1991; Warringa et al., 1992). Nishikawa et al. (1992) demonstrated significant amounts of CD69 expressed on eosinophils obtained from bronchoalveolar lavage fluid in patients with lung inflammation. However, CD69 was not found on peripheral blood eosinophils. CD69 could be induced on peripheral blood eosinophils by incubation with cytokines and other agents. Thus, CD69 appears to be a marker for activated eosinophils. We found little or no CD69 on eosinophils isolated by either technique (Table 1). Thus, based on these data Percoll-isolated eosinophils do not appear to be more activated than MACS-isolated eosinophils.

The positive and negative selection techniques evaluated contain several different steps including dextran sedimentation and Ficoll-Paque and Percoll gradients. Hoekstra et al. (1994) determined that dextran sedimentation could induce an increased percentage of hypodense eosinophils in the circulation of children with asthma. The effects of dextran on eosinophil density could be due to the release of specific granules from eosinophils (Berends et al., 1994). Similarly, Watson et al. (1992) proved that an isolation procedure that specifically included a combined dextran/ficoll step to obtain purified neutrophils increased both receptor expression and oxidase function. We determined that neither dextran sedimentation alone nor in combination with Ficoll-Paque caused MACS-isolated eosinophils to migrate to the level of standard Percoll-isolated eosinophils (Fig. 5) Exposing these cells to discontinuous Percoll gradients did not stimulate migration either (Fig. 5). Finally, the reverse was also true. That is, MACS-isolated eosinophils placed on Percoll gradients did not migrate (Fig. 3).

Since none of the Percoll-isolation steps stimulated MACS-isolated eosinophils to respond to IL-8, we then examined the MACS-isolation steps on Percoll-isolated eosinophils to determine if any change occurred. Indeed, when Percoll-isolated eosinophils were incubated with CD16 MicroBeads, these eosinophils did not migrate to IL-8 (Fig. 6). This inhibition of migration was equal to the typical nonexistence of migration of MACS-isolated eosinophils.

This inhibition of migration of Percoll-isolated eosinophils further isolated with the MACS technique was not due to the removal of the small percentage of contaminating neutrophils. When eosinophil purity of Percoll-isolated preparations was as high as 92%, migration was still significantly higher than that of MACS-isolated eosinophil prepa-
rations ranging from 86% to 100% purity. These data are consistent with studies of Beeson and Bass (1977) showing that varying concentrations of neutrophils and eosinophils did not influence the chemotactic responses to either cell type.

We also questioned whether the negative chemotactic responses of MACS-isolated eosinophils were due to the production of a soluble inhibitor released by neutrophils or eosinophils incubated with the CD16 MicroBeads. We found that supernatants collected from neutrophils and eosinophils incubated with CD16 MicroBeads did not affect migration of standard Percoll-isolated eosinophils. Moreover, sodium azide present in the CD16 MicroBeads preparation was determined not to alter the eosinophil responsiveness to IL-8.

Finally, we checked the effect of the magnetic column itself. We ran Percoll-isolated eosinophils through the magnetic column without any CD16 MicroBeads present. Migration was identical to Percoll-isolated eosinophils not run through the magnetic column.

In summary, we determined that eosinophils isolated by the positive selection technique of Percoll and the negative selection technique of MACS had significantly different migration patterns to IL-8. The migration of eosinophils was inhibited when Percoll-isolated eosinophils were further purified with CD16 MicroBeads. Through a series of experiments we determined that this inhibition was not due to purity of eosinophil preparation used, the loss of adhesion or activation markers, the presence of a soluble inhibitor or the effect of the magnet. Although we were unable to determine the reason for the inhibition and difference in migration, we demonstrated that the isolation technique used to isolate eosinophils does affect the migration to IL-8. Thus, the isolation methodology is the likely reason for discrepancies in the literature concerning IL-8's eosinophilic capacity.

We recognize that eosinophil chemotaxis studies performed in vitro can never exactly duplicate in vivo situations. However, IL-8 lung and nasal challenges have caused eosinophilia (Lagente et al., 1995; Collins et al., 1993; Burrows et al., 1991; Douglass et al., 1994). Therefore, since we and others (Erger and Casale, 1995; Sehmi et al., 1993; Bruijnzeel et al., 1993) have also demonstrated that Percoll-isolated eosinophils chemotactically respond to IL-8, we conclude that studies using eosinophils isolated by the positive isolation technique of Percoll correspond best to in vivo conditions.

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