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The Effects of Eotaxin on the Surface Adhesion Molecules of Endothelial Cells and on Eosinophil Adhesion to Microvascular Endothelial Cells

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Eosinophil recruitment occurs in tissues as the result of allergic diseases. Human eotaxin is thought to be specific to eosinophils. In this study, we examined the effects of human eotaxin on the expression of adhesion molecules on nasal microvascular endothelial cells and on eosinophil adhesion to endothelial cells. Eotaxin upregulated the expression of ICAM-1 and VCAM-1 on human nasal mucosal microvascular endothelial cells (HMMEC), but not human umbilical vein endothelial cells (HUVEC). The eotaxin-induced eosinophil adhesion to HMMEC was increased at 10 ng/ml and significantly increased at the concentration of 100 ng/ml. On HUVEC, however, eotaxin did not induce increases of eosinophil adhesion. Anti-ICAM-1 and anti-VCAM-1 mAbs significantly decreased eotaxin-induced eosinophil adhesion. These results suggest that eotaxin regulates eosinophil accumulation to the nasal mucosa through its effect on the adhesion molecules on microvascular endothelial cells.

Key Words: **endothelial cells; eosinophils; adhesion molecules; FACS (sorting or staining); chemokines**

Eosinophils are the key cells in allergic inflammatory conditions. Accumulation of eosinophils is seen in a variety of human diseases, including asthma and nasal allergy. The mechanism of leukocyte migration into tissues from the vasculature is not completely understood. Leukocyte migration is regulated in vivo by many cytokines and chemokines. The chemokines behave like chemoattractants to leukocytes and are classified into three families based on the sequence of conserved cystein residues in their primary structures. One of the C-C chemokines, eotaxin, was recently purified from bronchoalveolar lavage fluid of antigen-challenged guinea pigs (1, 2). Human eotaxin has recently been cloned and its cDNA has been sequenced (3, 4), and classified as a member of the C-C chemokine family. The C-C chemokines, macrophage inflammatory protein (MIP)-1α, regulated on activation, normal T cell expressed and secreted (RANTES) and monocyte chemoattractant protein (MCP)-3 have potent leukocytes chemotactic activity. These chemokines have a broad activity spectrum - on eosinophils, monocytes, neutrophils, and lymphocytes (5). However, eotaxin is known as a chemotactic factor specific to eosinophils because the eotaxin receptor, CCR-3, is expressed only on eosinophils (1, 3, 4, 6-10). Human eotaxin stimulates eosinophils, but it has not been reported to have an effect on endothelial cells. In this study, we examined the effects of eotaxin on the surface adhesion molecules of endothelial cells, as well as on eosinophil adhesion to microvascular endothelial cells.

MATERIALS AND METHODS

Reagents. RANTES, IL-4 and TNF-α were purchased from R&D systems (Minneapolis, MN).

Preparation of recombinant eotaxin protein. To isolate a guinea pig eotaxin cDNA, we designed the following degenerate oligonucleotide primers based on the published amino acid (aa) sequence of guinea pig eotaxin protein (2): +5′-gcgaattcAT(C/T/C)-CC(C/G/T/A)-AG(C/T)GCITG(C/T)TG(C/T)TT-3′ and −5′-gcgaattcTT(C/G/T/A)GG-(G/C)TCIGC(G/A)CA(G/T/A)ATCAT(C/T)TT-3′, where the lower case letters represent sequence included to facilitate subcloning of PCR products. The primers were used in an reverse transcriptase-PCR

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Abbreviations used: HMMEC, human mucosal microvascular endothelial cells; RANTES, regulated on activation, normal T cell expressed and secreted; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; mAb, monoclonal antibody.

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protocol to amplify from poly(A)$^+$ RNA prepared from phytohemagglutinin-stimulated guinea pig thymocytes, a 148-bp fragment corresponding to nucleotides 79-227 of the guinea pig eotaxin open reading frame (ORF) (21). A cDNA was then isolated from a phytohemagglutinin-activated guinea pig thymocyte library (22) using the fragment as probe. The guinea pig eotaxin cDNA, which is essentially identical to the one later reported (21), was then used to screen a human lymphocyte genomic library (Stratagene, La Jolla, CA) under low stringency condition (final wash in 0.5 × SSC at 50°C). Hybridizing restriction fragments were subcloned into pBluescript II KS (Toyobo, Tokyo, Japan) and sequenced. A 217-bp genomic fragment corresponding to exon 3 of a novel human chemokine gene was then used to screen a human small intestine cDNA library (Clontech). A positive phage clone designated 141 was converted to plasmid following the manufacturer's instructions. The cDNA inserted was excised by *Xho*- I - and *Xba*- I - digestion, subcloned into pBluescript II KS, and sequenced on both strands. Methods for analyzing human genomic DNA and RNA, and for chromosome mapping by PCR were as described (22). The PCR primers specific for human eotaxin were: +5′-CCTCTCACGCCAAAGCTCACA-3′ and −5′-TAGGCAACACTCAGGCTCTGG-3′. The clone 141 ORF was amplified by PCR using specific primers incorporating *Not*- I - and *Xba*- I - cloning sites, and cloned into the baculovirus transfer vector pVL1392 to make pVLEOS-1. Cotransfection of Tn5B1-4 insect cells with BacPAK6 and pVLEOS-1 DNA and isolation of recombinant viruses were carried out according to the manufacturer's instructions. All of the following protein purification steps were carried out in 20 mM sodium phosphate buffer at pH 7.5 containing the indicated NaCl. Growth medium was collected from Tn5B1-4 cells 48 h after infection with recombinant baculovirus. The medium was dialyzed against 150 mM NaCl, and applied to HiTrap Heparin (Pharmacia Biotech AB, Uppsala, Sweden) pre-equilibrated with 150 mM NaCl. The column was washed with 10 volumes of 400 mM NaCl, and bound protein was eluted with 600 mM NaCl. Eluted fractions were diluted with 2 volumes of NaCl - free buffer and applied onto HiTrap SP (Pharmacia Biotech AB, Uppsala, Sweden) pre-equilibrated with 300 mM NaCl. The column was washed with 300 mM NaCl and eluted with a linear NaCl gradient from 0.3 to 1 M. Fractions containing recombinant protein were pooled, diluted with 4 volumes of buffer, and concentrated in a Centricon 3. The protein concentration was determined by BCA kit, and purity was checked by silver staining after SDS-PAGE. The purity was > 95%. The signal peptide cleavage site was determined by N-terminal sequencing.

Antibodies. Monoclonal mouse anti-human intercellular adhesion molecule-1 (anti-Human ICAM-1, clone: Hu5/3, $IgG_{1\kappa}$), vascular cell adhesion molecule-1 (anti-Human VCAM-1, clone: 2G7, $IgG_{1\kappa}$), and endothelial cell-leukocyte adhesion molecule-1 (anti-Human E-selectin, clone: 7A9, $IgG_{1\kappa}$) were all purchased from Genzyme (Cambridge, MA). CD16 microbeads were purchased from Miltenyi Biotec, Inc. (Sunnyvale, CA).

Collection of inferior nasal mucosa. Turbinal mucosa samples were collected from 9 perennial allergy patients sensitized to house dust and/or mites (5 males and 4 females, mean age 30.8 ± 6.7 yrs) and 6 non-allergic hypertrophic rhinitis patients (3 males and 3 females, mean age 22.1 ± 5.4 yrs). These patients evidenced more than moderate nasal obstruction. Inferior nasal mucosa samples were obtained at the time of surgery for submucosal resection.

Purification of endothelial cells. HMMEC were isolated from human nasal mucosa using the methods reported by Fukuda et al (11). Surgically removed mucosa specimens were washed three times with a Ca^{2+} and Mg^{2+} -free buffered phosphate saline solution (PBS) containing 500 U/ml penicillin, 500 μg/ml streptomycin, and 6 μg/ml fungizon. In these samples, most of the epithelium was peeled off from the underlying connective tissue. Subsequently, the mucosal tissue specimens were cut into 4-mm square sections. The epithelium was then incubated separately for one hour at 37°C in PBS containing 0.05% collagenase (typeII) and 1% EDTA. These sections were placed into Dulbecco's modified Eagle's medium (DMEM) containing 20% fetal calf serum (FCS, GIBCO RBL, Grand Island, NY) in a culture dish after the specimens had been washed three times with PBS. Next, these samples were pressed with a mucosa elevator from the center of each dish toward the periphery as a means of releasing the endothelial cells. The cells were collected by centrifugation (400× g, 3 minutes), and resuspended in DMEM with added 20% FBS and antibiotics. The cells were then placed onto a 60-mm-diameter plastic culture dish coated with type I -rich collagen (Sigma, St.Louis, Mo), and incubated at 37°C under a humidified atmosphere of 5% CO_2 and 95% air. After incubation for one hour, the medium was discarded and the plate was washed three times with DMEM to remove floating cells. HMMEC were cultured in a MCDB107 medium (GIBCO RBL, Grand Island, NY) containing 10% FCS and 75 μg/ml endothelial cell growth supplement. Based on the morphology and positive reactions for anti-factor VIII antigen (clone: 4F9, Immunotech, Marseilles, France) in immunofluorescence staining tests, more than 95% of the HMMEC were shown to be endothelial cells. Materials contaminated with fibroblasts were excluded from the experiments. HUVEC were isolated from umbilical veins and cultured according to the methods as described (12). After two to three passages, the cells were seeded (10^5 cells/cm^2) in collagen-type- I -coated 12 well culture plates (Sumitomo Bakelite Co., Ltd, Tokyo, Japan).

Purification of eosinophils. Eosinophils were purified from peripheral blood obtained from 15 patients with nasal allergy (7 males and 8 females, mean age 31.2 ± 7.9 yrs) using density gradient centrifugation methods. The diagnosis of nasal allergy was made on the basis of a typical history of perennial nasal allergy symptoms, eosinophilia in nasal smears, and a positive radioallergo-sorbent test (RAST) to house dust and mites. The leukocyte fraction was obtained by dextran sedimentation. After the removal of mononuclear cells by the gradient centrifugation at room temperature using Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden), the eosinophils were isolated by negative immunomagnetic selection using CD16 microbeads and the MACS system (Miltenyi, Biotec, Germany) (13). Isolates consisting of > 95% eosinophils, with viability > 97% as judged by trypan blue exclusion, were used in this study. The cells were seeded (2×10^5 cells/cm^2) in collagen-type- I -coated-96 well culture plates (Sumitomo Bakelite Co., Ltd, Tokyo, Japan).

Flowcytometic analysis. HUVEC and HMMEC monolayers were cultured in collagen-type- I -coated-12 well tissue culture plates (Sumitomo Bakelite Co., Ltd, Tokyo, Japan) at 37°C under a humidified atmosphere of 5% CO_2 and 95% air. After reaching confluence, HUVEC and HMMEC were preincubated in the presence or absence of the indicated concentration of 1, 10, or 100 ng/ml of eotaxin, 10 ng/ml of IL-4, 10 ng/ml of TNF-α for 4, 8, 12, 18, 36, and 72 h at 37°C. The cells were then washed with FACS buffer (PBS containing 0.1% of NaN3 and 1.0% of BSA). HUVEC and HMMEC were harvested by tripsinization and the cells were suspended in FACS buffer. The cell suspensions were incubated with saturating concentrations of IgG mouse mAbs recognizing ICAM-1, VCAM-1, and E-selectin, respectively, for 30 min at 4°C. They were then washed free of unbound antibodies, and incubated with the 1:50 dilution of FITC-conjugated anti-mouse IgG (Southern Biotechnology Associates, Inc., Birmingham, AL) in FACS buffer for 30 min at 4°C. After washing, the cells were suspended in 0.5 ml of FACS buffer. The mean fluorescence of each cell population was then determined by flowcytometry (FACScan, Becton-Dickinson, Mountain View, CA), with quantitative determination of mean fluorescence intensity. The cell viability was > 97% as judged by trypan blue exclusion before and after experiments.

Eosinophil-endothelial cell adhesion assays. Details of the Eosinophil-endothelial cell adhesion assay has been described elsewhere (14). Briefly, HUVEC and HMMEC were seeded in collagen type- I -coated-96 well culture plates (Sumitomo Bakelite Co., Ltd, Tokyo, Japan). HUVEC and HMMEC monolayers were observed by phase-contrast microscopy. At confluence, endothelial cells were washed

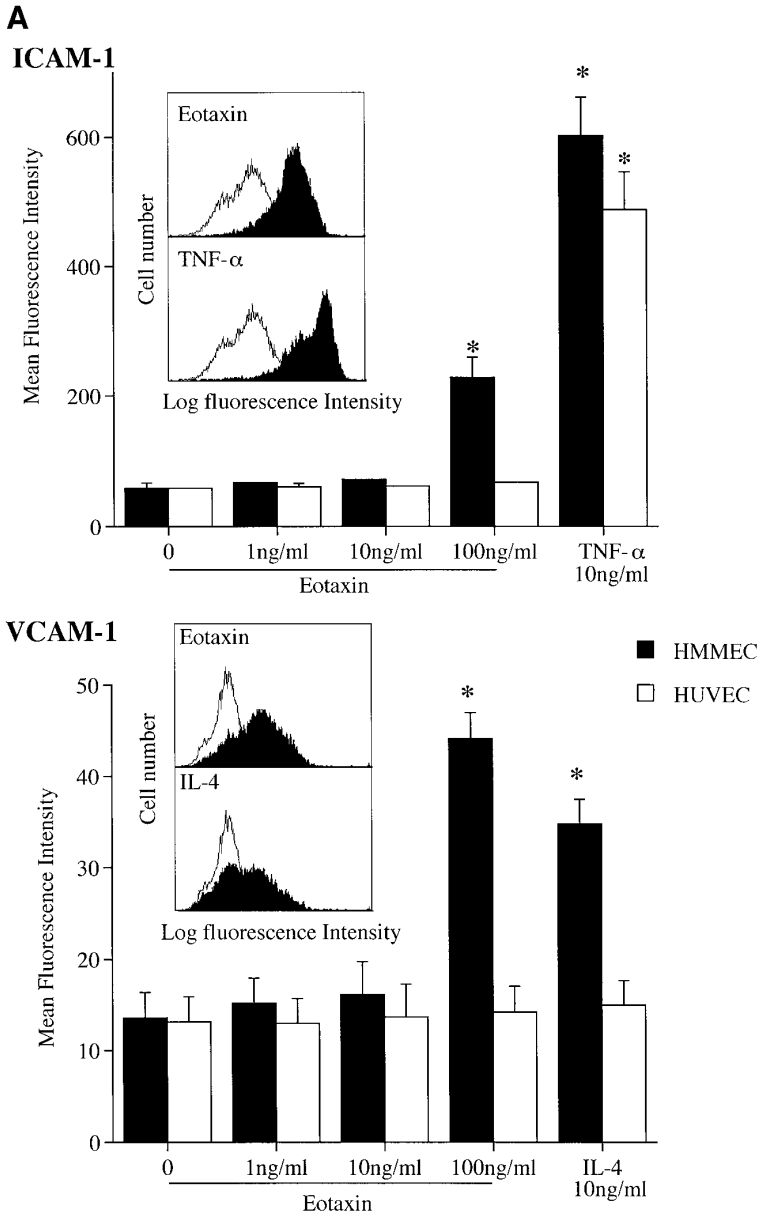

FIG. 1. A, flowcytometric analysis of the expression of surface adhesion molecules, ICAM-1 and VCAM-1 on HUVEC (open bars) and HMMEC (black bars). They were stained for ICAM-1 and VCAM-1 by the indirect FITC method with anti-ICAM-1 and anti-VCAM-1 mAbs, as well as FITC-labeled anti-mouse immunoglobulin G. Inset, representative flow cytometric histograms illustrating expression of ICAM-1 (18 h) or VCAM-1 (4 h) on unstimulated HMMEC (gray lines) or stimulated (black area) with eotaxin (100 ng/ml), TNF-α (10 ng/ml), and IL-4 (10 ng/ml). All values are means ± SEM of 12 experiments. *Indicates $p < 0.01$ vs unstimulated. B, time courses of induction of HMMEC expression of ICAM-1 and VCAM-1 by culture with medium (closed squares), eotaxin (100 ng/ml, closed circles), TNF-α (10 ng/ml, open circles), or IL-4 (10 ng/ml, open squares). All values are means ± SEM of 12 experiments.

three times with phosphate buffered saline (PBS) containing 10 mg/ml gentamicin (Sigma, St. Louis, MO). HUVEC and HMMEC (2.5×10^5 cells/well) were preincubated in the presence or absence, respectively, of the indicated concentration of eotaxin at concentrations of 1, 10, and 100 ng/ml for 8 h at 37°C. The eosinophils were labeled by incubation with the ^{35}S Protein Labeling Mix (Du Pont, Biotechnology Systems, Wilmington, DE) in RPMI without methionine and cystein (GIBCO RBL, Grand Island, NY) for 3 h at 37°C. HMMEC were washed by PBS, and labeled eosinophils were then added and were allowed to adhere to the monolayers for 1 h at 37°C. Monolayers with eosinophils were rinsed by PBS, and then lysed by the addition of 2% Triton X-100 (Sigma, St. Louis, MO). A liquid scintillator (SuperMix, Wallac, Finland) was then added. ^{35}S radioactivity was determined by Micro Beta (Pharmacia Biotech, Sweden). The number of adherent was calculated with each standard curve. All assays were performed in duplicate wells.

Statistical analysis. The results of the study were expressed as the mean value ± SEM. Statistical significance was determined by using the paired Wilcoxon test, defining $p < 0.05$ as statistically significant.

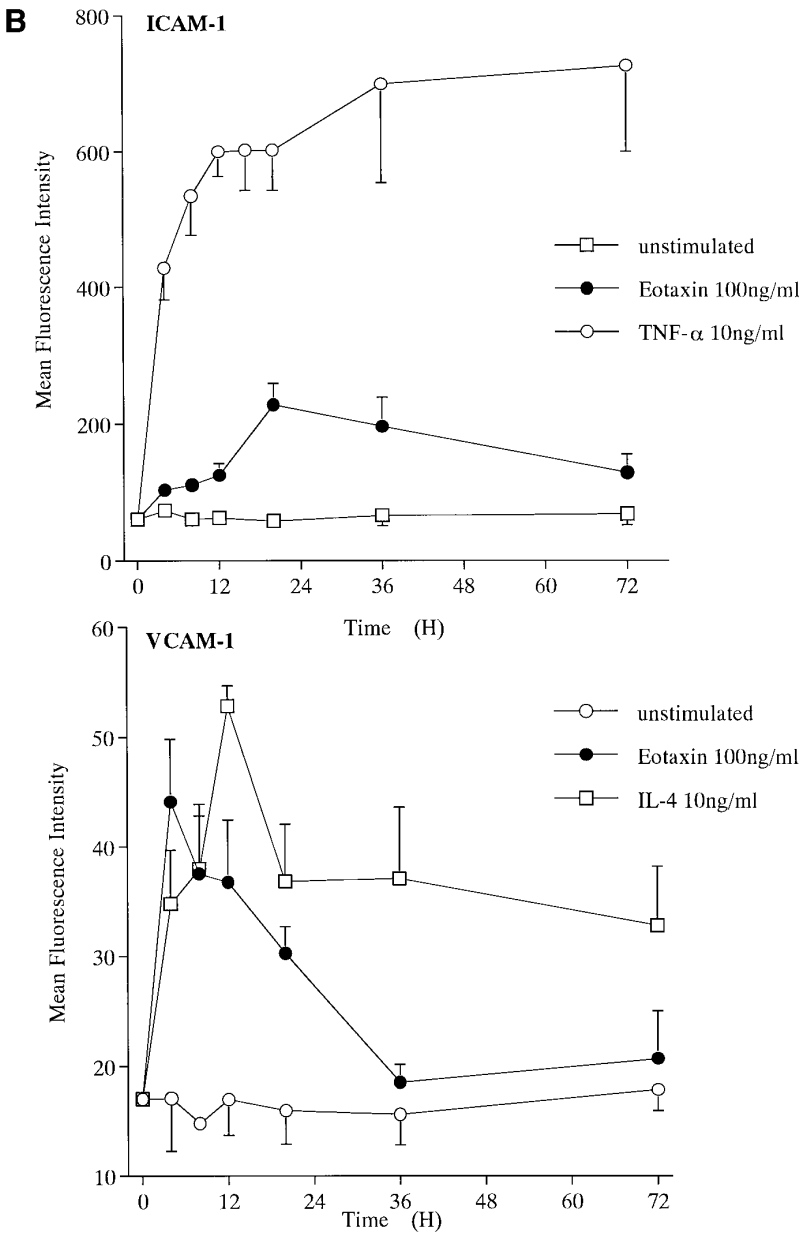

FIG. 2—*Continued*

RESULTS

Effects of eotaxin to the expression of surface adhesion molecules on HUVEC and HMMEC. As shown in Figure 1, eotaxin increased expression of ICAM-1 at 18 h (228.0 ± 31.2, $n = 12$; $p < 0.01$) and VCAM-1 at 4 h (44.1 ± 9.7, $n = 12$; $p < 0.01$) on HMMEC. The expression of ICAM-1 peaked at 18 h, and was sustained to 36 h, while the expression of VCAM-1 peaked at 4 h, but returned to the basal level at 36 or 72 h. However, on HUVEC, expression of these adhesion molecules was not changed. Culture of HUVEC and HMMEC for 4, 8, or 18 h with eotaxin 100 ng/ml failed to induce expression of E-selectin ($n = 5$). TNF-α significantly increased expression of ICAM-1 at 18h on both endothelial cells (601.8 ± 59.3: HMMEC, 487.5 ± 58.3: HUVEC). RANTES also did not significantly increase the expression of ICAM-1 and VCAM-1 at any time and dose (data not shown).

Eosinophil-endothelial cell adhesion experiments. The effects of eotaxin and TNF-α on eosinophil adhesion to HMMEC are shown in Figure 2. HMMEC were incubated with PBS, 10 ng/ml of TNF-α and 1, 10 or 100 ng/ml of eotaxin, and RANTES, respectively, for 8 h. Eosinophils were then allowed to adhere to HMMEC for 1 h, after which unbound cells were rinsed away, and adher-

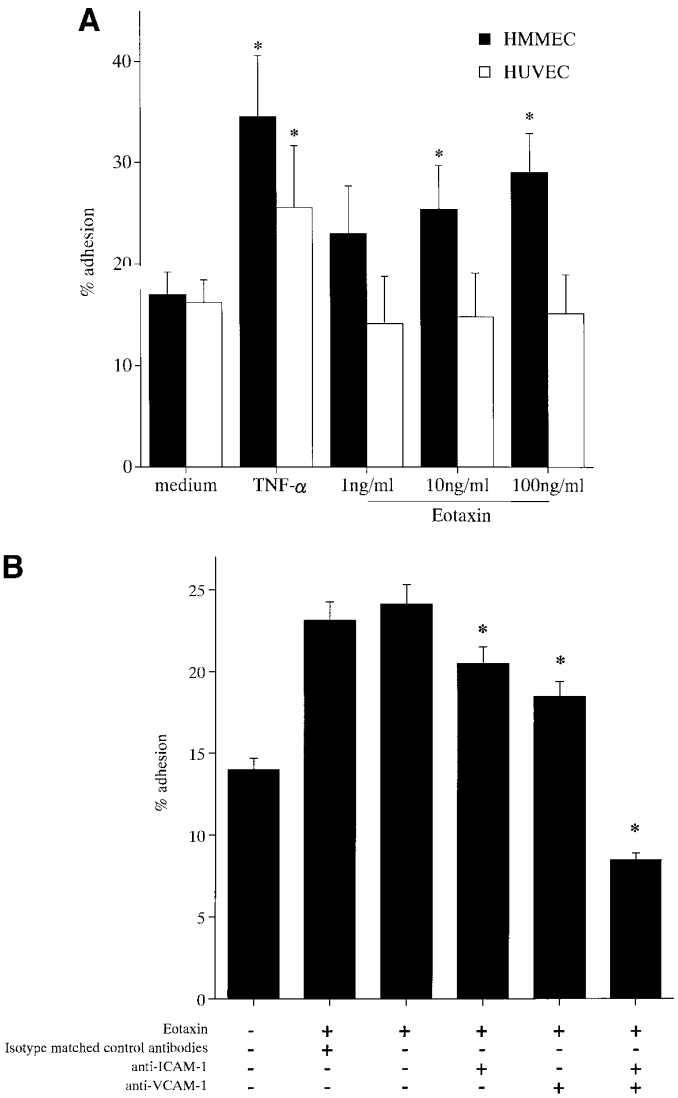

FIG. 2. A, effects of eotaxin on eosinophil adhesion to HMMEC (black bars) without treatment and HUVEC (open bars) or with pretreatment of TNF-α (10 ng/ml) and eotaxin (1, 10, 100 ng/ml). B, effects of anti-ICAM-1 and anti-VCAM-1 mAbs on eosinophil adhesion to eotaxin (100 ng/ml) stimulated HMMEC. Eosinophil adhesion to eotaxin-stimulated HMMEC in the presence of isotype matched control antibodies, anti-ICAM-1 mAb(Hu5/3, 10 μg/ml), anti-VCAM-1 mAb(2G7, 10 μg/ml), or their combinations. All values are means $\pm$ SEM 10 experiments. *Indicates $p < 0.01$ compared to isotype matched control antibodies.

ent eosinophils were detected as isotope counts. The basal rate of eosinophil adhesion to untreated HMMEC was 16.9 $\pm$ 2.2% (n = 10). Adhesion of Eosinophil to TNF-α-activated HMMEC was significantly increased (34.5 $\pm$ 6.1%, n = 10; $p < 0.01$). The adhesion rate following exposure of HMMEC by eotaxin at concentrations of 1 ng/ml (23.0 $\pm$ 4.7%, n = 10), 10 ng/ml (25.4 $\pm$ 4.3%, n = 10; $p < 0.01$) or 100 ng/ml (29.0 $\pm$ 3.8%, n = 10; $p < 0.01$) increased in a dose-dependent manner. RANTES slightly increased the adhesion rate at 100 ng/ml (25.1 $\pm$ 3.3%, n = 10; $p < 0.01$). On HUVEC, eotaxin did not induce increase the eosinophil adhesion.

Effects of anti-ICAM-1, VCAM-1 monoclonal antibodies on eosinophil-HMMEC adhesion. Neutralizing antibodies were added to HMMEC 15min before the addition of labeled eosinophils, and then washed. The eosinophil adhesion induced by 100ng/ml of eotaxin was diminished by anti-ICAM-1 mAb (10 μg/ml; 20.5 $\pm$ 5.4%, n = 5; $p < 0.01$) and anti-VCAM-1 mAb (10 μg/ml; 18.4 $\pm$ 4.8%, n = 5; $p < 0.01$). A combination of anti-ICAM-1 and anti-VCAM-1 mAbs significantly diminished the eotaxin-induced eosinophil adhesion (8.5 $\pm$ 1.5%, n = 5; $p < 0.01$).

DISCUSSION

In this study, we determined that eotaxin upregulates the expression of ICAM-1, and VCAM-1 on HMMEC, but not on HUVEC. We found that eotaxin rapidly augmented VCAM-1 expression at 4 h, but that the expression returned to the basal level at 36 h. On the other hand, the expression of ICAM-1 on HMMEC also increased by eotaxin, peaked at 18 h, and was sustained to 36 h. RANTES had no effect on the expression of these adhesion molecules on both HMMEC and HUVEC. This consistents with the observation that eotaxin rapidly induced pulmonary eosinophilia after antigen challenge in immunized mice, peaked at 6 h (15). Our previous study and another study demonstrated that IL-4 and IL-13 enhanced VCAM-1 expression on HMMEC, peaked at 18 h and sustained at 24 and 48 h (16). The maximal level of eotaxin-induced VCAM-1 expression was comparable with that seen with IL-4. Eotaxin and IL-4 had a different time course of VCAM-1 expression.

Particularly noteworthy is the fact that the expression of the adhesion molecule were increased on HMMEC, but not on HUVEC. We observed that the eotaxin receptor CCR-3 mRNA is detected in HMMEC (unpublished observation). In vivo transendothelial migration of eosinophils into extracellular spaces occurs in microvascular endothelial cells like HMMEC. The diameters of umbilical veins are larger than those of nasal mucosal microvasculars. In histochemical studies, eosinophil migration occurs on microvasculars. These findings suggest that CCR-3 is located on nasal microvascular endothelial cells, not on HUVEC.

Eotaxin increases the expression of ICAM-1 and VCAM-1 on HMMEC, with a significant expression at 100ng/ml. It has been reported that stimulation of eosinophils with eotaxin resulted in increased adhesion to human lung microvascular endothelial cells pretreated with TNF-α, whereas this adhesion was not increased in untreated endothelial cells (17).

In contrast, in this study we stimulated HMMEC with eotaxin. Eosinophil adhesion to eotaxin-activated HMMEC was increased in a dose-dependent manner. Eosinophil adhesion to eotaxin-activated HMMEC in-

creased at the concentration of 10 ng/ml, but no upregulation of the expression of the adhesion molecules occurred. This suggests that eotaxin augments the affinity of eosinophils to HMMEC at low concentrations, and upregulate the expression of the adhesion molecules of HMMEC at high concentrations. While adhesion to RANTES-activated HMMEC was only slightly increased at the concentration of 100 ng/ml. RANTES also binds to CCR-3, but the affinity of eotaxin to CCR-3 is believed to be higher than that of RANTES. Anti-ICAM-1 and anti-VCAM-1 mAbs reduced eotaxin-induced eosinophil adhesion. Moreover pretreatment with anti-ICAM-1 mAb in combination with anti-VCAM-1 mAb reduced the eosinophil adhesion to the basal level. It should be pointed out that the results we observed were eotaxin specific. At present, the specific antibody to CCR-3 is not available to us.

Another C-C chemokine, RANTES, is known to enhance eosinophilic adhesion without an increase in the eosinophil adhesion molecule expression (18). One study has reported that RANTES slightly increased CD11b and CD18 expression (19). This suggests that there exists another mechanism of eosinophil adhesion which is not related to adhesion molecules.

Eosinophils are exposed by many cytokines and chemokines in the intravascular space and in the tissues. The major cellular sources of eotaxin are thought to be the epithelial cells, endothelial cells, and activated infiltrating leukocytes such as eosinophils (4, 8, 20). Leukocytes and epithelial cells are immunoreactive for eotaxin on nasal polyps by immunohistochemistry (4). Eotaxin exposes eosinophils and endothelial cells in vivo. We measured the chemotactic effects of intermediated adhesion molecules on endothelial cells in vitro and observed that eotaxin prolongs the viability of eosinophils (unpublished observation). Eotaxin not only has a chemoattractant effect but also increases the number of adhesion molecules on microvascular endothelial cells. Further studies will doubtless reveal further eotaxin effects. In nasal allergy, eosinophils migrate into extravascular spaces from microvascular in the nasal mucosa, and activated eosinophils secrete the specific granules by eosinophil-degranulating agents. The discovery of the blocking agents for eotaxin-induced eosinophil recruitment may lead to an effective therapy for allergic disease such as nasal allergy.

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REFERENCES

1. Griffiths, J. D., Collins, P. D., Rossi, A. G., Jose, P. J., and Williams, T. J. (1993) *Biochem. Biophys. Res. Commun.* **197,** 1167–1172.
2. Jose, P. J., Griffiths, J. D., Collins, P. D., Walsh, D. T., Moqbel, R., Totty, N. F., Truong, O., Hsuan, J. J., and Williams, T. J. (1994) *J. Exp. Med.* **179,** 881–887.
3. Kitaura, M., Nakajima, T., Imai, T., Harada, S., Combadiere, C., Tiffany, H. L., Murphy, P. M., and Yoshie, O. (1996) *J. Biol. Chem.* **271,** 7725–7730.
4. Ponath, P. D., Qin, S., Ringler, D. J., Clark, L. I., Wang, J., Kassam, N., Smith, H., Shi, X., Gonzalo, J. A., Newman, W., Gutierrez, R. J., and Mackay, C. R. (1996) *J. Clin. Invest.* **97,** 604–612.
5. Wells, T. N., Power, C. A., Lusti-Narasimhan, M., Hoogewerf, A. J., Cooke, R. M., Chung, C. W., Peitsch, M. C., and Proudfoot, A. E. (1996) *J. Leuk. Biol.* **59,** 53–60.
6. Collins, P. D., Marleau, S., Griffiths, J. D., Jose, P. J., and Williams, T. J. (1995) *J. Exp. Med.* **182,** 1169–1174.
7. Daugherty, B. L., Siciliano, S. J., DeMartino, J. A., Malkowitz, L., Sirotina, A., and Springer, M. S. (1996) *J. Exp. Med.* **183,** 2349–2354.
8. Garcia, Z. E., Rothenberg, M. E., Ownbey, R. T., Celestin, J., Leder, P., and Luster, A. D. (1996) *Nature Medicine* **2,** 449–456.
9. Ponath, P. D., Qin, S., Post, T. W., Wang, J., Wu, L., Gerard, N. P., Newman, W., Gerard, C., and Mackay, C. R. (1996) *J. Exp. Med.* **183,** 2437–2448.
10. Uguccioni, M., Loetscher, P., Forssmann, U., Dewald, B., Li, H., Lima, S. H., Li, Y., Kreider, B., Garotta, G., Thelen, M., and Baggiolini, M. (1996) *J. Exp. Med.* **183,** 2379–2384.
11. Fukuda, K., Imamura, Y., Koshihara, Y., Ooyama, T., Hanamure, Y., and Ohyama, M. (1989) *Am. J. Otolaryngol.* **10,** 85–91.
12. Jaffe, E. A., Nachman, R. L., Becker, C. G., and Minick, C. R. (1973) *J. Clin. Invest.* **52,** 2745–2756.
13. Hansel, T. T., De Vries, I. J. M., Iff, T., Rihs, S., Wandzilak, M., Betz, S., Blaser, K., and Walker, C. (1991) *J. Immunol. Methods* **145,** 105–110.
14. Terada, N., Maesako, K., Hamano, N., Hohki, G., Ikeda, T., Sai, M., Yamashita, T., Fukuda, S., Wakita, A., Yoshimura, K., and Konno, A. (1997) *J. Immunol.* **158,** 5464–5470.
15. Maclean, J. A., Ownbey, R., and Luster, A. D. (1996) *J. Exp. Med.* **184,** 1461–1469.
16. Bochner, B. S., Klunk, D. A., Sterbinsky, S. A., Coffman, R. L., and Schleimer, R. P. (1994) *J. Immunol.* **154,** 799–803.
17. Burke-Gaffney, A., and Hellewell, P. G. (1996) *Biochem. Biophys. Res. Commun.* **227,** 35–40.
18. Kakazu, T., Chihara, J., Saito, A., Higashimoto, I., Yamamoto, T., Kurachi, D., and Nakajima, S. (1995) *Int. Arch. Allergy Immunol.* **108,** 9–11.
19. Alam, R., Stafford, S., Forsythe, P., Harrison, R., Faubion, D., Lett-Brown, M. A., and Grant, A. (1993) *J. Immunol.* **150,** 3442–3448.
20. Ganzalo, J. A., Jia, G. Q., Aguirre, V., Friend, D., Coyle, A. J., Jenkins, N. A., Lin, G. S., Katz, H., Lichtman, A., Copeland, N., Kopf, M., and Gutierrez, R. J. (1996) *Immunity* **4,** 1–14.
21. Jose, P. J., Adcock, I. M., Griffiths, J. D., Berkman, N., Wells, T. N. C., Williams, T. J., and Power, C. A. (1994) *Biochem. Biophys. Res. Commun.* **205,** 788–794.
22. Yoshida, T., Imai, T., Kakizaki, M., Nishimura, M., and Yoshie, O. (1995) *FEBS Lett.* **360,** 155–159.