

Allergens, IgE, mediators, inflammatory mechanisms

Detection of the chemokine RANTES and endothelial adhesion molecules in nasal polyps

Lisa A. Beck, MD,^a Cristiana Stellato, MD,^a L. Dawson Beall, MS,^b Thomas J. Schall, PhD,^c Donald Leopold, MD,^d Carol A. Bickel, MS,^a Fuad Barood, MD,^d Bruce S. Bochner, MD,^a and Robert P. Schleimer, PhD^a Baltimore and Rockville, Md., and Palo Alto, Calif.

Background: To better understand the mechanisms of eosinophil recruitment into the upper airways, we examined human nasal polyps for the expression of the chemotactic cytokine RANTES and the endothelial adhesion molecules E-selectin and vascular cell adhesion molecule-1 (VCAM-1).

Methods: Routine histologic examination and immunostaining with antibodies to RANTES, E-selectin, and VCAM-1 were performed on three types of tissues: nasal polyps, sinus mucosa, or turbinates from patients undergoing other elective procedures (S/T), and nasal biopsy specimens from nonallergic volunteers (NA). To further quantify the expression of endothelial adhesion molecules, some tissue samples were homogenized, and the resulting supernatants were assayed with sandwich ELISAs for VCAM-1 and E-selectin.

Results: Polyp eosinophil counts ranged from 19/mm² to 1818/mm² ($763 \pm 120/\text{mm}^2$, mean $\pm$ SEM) and were significantly higher than those found in the control tissues (5 ± 2 in S/T samples and 20 ± 9 in NA samples, $p < 0.002$). Immunochemical staining for RANTES was observed in 11 of 14 polyps; intense staining for RANTES (grade 3) was observed in six of 14 polyps. None of nine S/T samples or five NA samples demonstrated grade 3 staining. Staining with anti-RANTES was largely localized to airway and glandular epithelium. There was no significant correlation between counts of eosinophils or the combined total of eosinophils plus mononuclear cells and the intensity of epithelial RANTES staining in all nasal tissues. Staining for VCAM-1, as well as for E-selectin, was detected in 11 of 14 polyps and eight of 13 control tissues. VCAM-1 detected by ELISA in polyp tissues ($6.8 \pm 1.3 \mu\text{g}/\text{gm}$) was higher than that found in six S/T samples ($1.2 \pm 0.3 \mu\text{g}/\text{gm}$, $p < 0.005$) and in two NA samples ($1.8 \pm 0.02 \mu\text{g}/\text{gm}$, $p = 0.08$). E-selectin values in polyps ($1.4 \pm 0.3 \mu\text{g}/\text{gm}$) were not statistically different from those detected in six S/T samples ($0.5 \pm 0.2 \mu\text{g}/\text{gm}$) or two NA samples ($1.6 \pm 0.4 \mu\text{g}/\text{gm}$). Counts of eosinophils and eosinophils plus mononuclear cells displayed a strong correlation with VCAM-1 ELISA values ($p < 0.005$ and $p < 0.004$, respectively) but not with VCAM-1 staining. VCAM-1 staining correlated with EG2-positive eosinophils in nasal polyp tissues ($p < 0.01$). E-selectin staining did not correlate with either neutrophil or eosinophil counts.

Conclusions: These studies demonstrate that the chemokine RANTES is produced in vivo and is localized predominantly to nasal epithelium. Endothelial activation, as indicated by adhesion molecule expression, occurs in human nasal polyp tissues and in control tissues,

From the ^aDepartment of Medicine (Division of Immunology) Johns Hopkins University School of Medicine, Johns Hopkins Asthma and Allergy Center, Baltimore; ^bOtsuka America Pharmaceutical, Inc., Rockville; ^cDNAX, Palo Alto; and ^dthe Department of Otolaryngology-Head and Neck Surgery, Johns Hopkins University School of Medicine, Johns Hopkins Asthma and Allergy Center.

Supported by grants AI 01226, AI 21037, and AM 31891 from the National Institutes of Health.

Received for publication Apr. 27, 1995; revised Aug. 10, 1995; accepted for publication Sept. 29, 1995.

Reprint requests: Lisa A. Beck, MD, Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Room 3A.62, Baltimore, MD 21224-6801.

J Allergy Clin Immunol 1996;98:766-80.

Copyright © 1996 by Mosby-Year Book, Inc.

0091-6749/96 \$5.00 + 0 1/1/69881

possibly reflecting the continued antigen exposure of the nasal mucosa. The correlations found in this study suggest that expression of VCAM-1 plays a role in the selective recruitment of eosinophils and mononuclear cells into nasal polyp tissues and that RANTES may be more important in localizing eosinophils to the epithelium. (J Allergy Clin Immunol 1996;98:766-80.)

Key words: Chemotactic factors, RANTES, VCAM-1, E-selectin, eosinophil, nasal polyps, endothelial adhesion molecules, eosinophils, epithelium

Eosinophils are now recognized to be important effector cells in allergic diseases.¹⁻⁴ Although the mechanisms by which eosinophils are selectively recruited to sites of inflammation are not yet known, it has been hypothesized that these mechanisms involve the expression of the endothelial adhesion molecule, vascular cell adhesion molecule-1 (VCAM-1), which promotes the adhesion of eosinophils, lymphocytes, monocytes, and basophils, but not neutrophils, to the vascular endothelium. This selectivity is conferred by the counterligand for VCAM-1, very late activation antigen-4, which is present on all circulating leukocytes except neutrophils.⁵⁻⁹ Little is known about the role of endothelial activation in the cellular recruitment seen in nasal polyposis, a disease characterized by outgrowths of unknown causes, arising from the mucosa of the upper airways and paranasal sinuses and characterized by an infiltrate rich in eosinophils and lymphocytes. The expression of VCAM-1 is selectively induced by the cytokines IL-4 and IL-13.⁹⁻¹¹ Recently, IL-4 has been found in nasal polyp (NP) tissue and in the nasal mucosa of allergic subjects after allergen challenge, and IL-4 immunoreactivity has been found in mast cells from patients with perennial rhinitis.¹²⁻¹⁴ Because VCAM-1 is thought to promote the selective recruitment of eosinophils and lymphocytes, we speculated that polyp tissues may express more VCAM-1 than control tissues.^{6-8, 15} In contrast, E-selectin, another endothelial adhesion molecule confers some relative specificity for neutrophils, because neutrophils bear more of the counterligands for E-selectin (sialyl Lewis X or sialyl dimeric Lewis X antigens) on their cell surface than eosinophils.¹⁶ In a primate model of extrinsic asthma, antibodies to E-selectin blocked the early recruitment of neutrophils but not eosinophils after a single allergen challenge.¹⁷ Therefore we speculated that nasal polyposis, a condition characterized by a paucity of neutrophils, would not express more E-selectin than control tissues.

The development of tissue eosinophilia is also probably dependent on the presence of selective

Abbreviations used

dPBS:	Dulbecco's phosphate-buffered saline (pH 7.3) with calcium and magnesium
GM-CSF:	Granulocyte-macrophage-colony-stimulating factor
NA:	Nonallergic
NP:	Nasal polyp
S/T:	Sinus and turbinate
VCAM-1:	Vascular cell adhesion molecule-1
vWF:	von Willebrand factor

priming cytokines such as IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) and chemoattractants such as RANTES, macrophage inflammatory protein-1 α , and monocyte chemoattractant protein-3.^{4, 18-23} Unlike previously described chemoattractants (such as platelet activating factor, leukotriene B₄, C5a, IL-3, and GM-CSF), RANTES is a potent chemoattractant for eosinophils and T lymphocytes, but not for neutrophils, *in vitro*.^{18, 21, 24, 25} This finding was recently confirmed *in vivo* by a study demonstrating that recombinant human RANTES injected intradermally in dogs resulted in an eosinophil- and mononuclear-rich infiltrate with the conspicuous absence of neutrophils.²⁶ Preliminary studies in our laboratories suggest that a similar pattern of eosinophil infiltration occurs after cutaneous injection of RANTES in human volunteers.²⁷ Little is known about the role of RANTES in human allergic diseases. Because RANTES recruits the pattern of cells seen *in vivo* in allergic inflammation (e.g., eosinophils and mononuclear cells), we hypothesized that it was produced by polyp tissues.

To elucidate the role of the chemokine RANTES, as well as endothelial activation (expression of E-selectin and VCAM-1), in the formation of the eosinophil- and mononuclear cell-rich infiltrate characteristic of NPs, we have used immunohistochemical techniques to determine whether these proteins could be detected in NPs. We quantified the cellular infiltrate (neutrophils,

eosinophils, and mononuclear cells) in Giemsa-stained tissue sections. To further analyze the extent of adhesion molecule expression, we prepared tissue homogenates and measured levels of soluble E-selectin and VCAM-1 by using sensitive and specific sandwich ELISAs. We demonstrated the presence of immunoreactive RANTES on the nasal epithelium and glands of tissues from patients undergoing elective surgery for treatment of nasal polyposis. We also observed a correlation between increased expression of VCAM-1 and eosinophil/mononuclear cell counts, suggesting that this endothelial adhesion molecule plays a critical role in the selective recruitment of these cells into NP tissues.

METHODS

Patients

NP tissue was obtained during elective polypectomy in 17 patients with symptoms (7 men and 10 women) between the ages of 33 and 83 years (mean age, 51 years). None of the patients were known to have cystic fibrosis, immunodeficiency, or any other major illness. In three of the 17 cases only archival specimens were available, and therefore only immunohistochemical studies were performed. All but two subjects were using glucocorticoids before polypectomy (intranasal and/or systemic steroids). A chart review identified eight subjects as allergic, (on the basis of positive skin test reactivity), two as nonallergic (on the basis of a lack of skin test reactivity to a battery of common aeroallergens), and seven as unclassified because of the absence of skin testing. Four of the 17 were known to be aspirin-sensitive. Other tissue evaluated for comparison included sinus mucosal and turbinate (S/T) samples from five adult patients undergoing surgery for treatment of sinusitis and sphenoid sinus samples from three patients undergoing transsphenoidal resection of pituitary tumors. Nasal biopsies were also performed on seven nonallergic (NA) adult subjects. Subjects were considered NA if they had no symptoms suggestive of allergic rhinitis or asthma and were also unreactive to skin testing with six common aeroallergens (grass mix, ragweed, cat, dog, dust mite, tree mix). Informed written consent was obtained from all nonallergic subjects with the approval of the Institutional Review Board at the Johns Hopkins Bayview Campus. Tissues were immediately processed for staining and homogenization.

Tissue sections (5 μ m) from formalin-fixed and paraffin-embedded tissues were processed for both routine staining and immunohistochemistry. Eosinophil, neutrophil, and mononuclear cell counts were performed on sections stained with Wright-Giemsa stain. Immunohistochemistry was performed by using the Vectastain ABC-AP kit and the Vector Red Substrate Kit (Vector Laboratories, Burlingame, Calif.) with the addition of a permeabilization step. All reagents were made in accor-

dance with the manufacturer's instructions. The mouse monoclonal IgG_{2a} anti-human RANTES antibody, 3D2, was used at a concentration of 4.2 μ g/ml.²⁸ The specificity of this antibody was confirmed by the lack of cross-reactivity with other C-C and C-X-C chemokines in Western blot assays. A mouse IgG_{2a} myeloma protein was used at the same concentration as a negative control for this antibody (Coulter Immunology, Hialeah, Fla.). In addition, several NP samples were stained with other monoclonal antibodies specific for RANTES, (no. 21404.1 [IgG₁] at a concentration of 2.56 μ g/ml and no. 21438.1 [IgG_{2a}] at a concentration of 9.2 μ g/ml; R&D Systems, Inc., Minneapolis, Minn.). Polyclonal rabbit anti-VCAM-1 and anti-E-selectin were used at a concentration of 6 μ g/ml (supplied by W. Newman, Otsuka America Pharmaceutical, Inc., Rockville, Md.), and rabbit anti-human von Willebrand factor (vWF) (Dako A/S, Glostrup, Denmark) was used at a concentration of 1 μ g/ml. Rabbit IgG was used as the negative control for these polyclonal antibodies. The monoclonal antibody EG2 (Kabi Pharmacia, Uppsala, Sweden) was used at a concentration of 2 μ g/ml to stain the cleaved, actively secreted form of eosinophil cationic protein and thereby identified activated eosinophils; mouse IgG₁ myeloma protein was used as a negative control.²⁹ The bronchial epithelial cell line, BEAS-2B, stimulated with tumor necrosis factor- α was used as a positive control for RANTES; tonsils were used as a positive control for VCAM-1, E-selectin, and vWF; and allergen-challenged skin was used as a positive control for EG2. Tissue sections were deparaffinized in xylene and rehydrated through graded alcohol solutions with the final wash in 0.05 mol/L Tris-buffered saline pH 7.5. The sections stained for RANTES, E-selectin, and vWF and their controls were permeabilized by incubation with 0.25% trypsin (Biofluids, Inc., Rockville, Md.) at 37° C for 20 minutes, whereas those stained for VCAM-1 and its negative control were permeabilized with a citric acid buffer, Antigen Retrieval *Citra* system (Biogenix, San Ramon, Calif.). Tissue sections were then washed twice with Tris-buffered saline, and nonspecific binding was blocked by incubating the sections with normal goat serum (Vector Laboratories) for 30 minutes at room temperature. The primary antibody and the negative control were added and allowed to incubate at 4° C for 12 to 18 hours. After this incubation, the sections were washed with Tris-buffered saline, and biotinylated goat anti-mouse or anti-rabbit secondary antibody was added and allowed to incubate for 1 hour at room temperature. The avidin-biotin-alkaline phosphatase complex was added, and slides were incubated for 30 minutes at room temperature, followed by the addition of the chromogen, Vector Red, for 20 minutes at room temperature in the dark. The sections were counterstained with Gill-Baker-Mayer formula hematoxylin solution (Anderson Laboratories, Inc., Fort Worth, Texas) for 2 minutes, dehydrated through graded alcohol solutions and xylenes, and permanently mounted with Cytoseal 60 (Stephen

Scientific, Riverdale, N.J.). Nasal epithelial cells and glandular cells were readily identified histologically and were clearly distinguishable from inflammatory cells.

Quantification

Specimens were scored by two independent observers (L.A.B. and C.A.B.) in a blinded manner. The same location in each specimen was identified by coordinates on the microscope stage, and inflammatory cells and immunoreactive RANTES, VCAM-1, E-selectin, and vWF were quantified. The area counted consisted of three rows of eight reticles, each beginning directly below the epithelium; an Olympus BH-2 microscope (Olympus America, Inc., Melville, N.Y.) equipped with an Olympus WHK 10×/20L eyepiece and 40× objective was used for counting. This count was multiplied by 3.5 to give the number of cells per square millimeter. Cells counted as mononuclear included lymphocytes and monocytes but not macrophages because cells that were larger than 12 μm and those that had a foamy cytoplasm were excluded. The immunohistochemical scores for the two endothelial adhesion molecules, VCAM-1 and E-selectin, were calculated by dividing the number of vessels staining for these endothelial adhesion molecules by the number of vWF-positive vessels in the same area of tissue by using adjacent sections and multiplying by 100. There was good correlation between the results of the two observers with regard to eosinophil ($p < 0.001$), mononuclear ($p < 0.05$), neutrophil ($p < 0.0001$), EG2⁺ cell ($p < 0.002$), E-selectin ($p < 0.0001$), and VCAM-1 counts ($p < 0.0002$).

The RANTES staining of the pseudostratified columnar epithelium was graded by the intensity of the stain as: 0, absent; 1, weak; 2, moderate; or 3, strong. The staining within the glandular epithelium was assessed by extent: 0, none; 1 up to 25% of all acini; 2, between 25% and 50% of all acini; or 3, more than 50% of all acini in the entire section. Some tissue sections had no glands, and therefore not all tissues had a score for extent of RANTES glandular staining. The two observers' assessments for intensity and extent of RANTES staining were identical.

Tissue homogenization and detergent solubilization

Representative samples from each specimen obtained directly from the operating room were washed with sterile Dulbecco's phosphate-buffered saline (pH = 7.3) with calcium and magnesium (dPBS) (Quality Biological, Inc., Gaithersburg, Md.) to remove any blood or blood clots. Specimens were either immediately processed as outlined below or stored at -80°C for later processing. Frozen tissue samples were lyophilized overnight and subsequently weighed. The tissue (up to 300 mg) was homogenized for 60 seconds in 0.85 to 2.0 ml of dPBS by using a Polytron PT 1200 homogenizer with a microprobe (Kinematica AG, Switzerland) at the highest speed. The specimen was kept on ice to minimize

protein denaturation from heat generated during homogenization. After homogenization, the probe was rinsed with an additional 0.5 ml of dPBS. Triton X-100 (Sigma Chemical Co., St. Louis, Mo.) was added to the homogenate to achieve a concentration of 1% (wt/vol), and the homogenate was stirred at 4°C for 4 to 6 hours. Detergent-solubilized homogenates were then centrifuged at 3000 g for 15 minutes to remove debris. Supernatants were stored in small aliquots at -80°C until they were assayed by ELISA.

ELISA

VCAM-1 ELISAs were performed with a commercially available kit (R&D Systems) that was sensitive to less than 2.0 ng/ml. E-selectin was quantified by using a sandwich ELISA (sensitivity < 100 pg/ml).³⁰ There is no cross-reactivity in this assay with either L- or P-selectin. These ELISAs each have an intraassay variation of less than 5% and interassay variation of less than 10%. ELISA results were expressed as nanograms per gram of freeze-dried tissue. Polyp extracts produced ELISA curves parallel to those of standards of either VCAM-1 or RANTES (not shown). dPBS with 1% Triton X-100 did not interfere with detection of VCAM-1 or E-selectin in the ELISA.

Statistics

Staining scores, ELISA values, and cell counts from the three clinical groups (NP, S/T, and NA) were analyzed by nonparametric analysis of variance with Kruskal-Wallis and Mann-Whitney U tests. Correlation coefficients were analyzed with the Spearman-Rank method. The software used for these calculations was Statview II for Macintosh computers (Abacus Inc., Berkeley, Calif.). Data were expressed as means $\pm$ SEM and p values less than 0.05 were considered significant.

RESULTS

Cellular infiltrate

Most NP tissues were characterized by large numbers of eosinophils in the subepithelium, with a mean of 763 ± 120 cells/mm². This was significantly more than that seen in the other two categories of tissues examined: S/T samples (5 ± 2 cells/mm², $p = 0.0001$) and NA samples (20 ± 9 cells/mm², $p = 0.002$) (Fig. 1). Over half (61% $\pm$ 14%) of the eosinophils in NP tissues were activated as demonstrated by EG2⁺ staining. In most NP tissues the eosinophils appeared to be preferentially localized in the vicinity of the surface epithelium and the mucinous glands (Fig. 2, A). NP tissue tended to have more mononuclear cells (191 ± 33 cells/mm²) than NA tissue (82 ± 28 cells/mm², $p < 0.05$) and S/T tissue (111 ± 36 cells/mm², $p = 0.06$). There were no differences in the number of neutrophils seen in the subepithe-

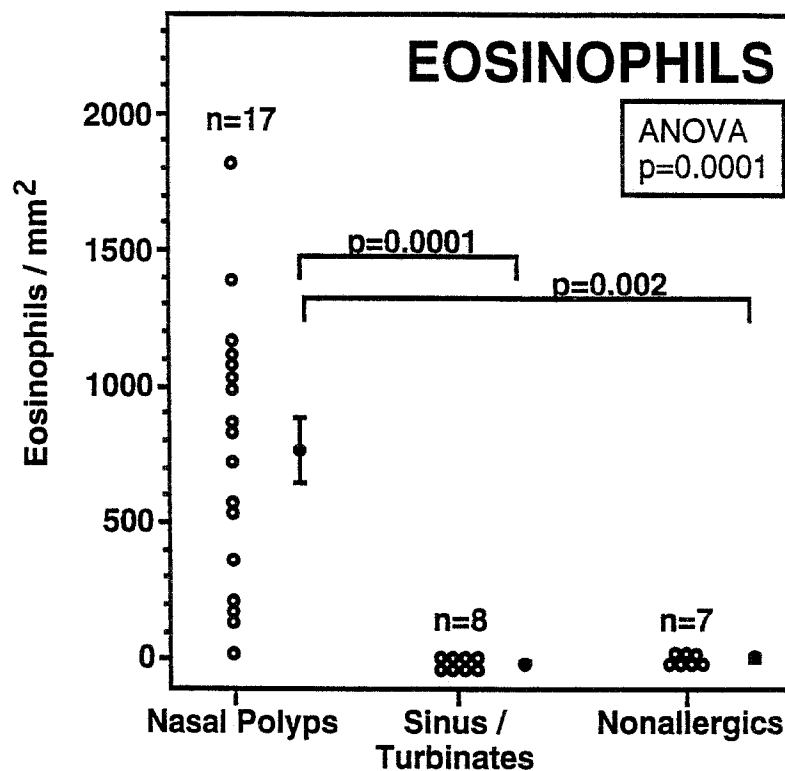


FIG. 1. Eosinophil counts in tissues from patients undergoing surgery for NPs and S/T disorders and in biopsy specimens of NA individuals. The degree of tissue eosinophilia was significantly greater in NP tissues when compared with S/T and NA samples.

lium among NP (67 ± 32 cells/mm²), S/T (120 ± 96 cells/mm²), or NA (69 ± 19 cells/mm²) samples.

RANTES expression

Staining of surface and glandular epithelium for RANTES was found in all tissue groups (Fig. 3). Although detectable RANTES staining was seen in the nasal epithelium of 11 of 14 NP, 5 of 8 S/T, and 3 of 7 NA tissues, intense staining (grade 3) was only seen in NP specimens (6 of 14) and not

S/T (0 of 8) or NA (0 of 7) tissues (Fig. 2, B and C). Although the average staining scores were not significantly different among these three groups of tissues, grade 3 staining was seen significantly more often in NP tissues than in NA and S/T tissues ($p < 0.03$). The surface epithelial cells demonstrated largely cytoplasmic staining with little to no staining seen in goblet cells. The epithelial stain intensity varied significantly within individual sections. We noticed that localization of the most intense

FIG. 2. Giemsa-stained section of NP tissue ($\times 150$ magnification) demonstrating prominent accumulations of eosinophils adjacent to the pseudostratified columnar epithelium (*left*). An adjacent section stained with EG2 antibody to identify activated eosinophils (*right*). Eosinophils can also be seen within the epithelium (**A**). RANTES-stained NP epithelium ($\times 200$) with a mouse monoclonal antibody (3D2); a representative grade 3 is shown. There was no staining with the control antibody (*insert*) (**B**). The epithelium of S/T tissues ($\times 300$) also stained for RANTES (grade 1) but always less intensely than that seen in NP. No staining was seen with the control antibody (*insert*) (**C**). RANTES staining (grade 3) was also noted on glandular epithelium of NP tissues. The negative control demonstrated no staining (*insert*) (**D**). RANTES staining was occasionally noted in vascular endothelium ($\times 300$) and vascular smooth muscle cells ($\times 400$) (**E** and **F**, respectively). VCAM-1 expression ($\times 200$) on vascular endothelium and perivascular mononuclear cells was seen occasionally in NP tissue, with no endothelial staining when rabbit immune serum was used (*insert*) (**G**). E-selectin expression in NP tissue ($\times 300$). No endothelial staining was seen in the control (*insert*) (**H**).

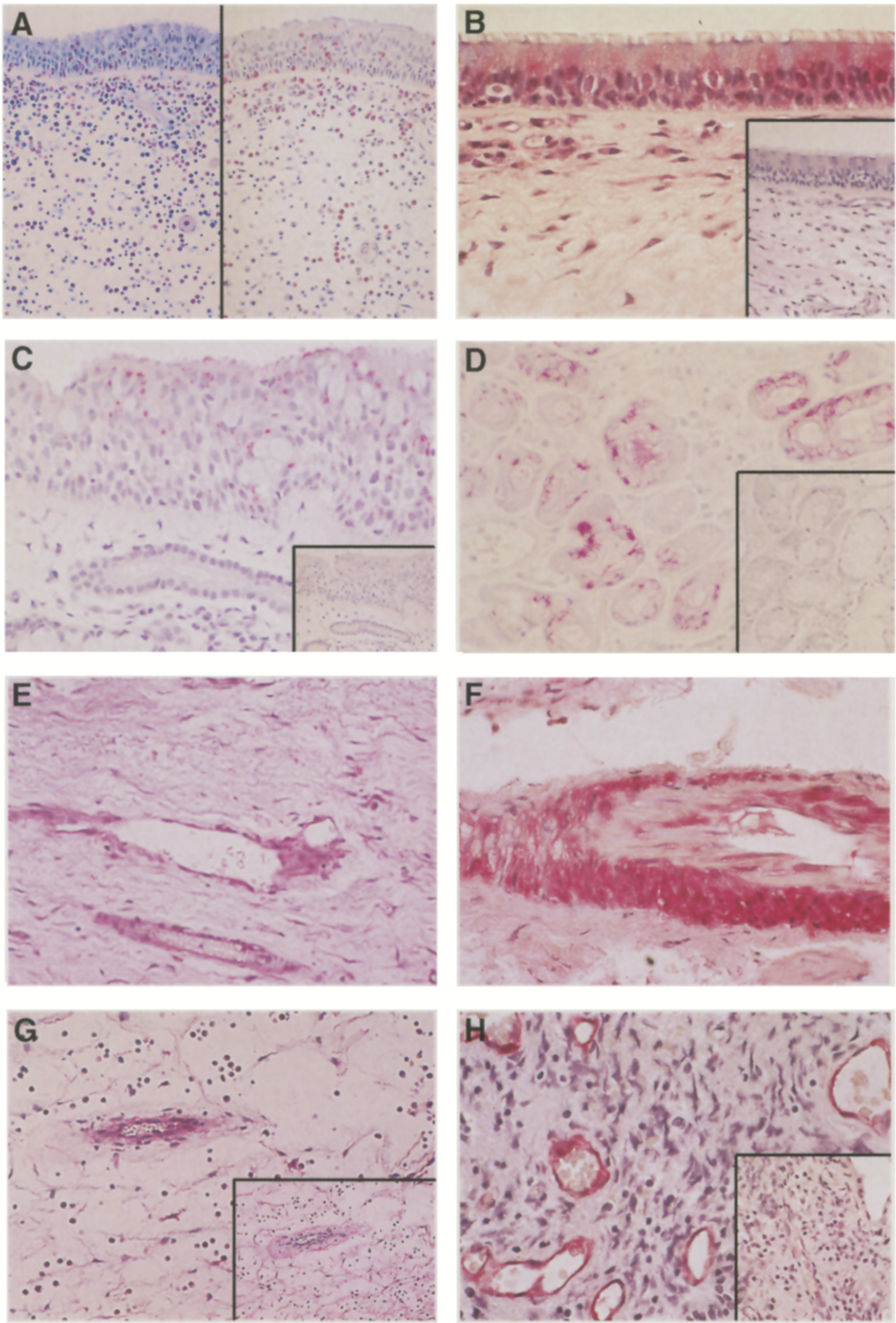


FIG. 2. For legend see opposite page.

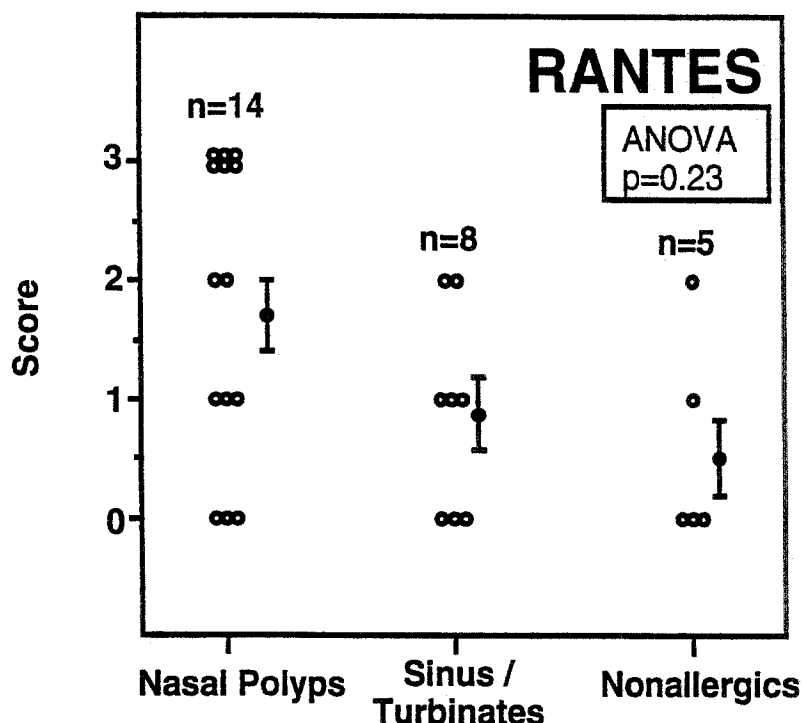


FIG. 3. RANTES staining score in NP, S/T, and NA nasal biopsy tissues. The RANTES score is based on intensity of epithelial staining according to a visual analog scale of 0 to 3 in increments of 1.0. Individual tissue values are the average of two counts performed by two blinded investigators.

staining occurred at sites with the most prominent sub- and intraepithelial eosinophilia. No staining was seen with control mouse IgG_{2α} antibody (Fig. 2, *B* and *C*), confirming the specificity of the mouse monoclonal antibody, 3D2. Other anti-RANTES antibodies (no. 21404.1 and no. 21438.1) demonstrated similar, although less intense, staining than that seen with 3D2 (not shown). Both the intraluminal mucin and the glandular epithelium were immunoreactive for RANTES in nine of 14 NP specimens (Fig. 2, *D*). RANTES staining was sometimes observed in vascular endothelial cells (Fig. 2, *E*), smooth muscle cells (Fig. 2, *F*), and some mononuclear cells in the lamina propria (not shown).

Endothelial adhesion molecule expression and quantification

The fraction of vessels staining with VCAM-1 ($16\% \pm 4\%$) and E-selectin ($16\% \pm 5\%$) in NP samples was determined (Fig. 4, *A* and *B*). Although there was a trend for greater VCAM-1 staining in NP samples, there was no significant difference in the percentage of vessels staining for these two endothelial adhesion molecules among all three tissue groups (NP, S/T, and NA).

VCAM-1 immunoreactivity was seen on endothelium (Fig. 2, *G*) and perivascular mononuclear cells, whereas E-selectin staining localized to endothelium only (Fig. 2, *H*). No endothelial staining was seen with the rabbit immune serum used as a negative control (Fig. 2, *G* and *H*). To further quantify the expression of E-selectin and VCAM-1, we homogenized and detergent-solubilized freeze-dried tissue samples and assayed the resulting supernatants for soluble E-selectin and VCAM-1 by ELISA (Fig. 5, *A* and *B*). The amount of VCAM-1 isolated from NP samples (6842 ± 1324 ng/gm) was greater than that found in S/T samples (1194 ± 279 ng/gm, $p < 0.005$) and NA samples (1820 ± 231 ng/gm, $p < 0.08$). These values are much greater than could be accounted for from blood trapped in these specimens (average freeze-dried weight = 68 mg) because the mean serum value of VCAM-1 from normal individuals ranges from 395 to 714 ng/ml (R&D Systems, Inc.). There were no significant differences among the levels of E-selectin found in NP samples (1436 ± 349 ng/gm), S/T samples (478 ± 234 ng/gm), and NA samples (1615 ± 351 ng/gm). Again, it seems unlikely that the levels of E-selectin found in these samples could be accounted

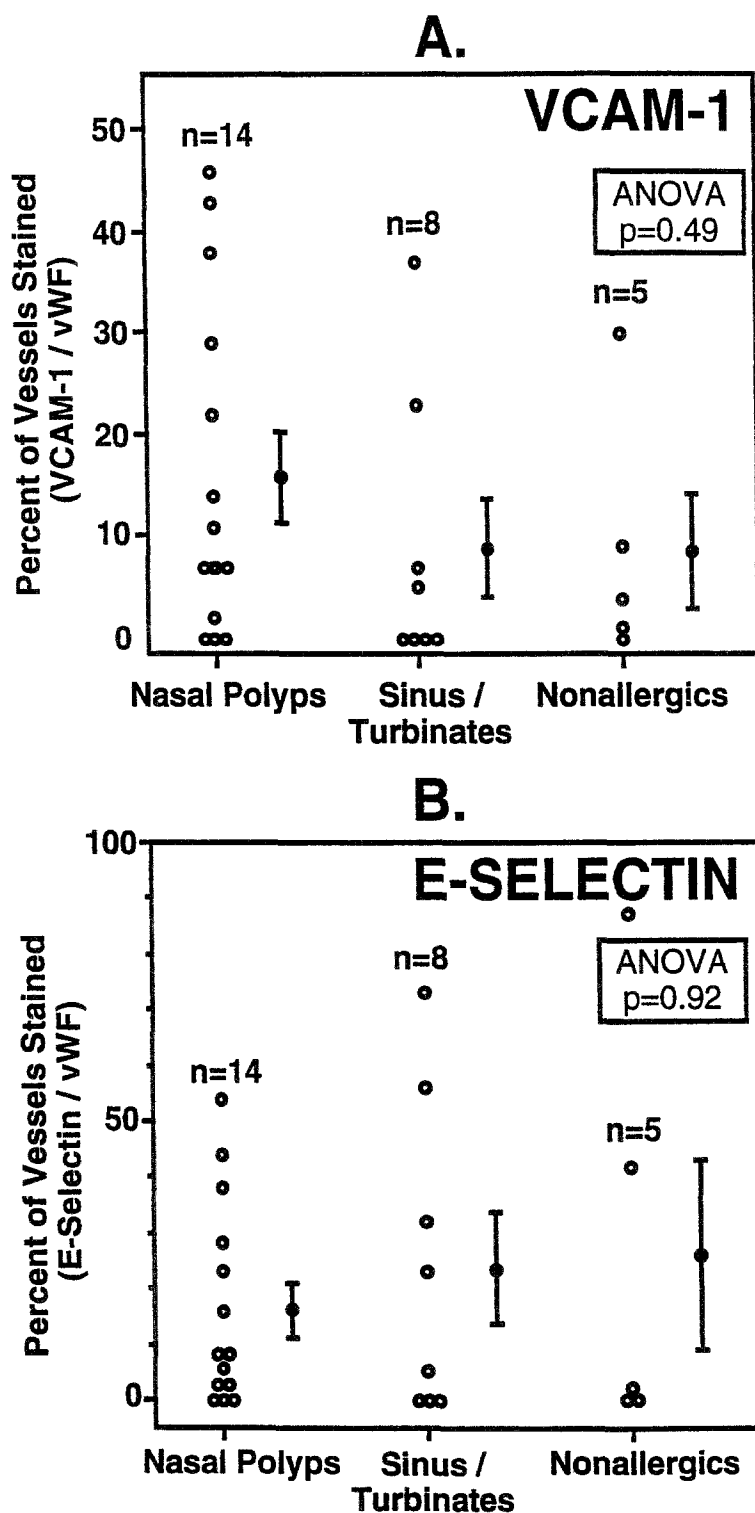


FIG. 4. Endothelial staining for VCAM-1 (**A**) and E-selectin (**B**) in NP, S/T, and NA nasal biopsy tissues. Immunohistochemical scores for the two endothelial adhesion molecules were calculated by dividing the number of VCAM-1- or E-selectin-positive vessels by the number of vWF-positive vessels in the same area of tissue by using adjacent tissue sections.

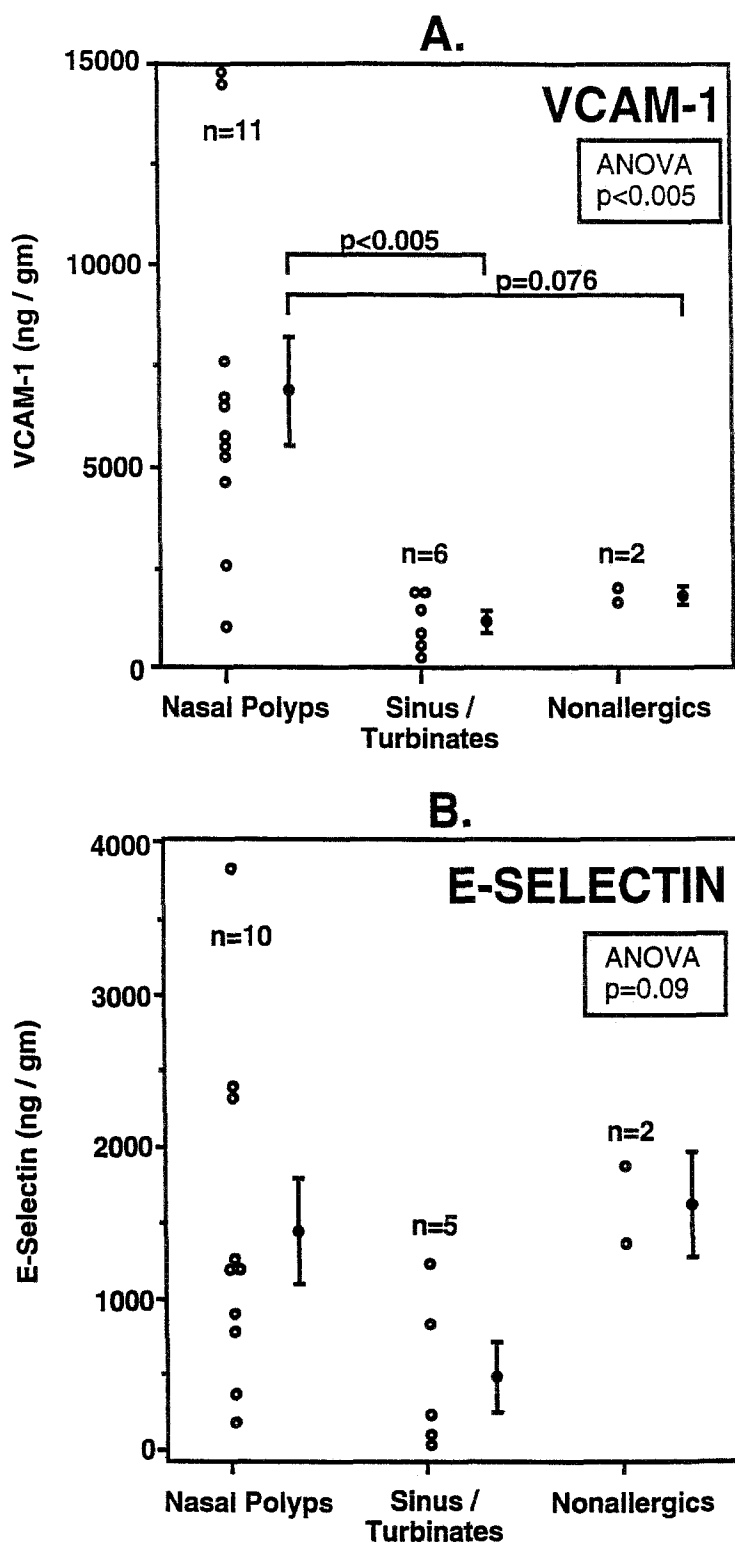


FIG. 5. ELISA for soluble VCAM-1 and E-selectin in tissue homogenates from NP, S/T, and NA nasal biopsy specimens. **A.** Quantity of VCAM-1 detected in tissue homogenates from NPs was significantly greater than that seen in S/T samples. **B.** There was a trend toward more E-selectin in NPs, but this did not reach statistical significance. ELISA results are reported as nanograms of protein per gram of freeze-dried tissue. Dilutions of samples were parallel to the standard curves in each ELISA, suggesting that immunoreactive material detected was indeed VCAM-1 or E-selectin.

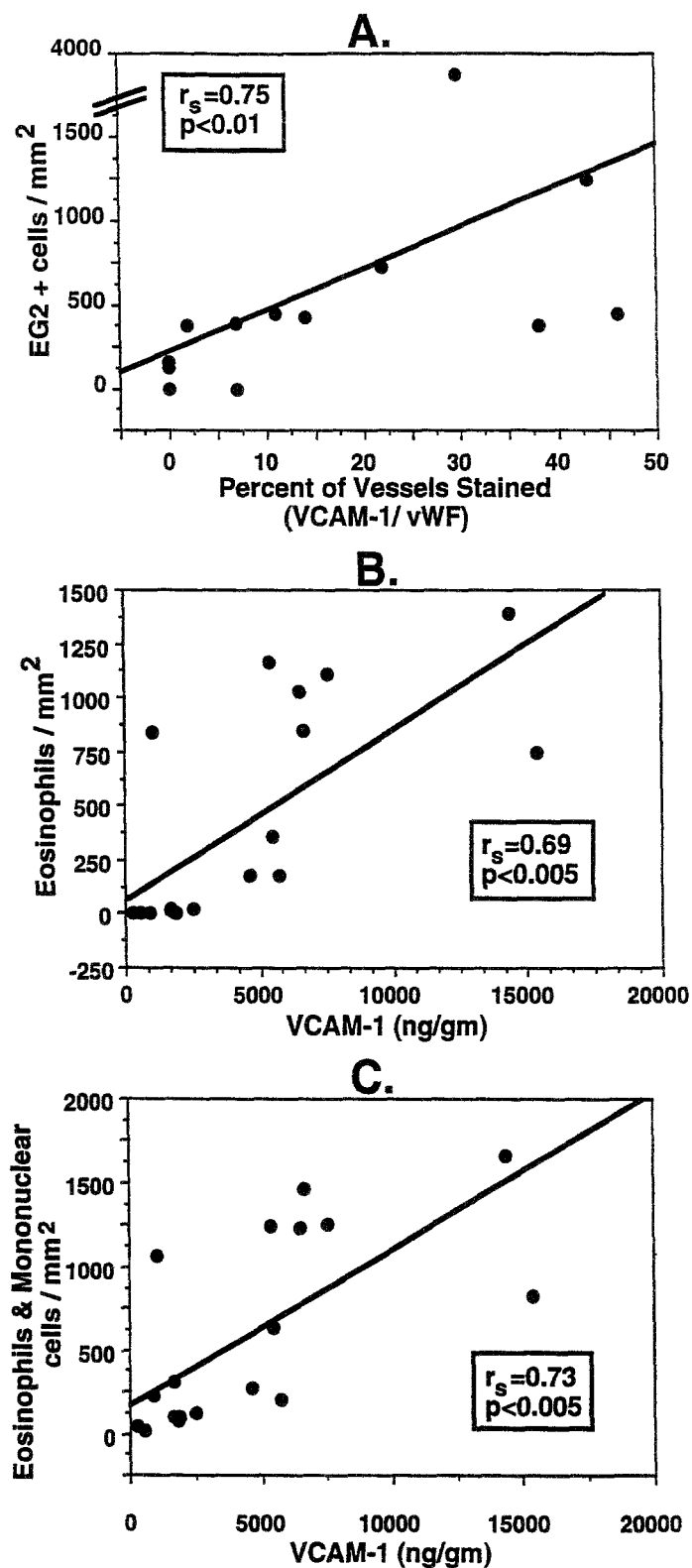


FIG. 6. Correlations between cellular infiltrates seen in nasal tissues and VCAM-1 expression as measured by immunohistochemistry and ELISA. A, $n = 14$. B, C, $n = 20$.

for by serum content because serum levels range from 0.13 to 2.8 ng/ml.³¹

Relationship between leukocyte infiltration and RANTES, VCAM-1, and E-selectin expression

There was a weak correlation between eosinophils and epithelial RANTES expression that did not reach statistical significance ($r_s = 0.32$, $p = 0.13$). No correlation was seen between epithelial and/or glandular expression of RANTES and the EG2-positive eosinophil, neutrophil, or mononuclear cell counts. The proportion of VCAM-1-positive vessels correlated with the extent of EG2-positive eosinophil influx in NP tissues ($r_s = 0.75$, $p < 0.01$) (Fig. 6, A). The more quantitative VCAM-1 ELISA values revealed correlations between VCAM-1 and the number of eosinophils ($r_s = 0.69$, $p < 0.005$) (Fig. 6, B) and the combined number of eosinophils and mononuclear cells ($r_s = 0.73$, $p < 0.005$) (Fig. 6, C), but there was no correlation with neutrophil influx when all nasal tissues studied were examined. No significant correlation was seen between the proportion of E-selectin-positive vessels or the E-selectin ELISA values and any cellular phenotype.

DISCUSSION

Eosinophils are widely recognized as proinflammatory cells by virtue of the mediators that they release. Basic proteins found in their cytoplasmic granules, including major basic protein and eosinophil cationic protein, have been shown to damage both the upper and lower respiratory epithelium.^{32, 33} Other eosinophil mediators, including leukotriene C₄ and platelet activating factor, can enhance vascular permeability, constrict smooth muscle, stimulate mucus release, and mobilize leukocytes. Eosinophils are found in significantly higher numbers and are more frequently activated (as determined by EG2 staining) in NP tissue as compared with surrounding turbinates or normal nasal mucosa.³⁴ To better understand the mechanisms responsible for recruitment and activation of eosinophils, we studied the expression of the chemokine RANTES and two endothelial adhesion molecules, VCAM-1 and E-selectin, in NP tissues. We have shown that RANTES is present on the nasal epithelium of polyps and that NPs stain more intensely for RANTES than either S/T or NA samples. Interestingly, the proportion of VCAM-1-staining vessels was similar among all clinical samples, but the VCAM-1 detected from tissue

homogenates by ELISA was significantly higher in NPs than in S/T control tissues. Moreover, these values correlated with the eosinophil (and eosinophil plus mononuclear) cell counts. There was no difference in E-selectin staining or ELISA values from tissue homogenates among all clinical samples.

Consistent with the work of others, we found that the predominant cell type infiltrating NP tissues was the eosinophil and that the majority of these cells were activated as defined by EG2⁺ staining.³⁴⁻³⁶ A previous study found an EG2-eosinophil ratio of 0.79 in NP tissue, which decreased to 0.46 after 1 month of budesonide treatment.³⁶ These values are comparable to our value of 0.61. Mononuclear cells (lymphocytes and monocytes) were also present in greater numbers in NP tissues as compared with the mucosa of nonallergic individuals. Therefore NP tissues are characterized by a selective recruitment of eosinophils and mononuclear cells, but not neutrophils.

Our immunohistochemical results suggest that RANTES, a potent chemotactic factor for eosinophils, is localized to surface and glandular epithelium. It is noteworthy that the greatest accumulations of inflammatory cells are seen adjacent to the epithelium. We cannot exclude the possibility that the immunoreactive RANTES detected was produced by other cell types and then immobilized on nasal epithelium. Because chemokines are known to bind to proteoglycans, such as heparin sulfate and other extracellular matrix components, it is conceivable that sites of RANTES staining may represent immobilization and not production.³⁷⁻³⁹ However, we think this is unlikely on the basis of recent work in our laboratory on two bronchial epithelial cell lines; BEAS-2B and IB3, as well as nasal epithelial explant cells, clearly demonstrate that RANTES messenger RNA and protein are produced at high levels after activation by inflammatory cytokines such as tumor necrosis factor- α and interferon- γ .⁵⁰ This information, coupled with the intensity of the observed staining, the specific localization of RANTES immunoreactivity within the cells to the perinuclear space, and the lack of staining of adjacent goblet cells, leads us to believe that RANTES is produced by nasal epithelium and not merely adsorbed from other sources. A variety of other cell types are also known to produce RANTES including cultured T cells and macrophages, platelets, synovial fibroblasts, an acute myelomonocytic cell line (THP-1), a rhabdomyosarcoma cell line (RD-1), a mast cell leukemic line (HMC-1), a bronchial epithelial cell line (BEAS-

2B), and primary cultures of umbilical vein endothelial cells.^{18, 40-44, 50}

It is ironic that the epithelium itself may secrete a chemotactic agent (RANTES) responsible for recruiting the eosinophils, which may result in the loss of nasal epithelium that is seen in up to 93% of NP cases.⁴⁵ Growing evidence suggests that both upper and lower respiratory tract epithelial cells may contribute to inflammatory responses. In one study bronchial epithelial cells from asthmatic airways were shown to express another C-C chemokine, monocyte chemoattractant protein-1. Interestingly, the epithelial staining correlated significantly with the percent reduction in FEV₁.⁴⁶ Nasal epithelial cells may secrete a variety of cytokines including GM-CSF, IL-1 α , IL-6, and IL-8.⁴⁷⁻⁴⁹ It is interesting to speculate that antigen exposure may result in the release of a variety of chemokines and cytokines by epithelial cells, which then serve to recruit and enhance the survival of proinflammatory cells such as eosinophils.

Work currently in progress in our laboratory has demonstrated that the glucocorticoid budesonide is a potent inhibitor of RANTES gene expression and protein production in a bronchial epithelial cell line stimulated with tumor necrosis factor- α .^{40, 50} The ability of corticosteroids to inhibit RANTES secretion from nasal epithelium may explain, at least in part, the reduction in both total and activated eosinophils seen after treatment with intranasal steroids.³⁴ This finding may also provide an additional explanation for the reduction of eosinophils in nasal washings or bronchoalveolar lavage seen after corticosteroid treatment in antigen-challenged patients with allergy. It is noteworthy that we detected significant staining for RANTES in subjects with NPs, despite the fact that 15 of 17 received some preoperative glucocorticoids. Possibly, the use of inhaled and/or systemic steroids in these subjects may have diminished the RANTES immunoreactivity, and the absence of steroid exposure in the other subjects (S/T or NA samples) may have lessened the difference among these three groups. It is also possible that RANTES is more important in localizing eosinophils to the vicinity of the epithelium rather than recruiting these cells from the circulation. The overriding *in vitro* evidence demonstrating RANTES induction of eosinophil chemotaxis and eosinophil transendothelial migration coupled with the present finding that the intensity of RANTES staining and levels of RANTES protein were greater in polyps than control nasal tissue and that the site of RANTES expression coincided with the

areas of dense eosinophilia all support the hypothesis that this factor is an important inducer of eosinophil accumulation *in vivo*.⁵¹

We next questioned whether endothelial adhesion molecules may contribute to the enhanced recruitment of eosinophils in NPs. Using immunohistochemical techniques, we discovered similar degrees of VCAM-1 and E-selectin expression in NP, S/T, and NA samples. Interestingly, VCAM-1 staining correlated with the density of activated eosinophils (e.g., EG2⁺) in NP tissues but not in other groups. Our results differ from those of Symon et al.,⁵² who showed that 29% of vessels stained for E-selectin and less than 5% stained for VCAM-1 in polyp tissues. In additional studies, these authors demonstrated P-selectin staining of endothelium, and using the Stamper-Woodruff assay, concluded that P-selectin was primarily responsible for eosinophil-endothelial adhesion in NP tissues. Because both E-selectin and P-selectin support binding of both neutrophils and eosinophils to endothelium, these adhesion molecules are unlikely to explain preferential recruitment of eosinophils to an inflammatory site. It is unclear why such variability in endothelial adhesion molecule expression was noted by our two laboratories. The possibilities include heterogeneity of disease, treatment differences, staining technique, and antibody selection.

To further quantify VCAM-1 and E-selectin expression, we performed ELISAs on the homogenized, detergent-solubilized tissue supernatants. The utility of measuring soluble endothelial adhesion molecules in inflammatory diseases has been documented previously. Levels of soluble E-selectin, for example, are elevated in serum and bronchoalveolar lavage after an acute asthma attack and after segmental antigen challenge, respectively.^{30, 53} The fact that we detected more VCAM-1 in tissue homogenates than by immunohistochemistry would suggest that we are not only solubilizing VCAM-1 bound to the endothelial surface but also VCAM-1 found on other cells including macrophages, fibroblasts, and nerves.^{54, 55} NP tissues had significantly greater amounts of VCAM-1 than S/T tissues, and nasal tissue VCAM-1 levels correlated well with both the eosinophil and the eosinophil plus mononuclear cell counts. The correlation of eosinophil influx and VCAM-1 expression has been noted by other researchers.⁵⁵⁻⁵⁸ Bentley et al.⁵⁷ demonstrated that the intensity and extent of endothelial VCAM-1 staining correlated in bronchial biopsy specimens with the number of tissue eosinophils as measured by major basic protein

staining in patients with mildly atopic asthma after antigen challenge. A similar finding was noted in acute skin lesions of subjects with atopic dermatitis.⁵⁹ Furthermore, indirect evidence that VCAM-1 is important in eosinophil and mononuclear cell influx was obtained in sheep, mouse, and guinea pig models of inflammation by using antibodies to the VCAM-1 counterligand very late activation antigen-4.⁶⁰⁻⁶⁴ Finally, antibodies to VCAM-1 inhibited the formation of eosinophil and T-lymphocyte infiltrates in the trachea of allergen-challenged mice.⁶¹

This study demonstrates that two molecules, which have been proposed to promote eosinophil recruitment on the basis of *in vitro* studies, namely the chemokine RANTES and the adhesion molecule VCAM-1, are expressed *in vivo* in human NP tissues. The close correlation of the expression of VCAM-1 (in nanograms per gram) with tissue eosinophil numbers suggests that endothelial VCAM-1 expression is likely to be partly responsible for the cellular influx. The intense staining of epithelial cells for RANTES in proximity to the densest cellular infiltrates suggests that this protein may be playing a role in localizing infiltrating cells, including eosinophils.

We thank Dr. Steve Georas for his editorial assistance, Dr. Walter Newman for helpful advice, Dr. Robert Naclerio for providing tissue samples and helpful discussions, and Ms. Gina Gorgone for technical assistance. We also thank Ms. Bonnie Hebden for assistance in the preparation of the manuscript.

REFERENCES

- Gleich GJ. The eosinophil and bronchial asthma: current understanding. *J Allergy Clin Immunol* 1990;85:422-36.
- Bousquet J, Chané P, Lacoste JY, Barneon G, Ghavanian N, Enander I, et al. Eosinophilic inflammation in asthma. *N Engl J Med* 1990;323:1033-9.
- Kay AB. Eosinophils and asthma. *N Engl J Med* 1991;324:1514-5.
- Weller PF. The immunobiology of eosinophils. *N Engl J Med* 1991;324:1110-8.
- Elices MJ, Osborn L, Takada Y, Crouse C, Lohowskyj S, Hemler ME, et al. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell* 1990;60:577-84.
- Bochner BS, Luscinskas FW, Gimbrone MA Jr, Newman W, Sterbinsky SA, Derse-Anthony C, et al. Adhesion of human basophils, eosinophils, and neutrophils to IL-1-activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. *J Exp Med* 1991;173:1553-7.
- Dobrina A, Menegazzi R, Carlos TM, Nardon E, Cramer R, Zacchi T, et al. Mechanisms of eosinophil adherence to cultured vascular endothelial cells: eosinophils bind to the cytokine-induced endothelial ligand vascular cell adhesion molecule-1 via the very late activation antigen-4 integrin receptor. *J Clin Invest* 1991;88:22-6.
- Weller PF, Rand TH, Goelz SE, Chi-Rosso G, Lobb RR. Human eosinophil adherence to vascular endothelium mediated by binding to vascular cell adhesion molecule 1 and endothelial leukocyte adhesion molecule 1. *Proc Natl Acad Sci USA* 1991;88:7430-3.
- Schleimer RP, Sterbinsky SA, Kaiser J, Bickel CA, Klunk DA, Tomioka K, et al. Interleukin-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium: association with expression of VCAM-1. *J Immunol* 1992;148:1086-92.
- Thornhill MH, Kyan-Aung U, Haskard DO. IL-4 increases human endothelial cell adhesiveness for T cells but not for neutrophils. *J Immunol* 1990;144:3060-5.
- Bochner BS, Klunk DA, Sterbinsky SA, Coffman RL, Schleimer RP. Interleukin-13 selectively induces vascular cell adhesion molecule-1 (VCAM-1) expression in human endothelial cells. *J Immunol* 1995;154:799-803.
- Durham SR, Ying S, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte-macrophage colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. *J Immunol* 1992;148:2390-4.
- Bradding P, Feather IH, Wilson S, Bardin PG, Heusser CH, Holgate ST, et al. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. *J Immunol* 1993;151:3853-65.
- Finotto S, Ohno I, Marshall JS, Gaudie J, Denburg A, Dolovich J, et al. TNF- α production by eosinophils in upper airways inflammation (nasal polyposis). *J Immunol* 1994;153:2278-89.
- Walsh GM, Mermoud J, Hartnell A, Kay AB, Wardlaw AJ. Human eosinophil, but not neutrophil, adherence to IL-1-stimulated human umbilical vascular endothelial cells is $\alpha 4\beta 1$ (very late antigen-4) dependent. *J Immunol* 1991;146:3419-23.
- Bochner BS, Sterbinsky SA, Bickel CA, Werfel S, Wein M, Newman W. Differences between human eosinophils and neutrophils in the function and expression of sialic acid-containing counterligands for E-selectin. *J Immunol* 1994;152:774-82.
- Gundel RH, Wegner CD, Torcellini CA, Clarke CC, Haynes N, Rothlein R, et al. Endothelial leukocyte adhesion molecule-1 mediates antigen-induced acute airway inflammation and late-phase airway obstruction in monkeys. *J Clin Invest* 1991;88:1407-11.
- Schall TJ. Biology of the RANTES/SIS cytokine family. *Cytokine* 1991;3:165-83.
- Dahinden CA, Geiser T, Brunner T, von Tscharner V, Caput D, Ferrara P, et al. Monocyte chemoattractant protein 3 is a most effective basophil- and eosinophil-activating chemokine. *J Exp Med* 1994;179:751-6.
- Warringa RA, Koenderman JL, Kok PTM, Kreukniet J, Bruijnzeel PLB. Modulation and induction of eosinophil chemotaxis by granulocyte-macrophage colony-stimulating factor and interleukin-3. *Blood* 1991;77:2694-700.
- Rot A, Krieger M, Brunner T, Bischoff SC, Schall TJ, Dahinden CA. RANTES and macrophage inflammatory protein 1 α induce the migration and activation of normal human eosinophil granulocytes. *J Exp Med* 1992;176:1489-95.
- Rand TH, Cruikshank WW, Center DM, Weller PF. CD4-mediated stimulation of human eosinophils: lym-

- phocyte chemoattractant factor and other CD4-binding ligands elicit eosinophil migration. *J Exp Med* 1991;173:1521-8.
23. Jose PJ, Griffiths-Johnson DA, Collins PD, Walsh DT, Moqbel R, Totty NF, et al. Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J Exp Med* 1994;179:881-7.
24. Taub DD, Conlon K, Lloyd AR, Oppenheim JJ, Kelvin DJ. Preferential migration of activated CD4+ and CD8+ T cells in response to MIP-1 α and MIP-1 β . *Science* 1993;260:355-8.
25. Resnick MB, Weller PF. Mechanisms of eosinophil recruitment. *Am J Respir Cell Mol Biol* 1993;8:349-55.
26. Meurer R, Van Riper G, Feeney W, Cunningham P, Hora D Jr, Springer MS, et al. Formation of eosinophilic and monocytic intradermal inflammatory sites in the dog by injection of human RANTES but not human monocyte chemoattractant protein 1, human macrophage inflammatory protein 1 α , or human interleukin 8. *J Exp Med* 1993;178:1913-21.
27. Beck L, Bickel C, Sterbinsky S, Stellato C, Hamilton R, Rosen H, et al. Injection of human subjects with RANTES causes dermal infiltration of eosinophils (EOS) and mononuclear cells (MNC). *FASEB J* 1995;9:A804.
28. Devergne O, Marfaing-Koka A, Schall TJ, Leger-Ravet M-B, Sadick B, Peuchmaur M, et al. Production of the RANTES chemokine in delayed-type hypersensitivity reactions: involvement of macrophage and endothelial cells. *J Exp Med* 1994;179:1689-94.
29. Tai PC, Spry CJ, Peterson C, Venge P, Olsson I. Monoclonal antibodies distinguish between storage and secreted forms of eosinophil cationic protein. *Nature* 1984;309:182-4.
30. Georas SN, Liu MC, Newman W, Beall D, Stealey BA, Bochner BS. Altered adhesion molecule expression and endothelial activation accompany the recruitment of human granulocytes to the lung following segmental antigen challenge. *Am J Respir Cell Mol Biol* 1992;7:261-9.
31. Newman W, Beall LD, Carson CW, Hunder GG, Graben N, Randhawa ZI, et al. Soluble E-selectin is found in supernatants of activated endothelial cells and is elevated in the serum of patients with septic shock. *J Immunol* 1993;150:644-54.
32. Gleich GJ, Flavahan NA, Fujisawa T, Vanhoutte PM. The eosinophil as a mediator of damage to respiratory epithelium: a model for bronchial hyperreactivity. *J Allergy Clin Immunol* 1988;81:776-81.
33. Gundel RH, Letts LG, Gleich GJ. Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates. *J Clin Invest* 1991;87:1470-3.
34. Stoop AE, van der Heijden H, Biewenga J, van der Baan S. Eosinophils in nasal polyps and nasal mucosa: an immunohistochemical study. *J Allergy Clin Immunol* 1993;91:616-20.
35. Kakoi H, Hiraide F. A histological study of formation and growth of nasal polyps. *Acta Otolaryngol* 1987;103:137-44.
36. Kanai N, Denburg J, Jordana M, Dolovich J. Nasal polyp inflammation. Effect of topical nasal steroid. *Am J Respir Crit Care Med* 1994;150:1094-100.
37. Tanaka Y, Adams DH, Hubscher S, Hirano H, Siebenlist U, Shaw S. T-cell adhesion induced by proteoglycan-immobilized cytokine MIP-1 β . *Nature* 1993;361:79-82.
38. Hadley TJ, Lu Z, Wasniowska A, Martin W, Peiper SC, Hesselgesser J, et al. Postcapillary venule endothelial cells in kidney express a multispecific chemokine receptor that is structurally and functional identical to the erythroid isoform, which is the Duffy blood group antigen. *J Clin Invest* 1994;94:985-91.
39. Gilat D, Hershkovich Y, Mekori A, Vlodavsky I, Lider O. Regulation of adhesion of CD4+ T lymphocytes to intact or heparinase-treated subendothelial extracellular matrix by diffusible or anchored RANTES and MIP-1 β . *J Immunol* 1994;153:4899-906.
40. Stellato C, Beck LA, Klunk DA, Schall TJ, Ono SJ, Schleimer RP. Differential regulation by glucocorticoids (GC) of RANTES production in human epithelial and endothelial cells. [abstract]. *J Allergy Clin Immunol* 1995;95:299.
41. Kameyoshi Y, Dorschner A, Mallet AI, Christophers E, Schroder JM. Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. *J Exp Med* 1992;176:587-92.
42. Rathanaswami P, Hachicha M, Sadick M, Schall TJ, McCoil SR. Expression of the cytokine RANTES in human rheumatoid synovial fibroblasts. *J Biol Chem* 1993;268:5834-9.
43. Selvan RS, Butterfield JH, Krangel MS. Expression of multiple chemokine genes by a human mast cell leukemia. *J Biol Chem* 1994;269:13893-8.
44. Stellato C, Beck LA, Klunk DA, Schall TJ, Ono SJ, Schleimer RP. Differential regulation by glucocorticoids (GC) of RANTES production in human epithelial and endothelial cells [abstract]. *J Allergy Clin Immunol* 1995;95:299.
45. Wladislawosky-Waserman P, Kern EB, Holley KE, Eisenbrey AB, Gleich GJ. Epithelial damage in nasal polyps. *Clin Allergy* 1984;14:241-7.
46. Sousa AR, Lane SJ, Nakhosteen A, Yoshimura T, Lee TH, Poston RN. Increased expression of the monocyte chemoattractant protein-1 in bronchial tissue from asthmatic subjects. *Am J Respir Cell Mol Biol* 1994;10:142-7.
47. Denburg JA, Gauldie J, Dolovich J, Ohtoshi T, Cox G, Jordana M. Structural cell-derived cytokines in allergic inflammation. *Int Arch Allergy Appl Immunol* 1991;94:127-32.
48. Kenney JS, Baker C, Welch MR, Altman LC. Synthesis of interleukin-1 α , interleukin-6, and interleukin-8 by cultured human nasal epithelial cells. *J Allergy Clin Immunol* 1994;93:1060-7.
49. Cox G, Ohtoshi T, Vanceri C, Denburg A, Dolovich J, Gauldie J, et al. Promotion of eosinophil survival by human bronchial epithelial cells and its modulation by steroids. *Am J Respir Cell Mol Biol* 1991;4:525-31.
50. Stellato C, Beck LA, Gorgone G, Proud D, Schall TJ, Ono SJ, et al. Expression of the chemokine RANTES by a human bronchial epithelial cell line: modulation by cytokines and glucocorticoids. *J Immunol* 1995;155:308-15.
51. Ebisawa M, Yamada T, Bickel C, Klunk D, Schleimer RP. Eosinophil transendothelial migration induced by cytokines. III. Effect of the chemokine RANTES. *J Immunol* 1994;153:2153-60.
52. Symon FA, Walsh GM, Watson SR, Wardlaw AJ. Eosinophil adhesion to nasal polyp endothelium is P-selectin-dependent. *J Exp Med* 1994;180:371-6.
53. Kobayashi T, Hashimoto S, Amemiya IKE, Yamaguchi M, Yachi A, Horie T. Elevation of serum soluble intercellular adhesion molecule-1 (sICAM-1) and sE-selectin levels in bronchial asthma. *Clin Exp Immunol* 1994;96:110-5.
54. Rice GE, Munro JM, Corless C, Bevilacqua MP. Vascular and nonvascular expression of ICAM-110. *Am J Pathol* 1991;138:385-93.

55. Kyan-Aung U, Haskard DO, Poston RN, Thornhill MH, Lee TH. Endothelial leukocyte adhesion molecule-1 and intercellular adhesion molecule-1 mediate the adhesion of eosinophils to endothelial cells in vitro and are expressed by endothelium in allergic cutaneous inflammation in vivo. *J Immunol* 1991;146:521-8.
56. Schleimer RP, Bochner BS. Letter to the editor. *J Immunol* 1991;147:380-1.
57. Bentley AM, Durham SR, Robinson S, Menz G, Storz C, Cromwell O, et al. Expression of endothelial and leukocyte adhesion molecules intercellular adhesion molecule-1, E-selectin, and vascular cell adhesion molecule in the bronchial mucosa in steady-state and allergen-induced asthma. *J Allergy Clin Immunol* 1993;92:857-68.
58. Lee B-J, Naclerio RM, Bochner BS, Taylor RM, Lim MC, Baroody FM. Nasal challenge with allergen upregulates the local expression of vascular endothelial adhesion molecules. *J Allergy Clin Immunol* 1994;94:1006-16.
59. Wakita H, Sakamoto T, Tokura Y, Takigawa M. E-selectin and vascular cell adhesion molecule-1 as critical adhesion molecules for infiltration of T lymphocytes and eosinophils in atopic dermatitis. *J Cutan Pathol* 1994;21:33-9.
60. Weg VB, Williams TJ, Lobb RR, Nourshargh S. A monoclonal antibody recognizing very late activation antigen-4 inhibits eosinophil accumulation in vivo. *J Exp Med* 1993;177:561-6.
61. Nakajima H, Sano T, Nishimura T, Yoshida S, Iwamoto I. Role of vascular cell adhesion molecule-1/very late activation antigen-4 and intercellular adhesion molecule-1/lymphocyte function-associated antigen-1 interactions in antigen-induced eosinophil and T-cell recruitment into the tissue. *J Exp Med* 1994;179:1145-54.
62. Baron JL, Reich EP, Visintin I, Janeway JCA. The pathogenesis of adoptive murine autoimmune diabetes requires an interaction between $\alpha 4$ -integrins and vascular cell adhesion molecule-1. *J Clin Invest* 1994;93:1700-8.
63. Terada N, Konno A, Togawa K. Biochemical properties of eosinophils and their preferential accumulation mechanism in nasal allergy. *J Allergy Clin Immunol* 1994;94:629-42.
64. Abraham WM, Sielczak MW, Ahmed A, Cortes A, Lauredo ET, Kim J, et al. $\alpha 4$ -Integrins mediate antigen-induced late bronchial responses and prolonged airway hyperresponsiveness in sheep. *J Clin Invest* 1994;93:776-87.