

Differential expression of vascular cell adhesion molecule mRNA and protein in nasal mucosa in response to IL-1 or tumor necrosis factor

Mark S. Weinberger, MD,^a Terence M. Davidson, MD,^a and
David H. Broide, MB, ChB^b San Diego, Calif.

IL-1 and tumor necrosis factor (TNF) are cytokines that share many overlapping functions, including induction of expression of the vascular cell adhesion molecule (VCAM) by endothelial cells. However, because most studies of cytokine induction of adhesion molecules have used human umbilical vein endothelial cells (HUVECs) and not microvascular endothelial cells, the functional significance of such observations to sites of allergic inflammation, such as the nasal mucosa, are at present unknown. We have therefore used nasal mucosa to compare the functional response of these microvascular endothelial cells with HUVECs. HUVECs or nasal mucosal explants were stimulated in vitro with varying concentrations of IL-1 or TNF for 0 to 48 hours, and VCAM mRNA and protein expression were determined by means of immunostaining and in situ hybridization. TNF and IL-1 were equivalent in their ability to induce VCAM mRNA and protein expression by HUVECs. In contrast, TNF was significantly more potent than IL-1 in inducing VCAM mRNA and protein expression by nasal mucosal microvascular endothelial cells. The recovery of significant amounts of IL-1 after incubation of recombinant IL-1 with nasal mucosa, as well as the ability of IL-1 to induce intercellular adhesion molecule expression by nasal mucosa, suggests that neither degradation of IL-1 nor downregulation of IL-1 receptors in nasal mucosa is likely to explain the inability of IL-1 to induce VCAM expression by nasal mucosa. These studies suggest that microvascular endothelial cells in the nasal mucosa differ functionally from HUVECs and that TNF may be more important than IL-1 in induction of VCAM expression in the nasal mucosa. (J ALLERGY CLIN IMMUNOL 1996;97:662-71.)

Key words: Nasal mucosa, IL-1, tumor necrosis factor, vascular cell adhesion molecule, intercellular adhesion molecule

The accumulation of eosinophils in tissues has been noted in clinical, postmortem and experimental models of allergic inflammation.¹ The activated eosinophil plays a pivotal proinflammatory role by generating lipid mediators (platelet activating fac-

Abbreviations used

ICAM:	Intercellular adhesion molecule
IL-1ra:	Interleukin 1 receptor antagonist
HUVEC:	Human umbilical vein endothelial cells
TNF:	Tumor necrosis factor
VCAM:	Vascular cell adhesion molecule
VLA:	Very late antigen

From the ^aDivision of Head and Neck Surgery and the ^bDepartment of Medicine, University of California, San Diego. Supported by grants AI29974, AI33977, AI31595 (Asthma and Allergic Disease Center), and T32 DC 00032 (Chemosensory Research Training fellowship) from the National Institutes of Health. Dr. Weinberger is the recipient of the American Academy of Otolaryngology-Head and Neck Surgery Research Training Award.

Received for publication July 12, 1994; revised Apr. 14, 1995; accepted for publication Apr. 18, 1995.

Reprint requests: David Broide, MB, ChB, Division of Allergy and Immunology, University of California, San Diego, 9500 Gilman Drive, San Diego, CA 92093-0635.

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0091-6749/96 \$5.00 + 0 1/1/65631

tor and leukotriene C₄), as well as cytokines and toxic granule proteins.¹⁻⁴ The preferential recruitment of eosinophils, as opposed to neutrophils, to sites of allergic inflammation suggests that a particular profile of cytokines or mediators that preferentially recruit eosinophils are released at sites of allergen challenge.⁵ These cytokines and mediators may preferentially recruit eosinophils by in-

ducing eosinophil chemotaxis (RANTES)⁶ as opposed to neutrophil chemotaxis (IL-8)⁷ and/or induce expression of adhesion molecules on endothelial cells (vascular cell adhesion molecule [VCAM]), which preferentially bind circulating eosinophils (very late antigen [VLA]-4⁺) as opposed to neutrophils (VLA-4⁻).⁸⁻¹¹ Eosinophil, like neutrophil, adhesion to endothelium *in vivo* is a multistep process.¹²⁻¹⁵ Initial eosinophil intravascular rolling and firm adhesion to endothelium is followed by sequential eosinophil diapedesis between endothelial cells and chemotaxis into tissues. The initial step of eosinophil adhesion to endothelium is the rolling of eosinophils on inflamed endothelium. *In vivo* studies in which videomicroscopy is used have demonstrated that eosinophils use both selectins (L-selectin) and integrins (VLA-4) as rolling receptors.¹⁵ The recognition of selectins as leukocyte rolling receptors has been extensively documented.¹²⁻¹⁵ The recent demonstration that integrins such as VLA-4 can function as rolling receptors has now been extended from initial studies in eosinophils¹⁵ to studies of lymphocytes.¹⁶ *In vitro* studies have shown that endothelial expressed adhesion molecules such as P-selectin, E-selectin, VCAM, and intercellular cell adhesion molecule (ICAM)¹⁷⁻¹⁹ can all bind to counterreceptors expressed by eosinophils.

The role of cytokines in the adhesion of eosinophils to endothelium is suggested from *in vitro* and *in vivo* studies demonstrating that (1) *in vitro* cytokines induce expression of adhesion molecules that bind eosinophils to endothelium^{8, 18, 19}; (2) at sites of allergic eosinophilic inflammation *in vivo* cytokine mRNA and proteins capable of inducing adhesion molecule expression can be detected;^{2, 20-23} and (3) cytokine transgenic mice (IL-4 transgenic mice) develop significant tissue eosinophilia,²⁴ presumably through IL-4 induction of VCAM expression by endothelial cells. However, because several cytokines can induce adhesion molecule expression by endothelium (IL-1, IL-4, TNF, interferon- γ),²⁵ the importance of the detection of an individual cytokine *in vivo* at sites of allergic inflammation is not readily apparent.

IL-1 and TNF are cytokines that share many overlapping functions, including induction of adhesion molecule expression by endothelial cells.^{26, 27} VCAM is one endothelial cell adhesion molecule that is regulated by both IL-1 and TNF.^{28, 29} Because VCAM binds to VLA-4-positive cells (eosinophils, lymphocytes, and basophils),^{10, 29} but not to VLA-4-negative neutrophils,¹¹ its endothelial expression may

be important in recruiting a particular profile of inflammatory cells to sites of allergic inflammation.

Both IL-1 and TNF are released at sites of allergic inflammation.^{23, 30} In addition, low levels of VCAM protein assessed by immunostaining have been noted in nasal mucosa from patients with allergic rhinitis.³¹ To determine whether IL-1 and TNF share a similar capacity to induce VCAM expression in microvascular endothelial cells derived from nasal mucosa, explants were stimulated for 0 to 48 hours with either IL-1 or TNF, and VCAM mRNA and protein expression were assessed by *in situ* hybridization and immunocytochemistry.

METHODS

Human umbilical vein endothelial cells

Human umbilical vein endothelial cells (HUVECs) (Clonetics, San Diego, Calif.) were grown to confluence before use. No cell lines were used after more than three serial passages.

Nasal mucosal explants

Nasal mucosa were obtained from the inferior turbinate of patients ($n = 7$) undergoing endoscopic sinus surgery in the Division of Head and Neck Surgery at the University of California at San Diego. Patients with perennial rhinitis who had no evidence of sinus infection and had discontinued intranasal corticosteroids at least 2 weeks before surgery were studied in a protocol approved by the university's Human Subjects Committee. The diagnosis of perennial rhinitis was based on medical history, results of physical examination, sinus computed tomographic scan, and an IgE RAST or immediate hypersensitivity skin test reaction to environmental inhalant allergens. Nasal mucosa obtained at surgery were extensively washed and cut into 5 mm³ sections. Fragments of nasal mucosa were incubated at 37°C in humidified 5% CO₂/95% air in Medium 199 supplemented with 20% fetal bovine serum, penicillin (100 μ /ml), streptomycin (100 μ g/ml), heparin (100 μ g/ml), endothelial cell growth supplement (20 μ g/ml), and 10 mmol/L L-glutamine (Gibco, Grand Island, N.Y.) for 0 to 48 hours in the presence or absence of either recombinant IL-1 β (0 to 10 ng/ml) (R & D Systems, Minneapolis, Minn.) or recombinant TNF (0 to 10 ng/ml) (Endogen, Boston, Mass.). Equal fragments of nasal mucosa (5 mm²) from the same patient were used in all experiments that compared TNF and IL-1 induction of VCAM. In selected experiments the bioactivity of recombinant TNF was neutralized for 1 hour with a polyclonal anti-human TNF antibody (Endogen) before addition of the TNF to nasal mucosa. Tissues were snap-frozen in Tissue-Tek OCT compound (Miles, Elkhart, Ind.) and stored at -70°C until cryosectioned and used for VCAM *in situ* hybridization and immunostaining.

Immunocytochemistry

VCAM immunocytochemistry was performed with the alkaline phosphatase method as previously described.² Nasal mucosa or HUVECs were immunostained with 5 μ g/ml of a mouse monoclonal IgG₁ anti-human VCAM antibody (R & D Systems) and a control species and isotype-specific IgG₁ antibody. Vascular endothelial cells were detected with 5 μ g/ml of a factor VIII mouse IgG₁ monoclonal antibody (Calbiochem, San Diego, Calif.) on adjacent tissue sections. In some experiments nasal mucosa and HUVECs were immunostained with 5 μ g/ml of a mouse monoclonal IgG₁ anti-human ICAM antibody (Genzyme, Boston, Mass.).

Quantitative analysis of HUVEC immunostaining

Slides of HUVECs immunostained with a mouse monoclonal anti-VCAM antibody were mounted on a light microscope, and the percentage of HUVECs expressing VCAM was determined by counting 50 individual cells in four random fields (a total of 200 cells/slide). HUVECs expressing a red color reaction were scored as positive, whereas HUVECs with no color reaction were scored as negative. The isotype control IgG antibody produced no visible color reaction.

Quantitative expression of nasal mucosa VCAM immunostaining with a computer image color analysis program

Quantitative color analysis of VCAM immunostaining (red color reaction) was performed with a Videometric 150 image analysis program (American Innovision, San Diego, Calif.).³² Slides, all immunostained under identical conditions, were mounted on a light microscope, and color images were digitized by videocamera. A negative (unstimulated HUVEC) and positive control slide (TNF-stimulated HUVEC) immunostained with the anti-VCAM or control monoclonal antibody were used to calibrate the program to detect shades of a red color reaction indicating VCAM expression.

The single-cell inner and outer layer of endothelium lining all visible blood vessels on each slide of nasal mucosa were circumferentially outlined on the slide image depicted on the computer monitor. The area of endothelium (between the inner and outer layer of endothelium) on each slide was determined by calibrating the computer program with a 1 μ m² area slide standard. In addition to calculating the total area of endothelium on each slide, the computer analysis program calculated the percent of the total area of endothelium outlined that was positive for a red color reaction indicative of VCAM expression. All immunostained slides were quantified under identical light microscope conditions, including magnification, gain, camera position, and background illumination. The isotype control antibody did not produce any color reaction. Results are expressed as the percent of a defined area of endothelial cells that expressed VCAM. The accuracy of the com-

puter program results was verified during each image analysis by direct light microscopic observation of the cytoplasmic color reaction developed by endothelial cells on each slide.

In situ hybridization

In situ hybridization with ³⁵S-labeled single-stranded VCAM sense or antisense RNA probes was performed as previously described.^{2,22} The VCAM probe³³ was kindly provided by Dr. Laurelee Osborn, Biogen, Cambridge, Mass. Slides were counterstained with bis benzimide (Sigma Chemical Co., St. Louis, Mo.), which fluorescently stains nuclei blue.

IL-1 receptor antagonist (IL-1ra) and IL-1 assay

Supernatants from unstimulated or IL-1-stimulated (10 ng/ml) HUVECs or nasal mucosa were assayed for IL-1ra and IL-1. IL-1ra was assayed with an immunoassay (R&D Systems). The assay is sensitive to an IL-1ra concentration of 22 pg/ml. There is no cross-reactivity or interference in the assay when up to 100 ng/ml of IL-1 α or IL-1 β are added to the assay.

IL-1 β was assayed with an IL-1 β sandwich ELISA (R&D Systems). The assay is sensitive to 0.3 pg/ml IL-1 β .

Statistical analysis

Statistical analysis was performed with Student's *t* test. Results are expressed as means $\pm$ SEM.

RESULTS

HUVEC expression of VCAM protein

Unstimulated HUVECs did not express VCAM protein as assessed by immunostaining. In contrast, HUVECs stimulated with either IL-1 (0 to 10 ng/ml) or TNF (0 to 10 ng/ml) resulted in equivalent amounts of VCAM expression (67% $\pm$ 12% VCAM-positive cells after stimulation with IL-1, 10 ng/ml for 6 hours; 64% $\pm$ 10% VCAM-positive cells after stimulation with TNF, 10 ng/ml for 6 hours) (*n* = 5). Dose-response studies (0 to 10 ng/ml) indicated that 1 to 10 ng/ml of either IL-1 or TNF induced maximum VCAM expression by HUVECs (data not shown).

Nasal mucosa expression of VCAM protein

Factor VIII immunostaining identified blood vessels in the nasal mucosa (Fig. 1). Unstimulated nasal mucosa expressed low levels of VCAM protein as assessed by immunocytochemistry in only two of seven patients. VCAM protein was induced in nasal mucosa by a 15- to 48-hour incubation with 10 ng/ml TNF (Fig. 1). Adjacent tissue sections immunostained with Factor VIII demonstrated

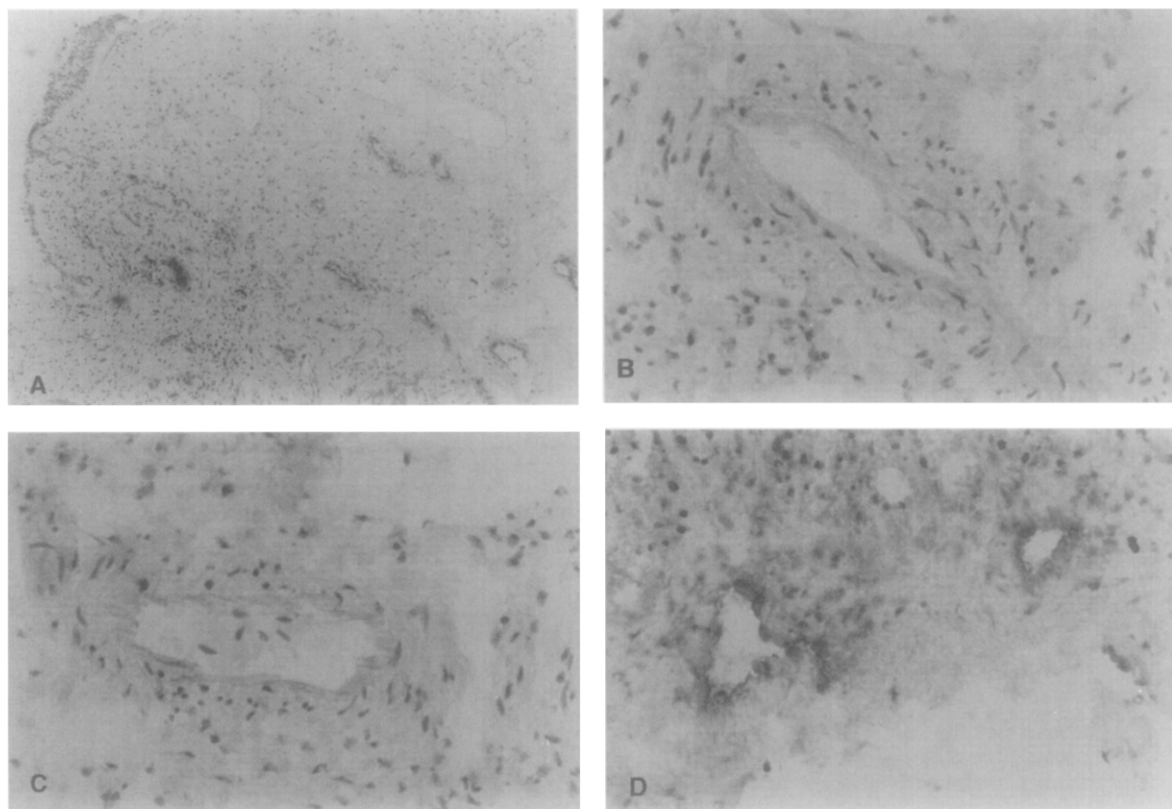


FIG. 1. VCAM protein expression by nasal mucosa. Human nasal mucosa were stimulated in vitro for 24 hours with IL-1, 10 ng/ml, or TNF, 10 ng/ml; and 5 μ m cryosections of nasal mucosa were immunostained with either a Factor VIII or VCAM antibody by the alkaline phosphatase method. **A**, Factor VIII-positive endothelial cells (magnification $\times 10$). **B-D**, High-power magnification views ($\times 40$) of nasal mucosa VCAM expression; **B**, before cytokine stimulation (time, 0 hour), **C**, after IL-1 stimulation (time, 24 hours), and **D**, after TNF stimulation (time, 24 hours). Only TNF induces VCAM expression (red color reaction). Immunostaining with control species and isotype-specific monoclonal antibodies produced no color reaction.

that the VCAM-positive cells in the nasal mucosa immunostained with Factor VIII.

In contrast to TNF, a similar concentration of IL-1 (10 ng/ml), which had induced expression of VCAM by HUVECs, had no effect on VCAM expression by nasal mucosa (Fig. 1). No blood vessels expressed VCAM 24 hours after stimulation with IL-1, 10 ng/ml, ($n = 7$ patients, 48 total blood vessels, three to 10 blood vessels counted per patient); $25.7\% \pm 4.5\%$ of blood vessels expressed VCAM 24 hours after stimulation with TNF, 10 ng/ml, ($n = 7$ patients, 54 total blood vessels, three to 10 blood vessels counted per patient) (Fig. 2). TNF dose-response studies (0 to 10 ng/ml) showed that 10 ng/ml was an optimal concentration for induction of VCAM by nasal mucosa. Studies with neutralizing antibodies to TNF demonstrated that the polyclonal anti-human TNF antibody inhibited TNF-induced VCAM expression by nasal mucosa by $73\% \pm 12\%$ ($n = 2$). The kinetics of VCAM

protein expression induced by TNF was maximum at 24 to 48 hours (Fig. 3). None of the concentrations of IL-1 tested (0 to 10 ng/ml) induced VCAM expression by nasal mucosa. Kinetic studies of VCAM expression with IL-1, 10 ng/ml, as a stimulus failed to induce significant VCAM expression at 15, 24 and 48 hours (Fig. 3). Studies of earlier IL-1 time points (4, 6, and 12 hours after IL-1 stimulation) also failed to provide evidence of VCAM expression by nasal mucosa (data not shown).

The kinetics of TNF-induced VCAM expression differed in HUVECs and nasal mucosa. Maximal VCAM expression induced by TNF was detected as early as 6 hours after stimulation of HUVECs compared with 24 hours after stimulation of nasal mucosa (Fig. 3). These kinetic differences in VCAM expression may relate to the slower penetration and binding of exogenously added TNF to blood vessels in explants of nasal mucosa, com-

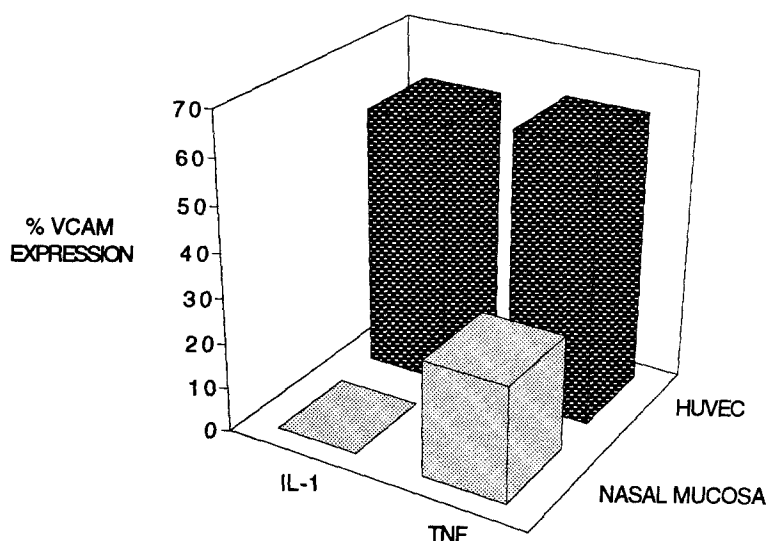


FIG. 2. Comparison of IL-1- and TNF-induced VCAM expression. Nasal mucosa or HUVECs were stimulated with equivalent concentrations of IL-1 or TNF (10 ng/ml), and VCAM expression was assessed by immunostaining. IL-1 (10 ng/ml) and TNF (10 ng/ml) induced equivalent degrees of VCAM expression by HUVECs (67% ± 12% VCAM-positive cells after IL-1 stimulation; 64% ± 10% VCAM-positive cells after TNF stimulation) ($n = 5$). In contrast, a 24-hour stimulation with TNF was significantly more potent than stimulation with IL-1 in inducing VCAM expression by nasal mucosa (0% of blood vessels express VCAM after IL-1 stimulation, $n = 7$ patients, 48 blood vessels; 25.7% ± 4.5% of blood vessels express VCAM after TNF stimulation, $n = 7$ patients, 54 blood vessels).

pared with HUVEC monolayers, or alternatively functional differences between HUVECs and nasal mucosa.

TNF (10 ng/ml) and IL-1 (10 ng/ml) both induced equivalent levels of ICAM expression by HUVECs (98% ± 1% of HUVECs are ICAM-positive after IL-1 stimulation; 99% ± 2% of HUVECs are ICAM-positive after TNF stimulation) ($n = 3$) and by nasal mucosa (51% ± 14% of blood vessels express ICAM 24 hours after IL-1 stimulation; 54% ± 6% of blood vessels express ICAM 24 hours after TNF stimulation) ($n = 3$). No color reaction was produced by the control IgG₁ antibody.

IL-1 receptor antagonist and IL-1 concentrations in HUVECs and nasal mucosa

Levels of IL-1 receptor antagonist (IL-1ra) and IL-1 in nasal mucosa were assayed to determine whether IL-1ra in nasal mucosa, degradation of IL-1 by proteases present in nasal mucosa, or both could account for the lack of IL-1 induction of VCAM by nasal mucosa. IL-1ra was not detected in unstimulated HUVEC supernatants (<20 pg/ml IL-1ra) ($n = 2$) or in HUVEC supernatants stimulated with 10 ng/ml IL-1 for 4 hours (<20 pg/ml

IL-1ra) or 24 hours (<20 pg/ml IL-1ra). Equivalent concentrations of IL-1ra were detected in unstimulated nasal mucosa (1056 ± 22 pg/ml IL-1ra) ($n = 2$) and nasal mucosa stimulated for 4 hours with 10 ng/ml IL-1 (955 ± 57 pg/ml IL-1ra). However, IL-1 (10 ng/ml) stimulation of nasal mucosa for 24 hours ($n = 2$) significantly increased levels of IL-1ra compared with unstimulated nasal mucosa (2775 vs 1056 pg/ml IL-1ra) ($p = 0.001$) and compared with 4-hour stimulation of nasal mucosa with IL-1 (2775 vs 955 pg/ml IL-1ra) ($p = 0.006$). Recombinant IL-1 (10 ng/ml) was added to nasal mucosa, and the percent recovery of IL-1 was assessed at 4 hours (95% of IL-1 added to nasal mucosa recovered) and at 24 hours (90% of IL-1 added to nasal mucosa recovered).

In situ hybridization

Unstimulated HUVECs did not express VCAM mRNA (Fig. 4, A). A 6-hour incubation with either IL-1 (10 ng/ml) or TNF (10 ng/ml) induced equivalent amounts of VCAM mRNA expression by HUVECs (Fig. 4, B). Unstimulated nasal mucosa did not express VCAM mRNA (Fig. 5, A). In contrast, low levels of VCAM mRNA were detected in TNF (Fig. 5, B and C), but not IL-1-stimulated nasal mucosa. VCAM mRNA was de-

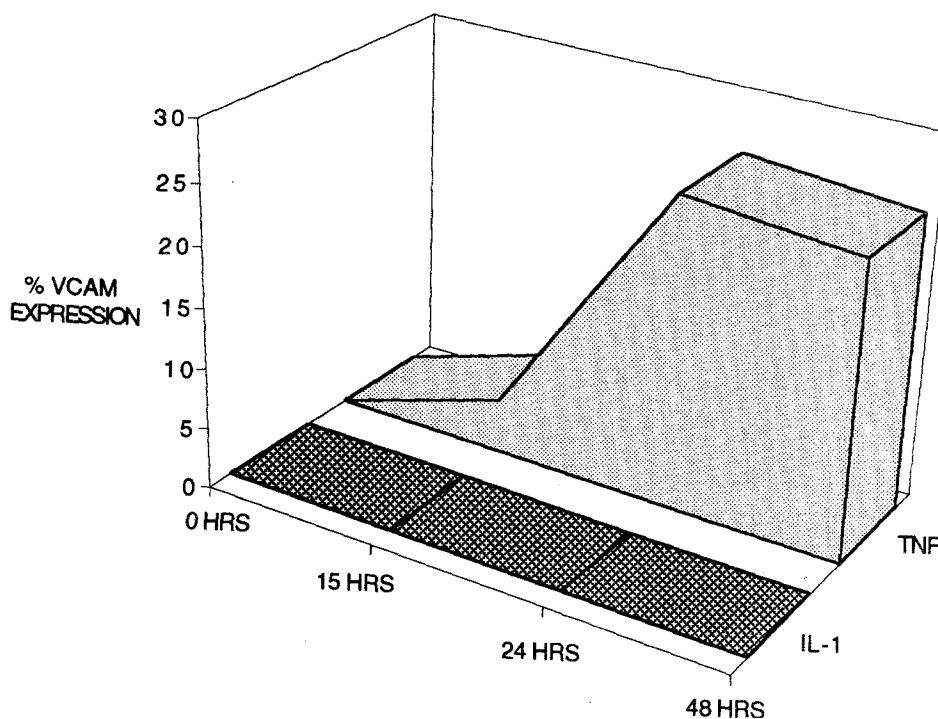


FIG. 3. Time course of VCAM expression in nasal mucosa after TNF stimulation. Nasal mucosa ($n = 3$) were stimulated with TNF (10 ng/ml) or IL-1 (10 ng/ml) for the periods indicated (0, 15, 24, and 48 hours), and VCAM protein expression was determined by immunostaining. Results are expressed as the percent of the total area of nasal mucosal blood vessels expressing VCAM corrected for any 0-hour baseline VCAM expression. TNF was significantly more potent than IL-1 in inducing VCAM expression by nasal mucosa at 15 hours ($4.5\% \pm 2.5\%$ vs 0% , $p = 0.01$), 24 hours ($25.7\% \pm 7.0\%$ vs 0% , $p = 0.01$) and 48 hours ($24.5\% \pm 8.4\%$ vs 0% , $p = 0.01$).

tected at 6 hours after stimulation, preceding VCAM protein expression, which peaked at 24 to 48 hours.

DISCUSSION

The expression by endothelium of cytokine-inducible adhesion molecules, such as VCAM, is likely to play an important role in localizing circulating inflammatory cells to sites of allergic inflammation in the nasal mucosa. Because allergic inflammatory sites are characterized by the presence of several cytokines^{2, 21-23, 30} (which may or may not share overlapping functions), the biologic significance of the detection of a particular cytokine(s) is not always evident. IL-1 and TNF are examples of cytokines that share many overlapping functions²⁶ and are detected at sites of allergic inflammation.^{23, 30} In this study we show that TNF is a more potent inducer of VCAM mRNA and protein expression in nasal mucosa microvascular endothelial cells as compared with IL-1. Although we could not detect IL-1-induced VCAM expression in nasal mucosa, we cannot exclude that IL-1 induced low levels of VCAM expression, which were not

noted visually or by computer analysis. Similar studies of HUVECs and human dermal microvascular endothelial cells have shown that TNF, but not IL-1 α or IL-4, induces VCAM in dermal microvascular endothelial cells.³⁴ Our studies of nasal mucosa suggest that as in the microvasculature of the skin, TNF is more important than IL-1 in regulating VCAM expression. The differences in induction of VCAM expression by IL-1 and TNF cannot be explained on the basis of lack of biologic activity of IL-1 because the same aliquots of IL-1, which did not induce VCAM expression on nasal mucosal endothelial cells, induced VCAM expression on HUVECs and ICAM on nasal mucosa. Dose-response and kinetic studies with IL-1 showed that the dose of IL-1 used or the kinetic expression of VCAM in nasal mucosa in response to IL-1 could not account for the differences in VCAM expression detected in nasal mucosa.

In vivo studies have also suggested that IL-1 may not play a prominent role in induction of VCAM expression. For example, intradermal injection of IL-1 induces significant expression of the adhesion molecule E-selectin, but not VCAM by dermal

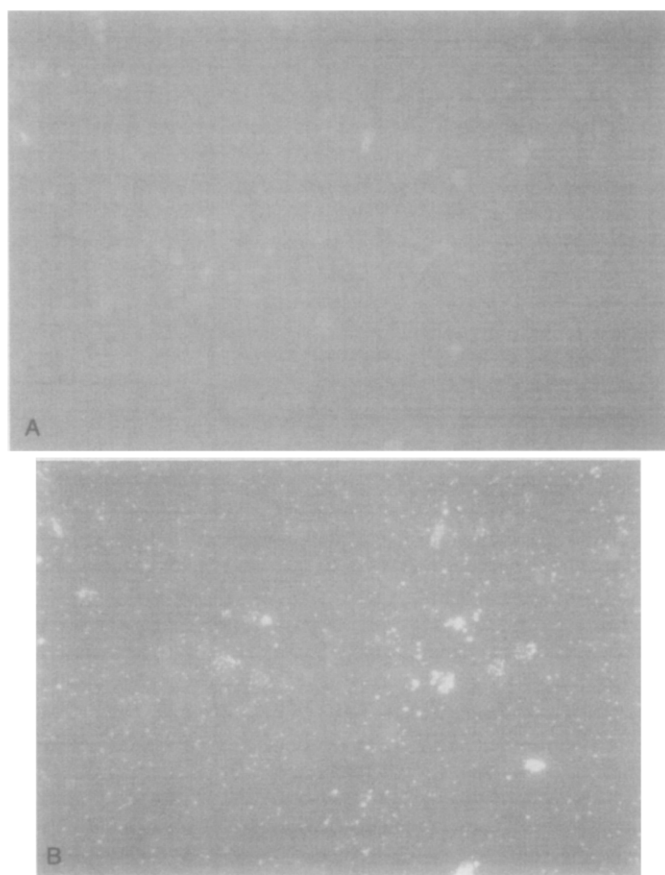


FIG. 4. VCAM mRNA expression by HUVECs. HUVECs were stimulated in vitro with either IL-1 or TNF, and VCAM mRNA expression was detected with ³⁵S-labeled sense and anti-sense VCAM RNA probes. Slides were counterstained with bis benzimide, which fluorescently stains nuclei blue. **A**, Dark field photomicrograph of unstimulated HUVECs demonstrating absence of hybridization to the ³⁵S-labeled VCAM antisense RNA probe. **B**, Dark field photomicrograph of HUVECs hybridized with the ³⁵S-labeled VCAM antisense RNA probe demonstrating expression of VCAM mRNA 6 hours after stimulation with TNF, 10 ng/ml. Similar results were obtained with IL-1 stimulation. The control sense ³⁵S-labeled VCAM RNA probe did not hybridize to IL-1 or TNF-stimulated HUVECs. (Magnification $\times 40$.)

microvascular endothelial cells.³⁵ Similarly, IL-4 injection into baboons does not induce significant VCAM expression.³⁶ In contrast, in vivo injection of TNF into baboons³⁶ is followed by VCAM-1 expression by endothelial cells. Interestingly, studies of skin explants have suggested differences in VCAM-1 expression in normal versus inflamed skin.³⁷ Although neither IL-1 nor TNF could induce VCAM-1 expression in normal skin explants, TNF (IL-1 was not tested) could induce VCAM-1 expression in inflamed skin explants derived from patients with unspecified dermatitis.³⁷ Thus it is possible that our nasal mucosa explants (derived from patients with perennial rhinitis) have been primed by inflammatory mediators or cytokines in vivo to respond preferentially to TNF as opposed to IL-1. In contrast to nasal mucosa, which prefer-

entially expressed VCAM in response to TNF, but not IL-1, inflamed skin explants³⁷ did not exhibit preferential VCAM expression in response to the cytokines tested (both TNF and IL-4 induced VCAM-1 expression; IL-1 was not studied). Further studies with normal and inflamed skin and nasal mucosa explants need to be performed to determine whether the differences observed are anatomic (skin vs nasal mucosa), dependent on the cytokine tested (IL-1, TNF, or IL-4), or dependent on the nature of the disease-associated inflammatory response (allergic or nonallergic inflammation). Overall, these studies suggest that certain cytokines may play a more prominent role in regulating VCAM expression, depending on the vascular bed studied.

Our study also indicates that VCAM expression

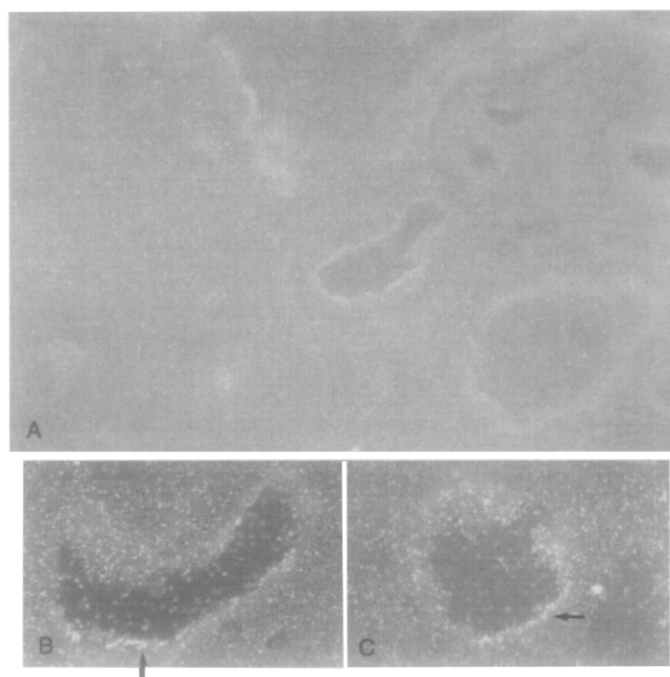


FIG. 5. VCAM mRNA expression by nasal mucosa. Nasal mucosa were cultured in vitro for 6 hours with 10 ng/ml TNF. Nasal mucosa were fixed in 4% paraformaldehyde and hybridized with an ^{35}S -labeled VCAM sense (control) or antisense probe. **A**, Dark field view shows that unstimulated nasal mucosa do not hybridize to the VCAM antisense probe. **B and C**, Dark field views of nasal mucosa stimulated with TNF (10 ng/ml) for 6 hours and hybridized with the VCAM antisense probe. Arrow indicates VCAM mRNA expression. In all the above experiments, the control sense VCAM probe did not hybridize to the nasal mucosa. (Magnification $\times 40$.)

is more readily detected in HUVECs as opposed to nasal mucosa. Similar studies of bronchial biopsy specimens obtained after allergen challenge from patients with asthma have shown much lower levels of VCAM expression ($\sim 2\%$ of total vessels positive by immunohistochemistry) compared with E-selectin ($\sim 36\%$ vessels positive) and ICAM ($\sim 70\%$ vessels positive).³⁸ Therefore studies of HUVEC expression of adhesion molecules might not always accurately predict levels of microvascular adhesion molecule expression at sites of allergic inflammation in the nasal mucosa or airway.

Potential mechanisms to explain the lack of induction of VCAM in nasal mucosa by exogenously added IL-1 include degradation of IL-1 by proteolytic enzymes present in nasal mucosa, competition for binding to IL-1 receptors by IL-1ra present in nasal mucosa, downregulation of IL-1 receptors by any IL-1 that may have been released in vivo in the nasal mucosa of subjects with perennial rhinitis, and differences in post-cytokine receptor signal transduction pathways. The recovery of significant amounts of IL-1 after incubation with nasal mucosa and the ability of IL-1 to induce ICAM expression by nasal mucosa suggest that

neither degradation of IL-1, downregulation of IL-1 receptors in nasal mucosa, nor the presence of IL-1ra (levels of IL-1ra a 1000-fold greater than IL-1 are generally required for IL-1ra to compete with IL-1 for IL-1 receptor sites)³⁹ is likely to explain the inability of IL-1 to induce VCAM expression by nasal mucosa. This suggests that differences in post-cytokine receptor signalling pathways may account for differences in VCAM expression. In this regard it is of interest that recent studies have shown that like IL-1, IL-4 is a relatively weak inducer of VCAM expression when compared with TNF in vitro.⁴⁰ Therefore in the microvasculature of the nasal mucosa of patients with perennial rhinitis, TNF is likely to play a more prominent role, compared with IL-1, in induction of VCAM expression by endothelial cells. However, cytokines that are weak inducers of VCAM alone (such as IL-4) can synergize with TNF in induction of VCAM expression by stabilization of VCAM mRNA transcripts.⁴⁰ As multiple cytokines are released at sites of allergic inflammation, the potential for positive, as well as negative, signals from several cytokines to influence VCAM expression needs to be considered when extrapolating in vitro observations to patients with allergic inflammation.

We thank Sue Sullivan for expert technical assistance, Dr. Allen F. Ryan and Mr. Duane Braumm for assistance with *in situ* hybridization experiments, Dr. L. Osborne (Biogen) for kindly providing the VCAM cDNA, and Crystal Goodman and Susan Chrismen for secretarial support during the preparation of this manuscript.

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