

# Respiratory pathophysiologic responses

## Cytokines and eosinophil-derived cationic proteins upregulate intercellular adhesion molecule-1 on human nasal epithelial cells

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**Background:** Allergic and nonallergic rhinitis with eosinophilia syndrome are characterized by tissue eosinophilia and nasal mucosal injury. Recently, it has been shown that the adherence of eosinophils and other leukocytes to epithelial cells is mediated by intercellular adhesion molecule-1 (ICAM-1) and related adherence-promoting glycoproteins.

**Methods:** In this study we examined the constitutive expression of ICAM-1 on human nasal epithelial cells (HNECs), and the effects of interferon- $\gamma$ , tumor necrosis factor- $\gamma$  eosinophil major basic protein, and eosinophil cationic protein on the regulation of ICAM-1 expression on these cells. Similar studies were performed with A549 pneumocytes as comparative epithelial cells.

**Results:** Constitutive expression of ICAM-1 was significantly higher on cultured HNECs than on A549 cells, although nasal epithelial cells in tissue specimens did not demonstrate detectable levels of ICAM-1. This spontaneous expression of ICAM-1 on cultured HNECs may explain the unique susceptibility of the nasal mucosa to rhinovirus infection, because ICAM-1 is the epithelial cell receptor for most rhinoviruses. Physiologic concentrations of major basic protein and eosinophil cationic protein stimulated significant upregulation of ICAM-1 on HNECs, which was comparable to that produced by interferon- $\gamma$  and tumor necrosis factor- $\alpha$ . In contrast, these eosinophil constituents did not stimulate ICAM-1 upregulation on A549 alveolar epithelial cells, although A549 cells did respond to interferon- $\gamma$  and tumor necrosis factor- $\alpha$ .

**Conclusion:** The observation that eosinophil products upregulate ICAM-1 on HNECs suggests a positive feedback mechanism, in which the products released from migrating eosinophils might promote additional HNEC-leukocyte adherence by enhancing interactions between leukocyte  $\beta_2$  integrins (CD11/18) and nasal epithelial ICAM-1. (*J ALLERGY CLIN IMMUNOL* 1993;92:527-36.)

**Key words:** ICAM-1, eosinophils, MBP, ECP, nasal epithelium

Substantial evidence indicates that eosinophils can directly damage tissues in allergic rhinitis, asthma, and other diseases associated with blood and tissue eosinophilia.<sup>1-3</sup> The reason for eosinophil accumulation in areas of injury in these diseases is only partially understood. Recent stud-

ies have shown that inflammatory leukocytes, including eosinophils, bind to adherence glycoproteins on endothelial and epithelial surfaces and that this is an essential prerequisite to leukocyte transmigration.<sup>4-6</sup> Such binding promotes leukocyte interactions with tissues, and the consequences may be either protective or harmful. A substantial body of literature suggests that eosinophil accumulation in nasal, sinus, and lung tissue is injurious and a causative factor in the pathogenesis of rhinitis, asthma, and sinusitis.<sup>7-14</sup> In this report we examine the possibility that upregulation of adhesion receptors on human nasal epithelial cells (HNECs) is one mechanism by which eosinophils and other inflammatory leukocytes bind to nasal epithelial membranes and subsequently produce tissue injury. To study this possibility and its relevance to allergic rhinitis

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**Abbreviations used**

|                 |                                                         |
|-----------------|---------------------------------------------------------|
| D-MEM/F-12:     | Dulbecco's modified Eagle's nutrient mixture/F-12 Ham's |
| ECP:            | Eosinophil cationic protein                             |
| ELAM-1:         | Endothelial leukocyte adhesion molecule-1               |
| HNECs:          | Human nasal epithelial cells                            |
| ICAM-1:         | Intercellular adhesion molecule-1                       |
| IFN- $\gamma$ : | Interferon- $\gamma$                                    |
| IL:             | Interleukin                                             |
| LFA-1:          | Lymphocyte function antigen-1                           |
| Mac-1:          | Macrophage-1                                            |
| MBP:            | Major basic protein                                     |
| PBS:            | Phosphate-buffered saline                               |
| PMNs:           | Polymorphonuclear leukocytes                            |
| TNF- $\alpha$ : | Tumor necrosis factor- $\alpha$                         |
| VCAM-1:         | Vascular cell adhesion molecule-1                       |

and/or the nonallergic rhinitis with eosinophilia syndrome, we cultured HNECs in vitro with cytokines known to promote intercellular adhesion molecule-1 (ICAM-1) expression on other cells<sup>15-17</sup> and measured the expression of ICAM-1 and the adherence of leukocytes to these epithelial cells. Further, we attempted to determine whether major basic protein (MBP) and eosinophil cationic protein (ECP), two eosinophil granule constituents, could upregulate ICAM-1 expression on HNECs and thereby produce positive feedback for leukocyte adherence. As a comparison, we performed similar studies with human A549 pneumocytes, a continuous cell line derived from a human bronchoalveolar carcinoma.<sup>18</sup>

**METHODS****Special reagents**

A549 cells, a continuous cell line derived from a human bronchoalveolar carcinoma, were obtained from the American Type Culture Collection (Rockville, Md.). Interferon- $\gamma$  (IFN- $\gamma$ ) and NuSerum were purchased from Collaborative Research Incorporated (Bedford, Mass.). The IFN- $\gamma$  activity was  $2.4 \times 10^7$  U/mg protein. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) was obtained from R & D Laboratories (Minneapolis, Minn.) at concentrations of 0.02 to 0.05 ng/ml. MBP and ECP were a gift of Dr. G. Gleich of the Mayo Clinic in Rochester, Minn. Monoclonal antibody to ICAM-1 (LB-2) was a gift of Dr. Edward Clark of the University of Washington in Seattle, Wash. Previous studies have shown that this antibody is specific to ICAM-1 in that it binds to purified ICAM-1, blocks the binding of lymphocyte function antigen-1 (LFA-1) positive cells to ICAM-1, and stains ICAM-1 on a variety of cell lines.<sup>19, 20</sup> o-Phenylenediamine and lipopolysaccharide (*Escherichia coli* 055:B5) were purchased from

Sigma Chemicals (St. Louis, Mo.). Hydrogen peroxide was purchased from Amersham Corporation (Arlington Heights, Ill.), and dispase was from Boehringer Mannheim Corporation (Indianapolis, Ind.). Phosphate-buffered saline (PBS) without  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ , Dulbecco's modified Eagle's nutrient mixture F-12 Ham's (D-MEM/F-12), L-glutamine, trypsin, ethylenediaminetetraacetic acid, fetal calf serum, antibiotics, and the chromogenic Limulus amoebocyte lysate (LAL) test kit for endotoxin measurement were obtained from Wako Bioproducts (Walkersville, Md.). Goat anti-mouse IgG/IgG-peroxidase conjugate was purchased from Cappel, Organon Teknica Corporation (Malvern, Pa.).

**Purification of human MBP and ECP**

Eosinophils from patients with hypereosinophilic syndrome were obtained by cytapheeresis. Human MBP and ECP were isolated and purified from these cells as described previously.<sup>13</sup> Briefly, purified eosinophil granules were solubilized in 0.01 mol/L HCl (pH 2.0) by sonication, the pH was adjusted to 3.0 with HCl, and the extract was centrifuged. The supernatant was fractionated at 4° C on a Sephadex G-50 column (Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with 0.025 mol/L sodium acetate buffer, pH 4.2, which contained 0.15 mol/L NaCl. Fractions from the third protein peak, which contained MBP, were pooled. Native MBP was prepared by pooling column fractions and freezing at -70° C. Human MBP concentrations were determined by absorbance at 277 nm with the extinction coefficient  $E_{1\text{cm}} = 26.3$ . The molecular weight of MBP, determined from its amino acid sequence, is 13,801.

ECP was purified from granules by sequential chromatography on a Sephadex G-50 column and by affinity chromatography on a heparin-Sepharose 6B-CL column (Sigma Chemical Co.). Fractions from the second protein peak and ascending limb of the third peak of the Sephadex G-50 column were pooled, dialyzed against PBS, concentrated, and chromatographed on heparin-Sepharose 6B-CL equilibrated with PBS at pH 7.4. Bound proteins were eluted with a sodium chloride gradient. ECP elutes at approximately 45,000 micromhos conductivity. Fractions containing ECP were pooled, dialyzed against PBS, and concentrated. ECP concentrations were determined by absorbance at 277 nm with the  $E_{1\text{cm}}$  of 15.6. The molecular weight of ECP was taken as 18,600.

**Human nasal epithelium specimens and tissue culture**

Nasal mucosal specimens were obtained from patients undergoing excision of turbinate tissue primarily for treatment of upper airway obstruction. All patients gave informed consent, and the study was approved by the University of Washington Human Subjects Committee.

Immediately after the surgery, the specimens were placed in D-MEM/F-12 supplemented with 10% NuSe-

rum, 2 mmol/L L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin sulfate, 2.5 µg/ml Fungizone, and 10 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid buffer (Complete medium) and transported to the laboratory. Specimens from patients with tumors, polyps, chronic rhinitis or sinusitis, or cystic fibrosis or specimens that did not appear normal on gross examination were not used.

Tissue specimens were rinsed three times in  $\text{Ca}^{++}$ - and  $\text{Mg}^{++}$ -free PBS with 200 U/ml penicillin, 200 µg/ml streptomycin, and 6 µg/ml Fungizone. The epithelium was peeled away from most of the underlying connective tissue, cut into approximately  $4 \times 4$  mm pieces, placed in 0.4% dispase in PBS without  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ , and incubated at 37° C for 30 minutes. The epithelium was then separated from any remaining connective tissue and placed in trypsin/ethylenediaminetetraacetic acid for 10 minutes at 37° C. The epithelial pieces were pipetted vigorously for 5 minutes, and the resulting cell suspension was transferred to a centrifuge tube. Fresh media containing 10% fetal calf serum was added to inactivate the trypsin, and 5 minutes of vigorous pipetting was repeated. The cell suspensions were pooled and centrifuged at 300 g for 5 minutes. The cell pellet was resuspended in D-MEM/F-12 medium plated in 6-well tissue culture dishes (Costar, Cambridge, Mass.) at a concentration of  $6 \times 10^5$  cells/well and incubated in humidified 95% air with 5%  $\text{CO}_2$  at 37° C. The cells were allowed to attach for 2 days, after which the medium with nonadherent cells was removed by aspiration and fresh medium was added. Thereafter, the cultures were fed twice weekly.<sup>21</sup> The cells were documented to be epithelial in nature by immunofluorescence staining and homogeneous by microscopy.<sup>21</sup>

## ELISA

The HNECs or A549 pneumocytes were plated in 96-well tissue culture plates at  $4 \times 10^4$  cells per well and incubated for 24 hours at 37° C in humidified 95% air and 5%  $\text{CO}_2$ . After this, 100 µl of medium containing TNF- $\alpha$  and/or IFN- $\gamma$  at varying concentrations was added for 16 to 72 hours. In certain experiments both cytokines were added in serial fashion. In other studies, MBP or ECP, in varying concentrations, was added for 24 hours. This duration of incubation was selected on the basis of preliminary time course studies. The plates were processed by washing each well three times with PBS followed by the addition of 100 µl of LB-2 antibody or medium as a control and incubated for a minimum of 60 minutes or overnight. The wells were again washed and then fixed with glutaraldehyde, after which peroxidase-labeled goat anti-mouse antibody (second antibody) or medium was added for 60 minutes at 37° C. After washing with PBS, o-phenylenediamine–0.3% hydrogen peroxide in citrate buffer pH 4.5 was added to each well, and the absorbance was read at 492 nm on an Automated EIA Reader EL309 (Bio-Tek Instruments, Burlington, Vt.). The results are expressed

as total absorbance minus the absorbance measured in wells to which only second antibody had been added.<sup>22</sup>

## Immunohistochemistry

The immunohistochemical methods used to characterize nasal biopsy specimens were modified from those of Ayars et al.<sup>21</sup> Tissue specimens were embedded in optimal cutting temperature embedding medium (Miles Inc., Elkhart, Ind.), frozen in liquid  $\text{N}_2$  and stored at –70° C. Sections of 8 µm in thickness were cut with a cryostat, air-dried on poly-L-lysine-coated slides, and fixed in acetone for 5 minutes at room temperature.

Tissue sections were incubated with various primary monoclonal antibodies for 1 hour at room temperature followed by three washes with PBS of 15 minutes each. The antibodies used were 35BH11, which stains a 54 kd keratin, 34BE12, which identifies 66 kd and 57 kd keratins and LB-2 to stain ICAM-1.<sup>19, 20, 23</sup> Specimens were then incubated for 30 minutes at room temperature in a dark humid chamber with the second antibody, fluorescein-isothiocyanate-conjugated IgG F(ab')<sub>2</sub> fragment specific goat anti-mouse or goat anti-human. The stained specimens were again washed three times with PBS and preserved with a 1:1 glycerol:PBS mounting medium on glass coverslips and stored at 4° C. Stained and mounted specimens were viewed with a Leitz Diaplan photomicroscope (Leica, Inc., Deerfield, Ill.) equipped with phase-contrast-transmitted light optics and with a mercury arc lamp for epifluorescence illumination. Photomicrographs were taken with Ektachrome 160T film (Eastman Kodak, Rochester, N.Y.).

## Isolation of polymorphonuclear leukocytes and assay of their adherence to HNECs

Polymorphonuclear leukocytes (PMNs) were prepared from normal heparinized blood samples with the standard techniques of Ficoll-Hypaque (Pharmacia Fine Chemicals) gradient separation, dextran sedimentation, and hypotonic lysis of erythrocytes.<sup>24</sup> Sterile, nonpyrogenic reagents were used in all experiments. Preparations were over 95% pure as determined by Wright stain and over 99% viable as determined by trypan blue exclusion.

PMNs ( $1 \times 10^6$ ) were labeled for 1 hour at 37° C with 1 µCi of sodium chromate 51 (New England Nuclear, Wilmington, Del.), washed three times, and then resuspended in Complete medium.

The assay of PMN adherence was modified from previously described methods with vascular endothelial cells.<sup>22</sup> Nasal epithelial cells were placed in 96-well plates (Costar) at  $4 \times 10^4$  cells per well and grown overnight until they reached visual confluence. One hundred microliters of TNF- $\alpha$  at 40 ng/ml or an equivalent volume of medium was added to the cells for 24 hours, the wells were gently washed with Complete medium and then replenished with medium alone or medium containing a 1:100 dilution of LB-2 antibody.

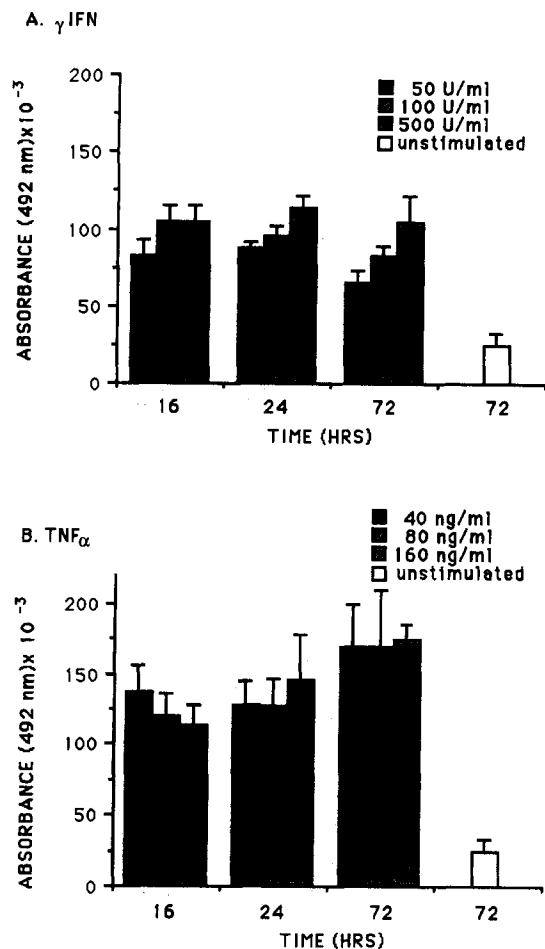


**FIG. 1.** Effects of IFN- $\gamma$  and TNF- $\alpha$  on ICAM-1 expression on HNECs. **A**, Effect of IFN- $\gamma$  added at three concentrations, 50, 100, and 500 U/ml for 16, 24, and 72 hours; **B**, effect of TNF- $\alpha$  added at concentrations of 40, 80, and 160 ng/ml for the same periods of culture. Constitutive expression, shown by the last bar, was measured at 72 hours. Results are expressed as mean  $\pm$  SEM absorbance with second antibody absorbance subtracted from a minimum of four and a maximum of 23 replicate studies.

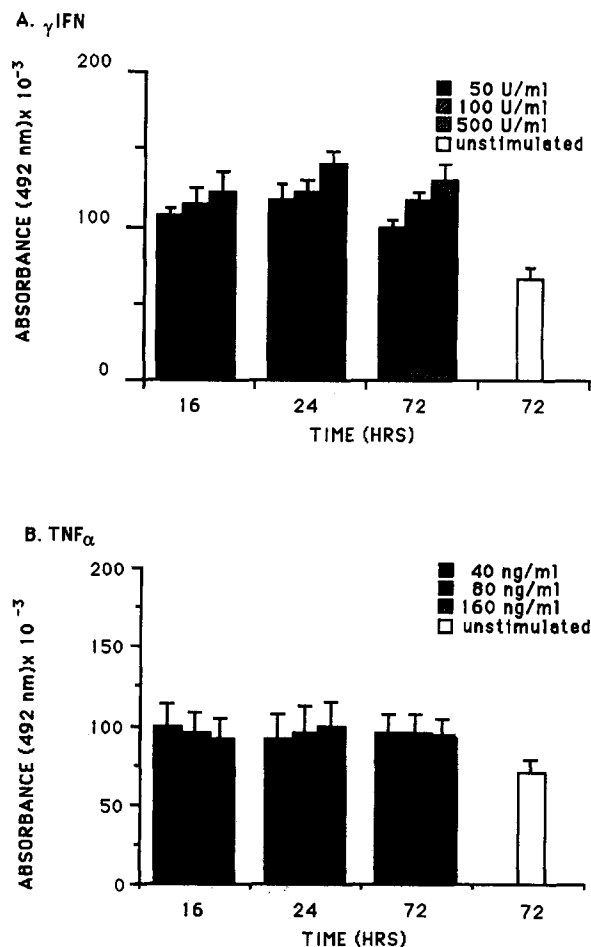


**FIG. 2.** Effects of IFN- $\gamma$  and TNF- $\alpha$  on ICAM-1 expression on A549 cells. **A**, Effect of IFN- $\gamma$  added at three concentrations, 50, 100, and 500 U/ml for 16, 24, and 72 hours; **B**, effect of TNF- $\alpha$  added at concentrations of 40, 80, and 160 ng/ml for the same periods of culture. Constitutive expression, shown by the last bar, was measured at 72 hours. Results are expressed as mean  $\pm$  SEM absorbance with second antibody absorbance subtracted from a minimum of four and a maximum of 16 replicate studies.

After a 30-minute incubation, the medium was decanted and 100  $\mu$ l of Complete medium containing  $1 \times 10^5$   $^{51}\text{Cr}$ -labeled PMNs was added to each well. This produced an approximate 2.5:1 PMN to epithelial cell ratio. After 60 minutes of coculture, the wells were washed three times with prewarmed Complete medium to remove nonattached PMNs, and the adherent PMNs were lysed with two washes of 1% Triton-X 100; the fluids were then pooled. Released  $^{51}\text{Cr}$  was counted with a gamma counter (Packard Instruments, Meriden, Conn.). All assays were performed in triplicate or quadruplicate, and the data are expressed as mean  $\pm$  SEM  $^{51}\text{Cr}$  release.

### Limulus amoebocyte lysate assay

To determine whether there was any contaminating endotoxin in the eosinophil products, we used a standardized chromogenic LAL test kit.<sup>25</sup> In brief, this involved incubating the solutions of ECP and MBP with the LAL sample for 6 minutes and then adding 25% vol/vol glacial acetic acid in water to stop the reaction. A colorimetric change occurred if endotoxin was present, and the absorbance of the sample was determined spectrophotometrically at 410 nm. The concentration of endotoxin was then calculated from a standard curve. The detection limit of the LAL assay is 0.001 ng/ml.

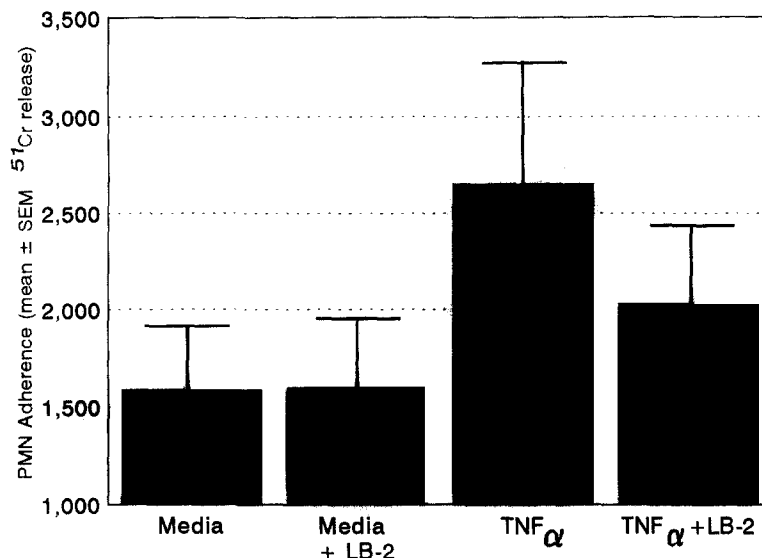


**FIG. 3.** Effect of TNF- $\alpha$  and LB-2 antibody on PMN adherence to HNECs. Cultured HNECs were treated with control medium or TNF- $\alpha$  at a concentration of 40 ng/ml for 24 hours, washed, and then treated with medium or a 1:100 dilution of LB-2 antibody for 30 minutes.  $^{51}\text{Cr}$ -labeled PMNs were added, and adherence was measured as described in Methods. TNF- $\alpha$  treatment of HNECs increased PMN adherence (bar 3 vs bar 1,  $p < 0.02$ ), and addition of excess LB-2 antibody reduced PMN adherence to TNF- $\alpha$ -stimulated HNECs (bar 4 vs bar 3,  $p < 0.02$ ). These data are the mean  $\pm$  SEM of five replicate studies.

## Statistics

Significance was determined by a paired, two-tailed  $t$  test, and  $p$  values equal to or less than 0.05 were considered significant.

## RESULTS

ICAM-1 was constitutively expressed on cultured HNECs at a high level; mean absorbance was  $0.700 \pm 0.009$  (Fig. 1). This was significantly greater than the constitutive expression of ICAM-1 on cultured A549 cells, which had a mean absorbance of  $0.025 \pm 0.008$  ( $p < 0.001$ ) (Fig. 2). To determine whether ICAM-1 could be upregulated on each of these epithelial cell types, we cultured them in vitro with IFN- $\gamma$  or TNF- $\alpha$  at various concentrations for 16 to 72 hours. Addition of IFN- $\gamma$  to HNECs produced significant ( $p < 0.001$  vs constitutive) upregulation of ICAM-1 expression at 16 hours, which did not increase further with time in culture. The increase induced by IFN- $\gamma$  was greatest at 500 U/ml; however, the dose-response curve was relatively flat over a 10-fold concentration range (Fig. 1, A). The effect of TNF- $\alpha$  was similar, demonstrating a flat dose-response curve, stimulation as early as 16 hours, and significant upregulation of ICAM-1

compared with constitutive expression ( $p < 0.01$ ) (Fig. 1, B). These cytokines had similar effects on ICAM-1 expression on A549 cells, producing significant upregulation at 16 hours ( $p < 0.001$  vs constitutive) for both cytokines, which did not increase further with longer duration in culture (Fig. 2). IFN- $\gamma$  produced maximal upregulation at 500 U/ml, whereas the effect of TNF- $\alpha$  was similar at all concentrations tested. TNF- $\alpha$  was less effective in stimulating ICAM-1 on HNECs ( $p < 0.01$  vs constitutive) than on A549 cells ( $p < 0.001$  vs constitutive). Furthermore, it should be noted that these cytokines produced an approximate fourfold to sevenfold increase of ICAM-1 expression on A549 cells but only an average twofold increase on HNECs because of the substantially higher constitutive expression of ICAM-1 on HNECs. Lastly, the combined addition of TNF- $\alpha$  (80 ng/ml for 48 hours) and IFN- $\gamma$  (500 U/ml for 72 hours) to HNECs produced no significant upregulatory effect (mean absorbance of  $0.124 \pm 0.006$ ) above that produced by IFN- $\gamma$  alone. There was, however, a synergistic effect of these cytokines on the upregulation of ICAM-1 on A549 cells. The mean absorbance of A549 cells stimulated with both IFN- $\gamma$  and TNF- $\alpha$  at the

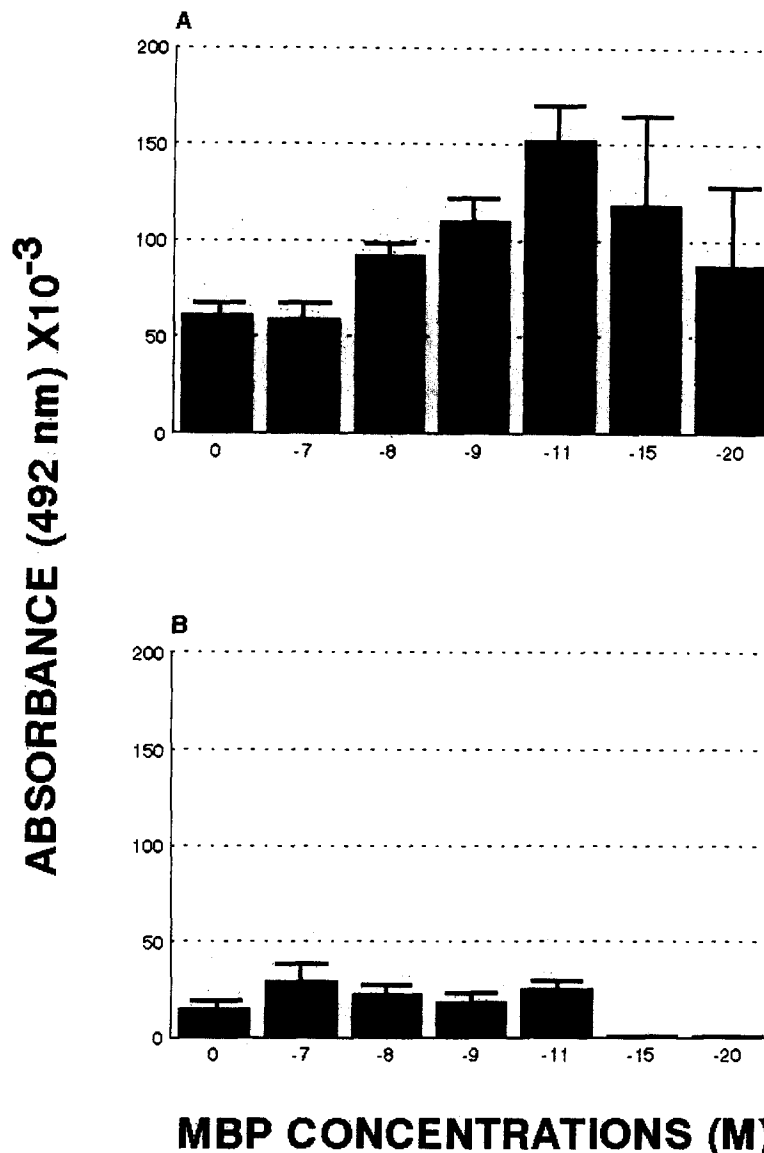


**FIG. 4.** Effect of MBP on ICAM-1 expression on HNECs (A) and A549 cells (B). Results are expressed as mean  $\pm$  SEM of 13 replicate studies. MBP produced significant upregulation of ICAM-1 on HNECs from  $10^{-8}$  to  $10^{-15}$  mol/L but had no significant effect on ICAM-1 expression on A549 cells.

above conditions was  $0.297 \pm 0.037$ , which was significantly greater than that of A549 cells stimulated with only IFN- $\gamma$  ( $p < 0.002$ ) or TNF- $\alpha$  ( $p < 0.05$ ).

In contrast to these findings with cultured HNECs, we detected no observable ICAM-1 on nasal epithelium in tissue specimens examined by immunofluorescence, although these cells did stain strongly with antibodies to cytokeratins (not shown).

To determine the functional relevance of ICAM-1 expression on HNECs we measured the adherence of human PMNs to HNECs, which had

been cultured with and without TNF- $\alpha$  and the ability of excess LB-2 to block PMN adherence (Fig. 3). HNECs stimulated with TNF- $\alpha$  demonstrated 66.7% greater adherence of PMNs than cells cultured in media only ( $p < 0.02$ ). Furthermore, addition of excess LB-2 anti-ICAM-1 antibody significantly reduced PMN adherence to HNECs stimulated with TNF- $\alpha$  ( $p < 0.02$ ). This blocking effect, however, was only partial, suggesting that non-ICAM-1 adhesion mechanisms may also be involved in leukocyte adherence to HNECs.

To determine whether eosinophil-derived cat-

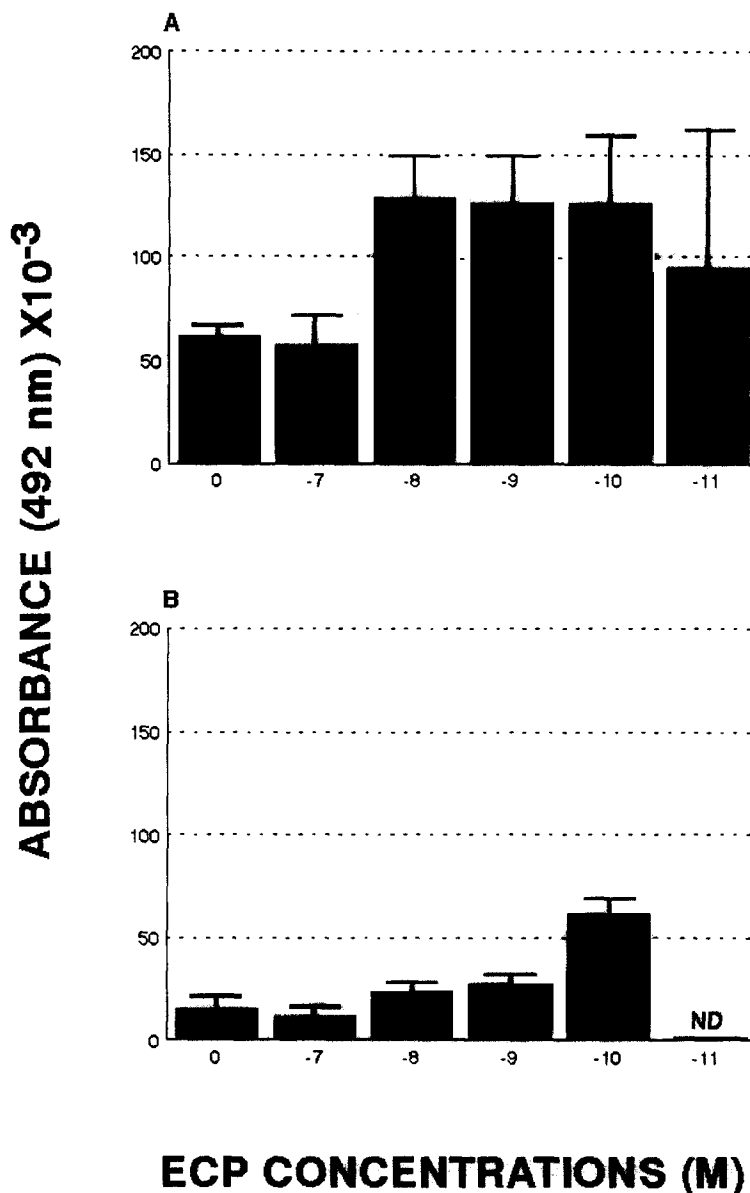


**FIG. 5.** Effect of ECP on ICAM-1 expression on HNE (A) and A549 cells (B). Results are expressed as mean  $\pm$  SEM of 13 replicate studies. ECP produced significant upregulation of ICAM-1 on HNECs from  $10^{-8}$  to  $10^{-10}$  mol/L but had no significant effect on ICAM-1 expression on A549 cells.

ionic proteins could regulate ICAM-1 expression on epithelial cells, we added MBP and ECP to cultured HNECs and A549 cells and measured ICAM-1 expression after 24 hours. MBP was studied over a concentration range of  $10^{-7}$  to  $10^{-20}$  mol/L (Fig. 4, A). In general, MBP stimulated ICAM-1 expression on HNECs over the middle range of concentrations and had an insignificant effect at the highest and lowest concentrations. Statistically significant upregulation of ICAM-1 on HNECs was observed from  $10^{-8}$  to

$10^{-15}$  mol/L MBP ( $p < 0.001$  at all concentrations except  $10^{-15}$  mol/L, which was  $p < 0.01$  vs constitutive). In contrast, there was no significant effect of MBP on ICAM-1 expression on A549 cells over the entire MBP concentration range studied (Fig. 4, B). The effect of ECP on the expression of ICAM-1 on HNECs was similarly examined at concentrations from  $10^{-7}$  to  $10^{-11}$  mol/L. Again, a plateau effect was observed with significant upregulation of ICAM-1 observed from  $10^{-8}$  to  $10^{-10}$  mol/L ( $p < 0.001$  at  $10^{-8}$  and  $10^{-9}$  mol/L,

0.002 at  $10^{-10}$  mol/L vs constitutive) (Fig. 5, A). ECP did not significantly upregulate ICAM-1 on A549 cells at any of the concentrations studied (Fig. 5, B).

Because endotoxin is known to stimulate ICAM-1 expression on a variety of cells, we tested our preparations of MBP and ECP for endotoxin contamination with an LAL test system and none was found. To further evaluate whether small quantities of endotoxin, which might contaminate the MBP or ECP preparations, could account for the observed effects of these eosinophil constituents, we added *E. coli* endotoxin for 24 hours at concentrations between 0.05 and 5 ng/ml to HNECs. These low levels did not stimulate ICAM-1 expression (data not shown). These findings and the fact that MBP and ECP had no effect on ICAM-1 expression on A549 cells make it most unlikely that the observed effects of MBP and ECP on HNECs can be attributed to contaminating endotoxin.

## DISCUSSION

The important findings in this article are that cultured HNECs can express a high constitutive level of ICAM-1 and that the expression of this receptor can be further upregulated by the eosinophil granule proteins MBP and ECP and the cytokines IFN- $\gamma$  and TNF- $\alpha$ . Furthermore, this receptor is functionally active, because HNECs expressing increased ICAM-1 demonstrated greater PMN adherence. These observations may explain the unique susceptibility of the nasal tissues to rhinovirus infections, because most rhinoviruses use ICAM-1 to adhere to epithelial cells.<sup>26, 27</sup> They also suggest that eosinophils might promote the adherence of additional leukocytes to HNECs through the upregulation of ICAM-1.

An extensive body of literature documents that epithelial and endothelial cells express membrane glycoprotein receptors, which mediate leukocyte adherence through interaction with counterreceptors on leukocytes.<sup>28-32</sup> The principal leukocyte adhesion receptor on epithelial cells is ICAM-1.<sup>28</sup> This is a 90 kd surface glycoprotein that is present at low constitutive levels on most epithelial and endothelial cells and upregulated by a number of stimuli such as interleukin-1 (IL-1), TNF- $\alpha$ , IFN- $\gamma$ , and endotoxins. The counterreceptors on leukocytes that bind to epithelial ICAM-1 are the CD11/CD18 adhesion molecules, of which there are three.<sup>28-32</sup> They are heterodimers that share a common  $\beta 2$  integrin chain and differ in their  $\alpha$  chain component. CD11a/CD18, also known as LFA-1, is a primary ligand for ICAM-1. LFA-1 is found on eosinophils, neutrophils, monocytes,

lymphocytes, and some macrophages and functions in T-cell activation and leukocyte adhesion to endothelial and epithelial cells. CD11b/CD18, known as Mac-1, is present on eosinophils, neutrophils, monocytes, macrophages, large granular lymphocytes, and some B cells. In neutrophils Mac-1 is present as a stored pool in secondary granules and can be mobilized to the cell surface within minutes after chemotactic stimulation. There is no such pool of LFA-1. The main functions of Mac-1 are to promote leukocyte adhesion, spreading, and respiratory burst activity after chemotactic stimulation. The counterreceptors for Mac-1 are iC3b, ICAM-1, fibrinogen, and factor X.<sup>31</sup> The third CD11/CD18 molecule is known as p150,95 or CR4, and its functions, although less well defined, appear to be related to neutrophil and monocyte adherence and phagocytosis.

To our knowledge there have been no prior studies of ICAM-1 expression and regulation on nasal epithelium, but nasal vascular endothelial cells and other respiratory epithelial cells have been shown to express this receptor. Montefort et al.<sup>33</sup> found increased expression of ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) on vascular endothelial cells in nasal biopsy specimens from patients with chronic rhinitis compared with specimens from normal control subjects. They also found greater numbers of LFA-1-positive cells in biopsy specimens from patients with rhinitis and a correlation between LFA-1 and ICAM-1 expression. Montefort et al.<sup>34</sup> also demonstrated that human bronchial epithelium, obtained by biopsy, expressed ICAM-1; however, they could not find a quantitative difference in ICAM-1 expression between tissues from patients with atopic asthma and normal subjects. Look et al.<sup>35</sup> found that cultured human tracheal epithelial cells and BEAS-2B virally transformed bronchial epithelial cells expressed ICAM-1 after incubation with IFN- $\gamma$  but not IL-1 $\beta$  or TNF- $\alpha$ . Of interest is that these investigators found high basal levels of ICAM-1 expression on BEAS-2B cells and late-passage human tracheal cells, similar to our observation of spontaneous ICAM-1 expression on cultured HNECs. In addition, Tosi et al.<sup>36</sup> have shown that SV40-immortalized human tracheal epithelial cells (9HTEo<sup>-</sup>) express high levels of ICAM-1, which is approximately sixfold greater than nontransformed cells, and that blocking ICAM-1 on 9HTEo<sup>-</sup> cells with specific monoclonal antibodies reduced PMN adherence by approximately 50%. Furthermore, they found that IL-1 and TNF- $\alpha$  increased ICAM-1 expression on primary tracheal epithelial cells by

three to four times and that treatment of full-thickness tracheal explants with these same cytokines increased epithelial cell expression of ICAM-1 as determined by immunohistochemistry.<sup>36</sup>

In related studies a number of investigators have examined the mechanisms of eosinophil adherence and sought explanations for the predominance of eosinophils at sites of allergic inflammation. Specifically, Lamas et al.<sup>4</sup> found that exposure of eosinophils to f-met-leu-phe, TNF- $\alpha$ , platelet activating factor and a phorbol ester increased adherence of these cells to vascular endothelial cells and gelatinized plates. They also found that treatment of endothelial cells with endotoxin, IL-1, and TNF- $\alpha$  increased eosinophil-endothelial adherence and that this was CD18-dependent because it was inhibited by a monoclonal antibody to the  $\beta$  subunit of the CD11/CD18 complex. Kimani et al.<sup>37</sup> verified that treatment of eosinophils with platelet activating factor and f-met-leu-phe increased the adherence of these cells to endothelial cells and showed that hypodense eosinophils had significantly higher spontaneous adherence and could be stimulated with platelet activating factor to a higher degree of adherence than normodense eosinophils. Kyan-Aung et al.<sup>38</sup> compared the adhesion of eosinophils and neutrophils to human umbilical vein endothelial cells stimulated with IL-1, TNF- $\alpha$ , or endotoxin. These three activators stimulated the adhesion of both types of leukocytes with similar dose- and time-dependent characteristics and antibodies to ICAM-1 and endothelial leukocyte adhesion molecule-1 (ELAM-1); each partially inhibited the adherence of the eosinophils and neutrophils. They also found that ICAM-1 and ELAM-1 were expressed with greater intensity at skin sites that had been challenged in vivo with antigen. These investigators were seeking an explanation for the preferential accumulation of eosinophils at tissue sites in allergic diseases; however, their findings failed to show that different adhesion characteristics between eosinophils and neutrophils were the explanation. In contrast, Dobrina et al.<sup>39</sup> have recently reported that several features of eosinophil adherence are distinct from those of neutrophils. Specifically, they found that unstimulated eosinophil adherence to endothelial cells was higher than that of neutrophils and that binding of eosinophils to endothelial cells involves VLA4 receptors on eosinophils and VCAM-1 on endothelial cells, which is not the case for neutrophil binding. These investigators also concluded that eosinophil adherence to endothelial cells did not involve ELAM-1 because

an anti-ELAM-1 monoclonal antibody did not inhibit adherence. Walsh et al.<sup>40</sup> have similarly reported that eosinophil but not neutrophil adherence to endothelial cells is mediated by the VLA4 ( $\alpha_4\beta_1$ )-VCAM-1 adhesion pathway.

On the basis of this evidence, it seemed likely that adherence glycoproteins were one mechanism by which eosinophils and other leukocytes adhered to nasal epithelium in allergic rhinitis and the nonallergic rhinitis with eosinophilia syndrome and might subsequently produce tissue damage. For this reason we measured the expression of ICAM-1 on HNECs and the effects of cytokines and eosinophil constituents on the up-regulation of ICAM-1 on these cells. Our results show that constitutive expression of ICAM-1 on cultured HNECs is high but can be further up-regulated by IFN- $\gamma$ , TNF- $\alpha$ , and eosinophil MBP and ECP. In contrast, nasal epithelial cells do not express membrane ICAM-1 in situ as shown by immunohistochemical staining of tissue specimens. Cultured A549 cells do not demonstrate a similar spontaneous expression of ICAM-1, nor do they upregulate this receptor in response to MBP or ECP. As noted earlier, other investigators have also found that cultured bronchial and tracheal epithelial cells express high basal levels of ICAM-1,<sup>35,36</sup> although this has not been observed with cultured endothelial cells and keratinocytes.<sup>17,23</sup> In summary, our findings suggest that: (1) rhinoviruses may attack nasal epithelial cells because these cells spontaneously express ICAM-1 and (2) eosinophils may enhance the adherence of additional leukocytes to HNECs by stimulating ICAM-1 expression.

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