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Sensory nerves in skin are capable of releasing multiple neuropeptides, which modulate inflammatory responses by activating specific cutaneous target cells. Extravasation of particular subsets of leukocytes depends upon the regulated expression of cellular adhesion molecules such as VCAM-1 on microvascular endothelial cells. We examined the direct effect of cutaneous neuropeptides on the expression and function of human dermal microvascular endothelial cell (HDMEC) VCAM-1. A significant increase in VCAM-1 immunostaining of microvascular endothelium was observed in vivo following capsaicin application to human skin. Multiple cutaneous sensory C-fiber-released neuropeptides were evaluated for their ability to induce VCAM-1 cell surface expression on HDMEC. Only substance P (SP) was found to be capable of inducing HDMEC VCAM-1 expression. This SP-mediated VCAM-1 induction appeared to be a direct effect that did not require the release of other HDMEC-derived soluble factors. Increased HDMEC VCAM-1 mRNA expression was detected 1 h after the addition of SP, with peak mRNA increase at 6–9 h postinduction. FACS studies demonstrated a 6.5-fold increase in endothelial cell surface VCAM-1 expression detectable 16 h after addition of SP, which was specifically blocked by a neurokinin-1 receptor antagonist. Increased VCAM-1 cell surface expression on SP-treated HDMEC resulted in a 4-fold increase in the functional binding of 51Cr-labeled MOLT-4 T cells. These data indicate that SP is capable of directly and specifically up-regulating functional endothelial VCAM-1 expression and thus may play a key role in modulating certain inflammatory responses in the skin. The Journal of Immunology, 1999, 162: 1656–1661.

A critical component of the initiation and evolution of localized inflammation is the homing and extravasation of leukocytes at sites of tissue injury (1), which is fundamentally directed by the expression of cell adhesion molecules (2). Leukocyte-endothelial adhesive interactions leading to extravasation are based on a sequential series of events requiring regulated expression of multiple adhesive proteins by endothelial cells, including E-selectin (3), ICAM-1 (4), and VCAM-1 (5). In the skin, the expression of specific combinations of adhesion molecules is closely regulated and correlates with the selective recruitment of leukocyte subtypes. The biological functions of adhesion molecules ICAM-1 and VCAM-1 are distinct. Unlike ICAM-1, which is widely distributed on APCs, epithelial cells, and fibroblasts, VCAM-1 is restricted to the vascular endothelium (6). We have previously demonstrated that human dermal microvascular endothelial cell (HDMEC)⁵ VCAM-1 gene expression is regulated by a number of cytokines (7–9).

A number of distinct neuropeptides are released by sensory C-fibers in the skin (10) where they exert a variety of modulatory actions on dermal and epidermal target cells. Substance P (SP) has been studied as the prototypic cutaneous neuropeptide because it has been shown to induce a wide range of inflammatory activities including vasodilation, immune cell activation, leukocyte infiltration, and cytokine secretion (reviewed in Refs. 11 and 12). SP mediates its effects on target cells by binding to cell surface G protein-coupled neurokinin receptors (NK-R) (13–15). In addition to SP, cutaneous C-fibers have also been shown to release several other neuropeptides including neurokinin A (NKA), neurokinin B (NKB), calcitonin gene-related peptide (CGRP), neurotensin (NT), and somatostatin (Sms) that may be important in certain neuroinflammatory processes in the skin (reviewed in Ref. 16).

There is recent evidence that cutaneous neuroinflammatory interactions include the ability of neuropeptides to induce adhesion molecules on HDMEC (11, 17). We have detected NK-1, NK-2, and NK-3 receptor mRNA in cultured HDMEC and positive immunostaining of the NK-1R on dermal microvascular endothelium in human skin (17). SP engagement of the NK-1R resulted in a rapid increase in HDMEC intracellular Ca²⁺ levels, which could be specifically blocked by a selective NK-1R antagonist, SP-induced HDMEC activation was followed by increased mRNA and...
cell surface expression of ICAM-1. In the current study, we examine the effect of cutaneous nerve-released neuropeptides on microvascular endothelial cell VCAM-1 expression and function. These studies demonstrate the direct and specific ability of SP to induce VCAM-1 expression and function on these cells, thus further supporting the role of effenter neurological signals in mediating specific cutaneous inflammatory responses.

Materials and Methods

Cells and reagents

HDMEC isolated from foreskins were obtained from the cell culture facility of the Emory Skin Disease Research Core Center (Atlanta, GA) (18). Experiments were conducted with cells in passages 3–5. HDMEC were cultured on a gelatinized (0.1%) surface in MCD1311 (Life Technologies, Gaithersburg, MD) supplemented with 10% normal human serum (Irvine Scientific, Santa Ana, CA), 5 ng/ml epidermal growth factor (Clonetics, San Diego, CA), 1 mg/ml hydrocortisone acetate (Sigma, St. Louis, MO), 100 U/ml penicillin, 250 μg/ml amphotericin B, and 10 μg/ml streptomycin (Life Technologies). SV40 large T Ag-transformed HDMEC (HMEC-1) were generated by the Emory Skin Disease Research Core Center (Atlanta, GA) (18). MOLT-4 T cells (derived from a patient with acute T cell leukemia; American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 medium supplemented with 10% FBS, 100 U/ml penicillin, 250 μg/ml amphotericin B, and 10 μg/ml streptomycin (Life Technologies). Lyophilized SP, NKA, NT, CGRP, and Soms (Peninsula Laboratories), Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS, 100 U/ml penicillin, 250 μg/ml amphotericin B, and 10 μg/ml streptomycin (Life Technologies). Lyophilized SP, NKA, NT, CGRP, and Soms (Peninsula Laboratories), Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS, 100 U/ml penicillin, 250 μg/ml amphotericin B, and 10 μg/ml streptomycin (Life Technologies). SV40 large T Ag-transformed HDMEC (HMEC-1) were generated by the Emory Skin Disease Research Core Center (19) and were cultured identically to primary isolated HDMEC. MOLT-4 T cells (derived from a patient with acute T cell leukemia; American Type Culture Collection, Manassas, VA) were cultured in RPMI 1640 medium supplemented with 10% FBS, 100 U/ml penicillin, 250 μg/ml amphotericin B, and 10 μg/ml streptomycin (Life Technologies). Lyophilized SP, NKA, NKB, CGRP, and Soms (Peninsula Laboratories, Belmont, MA) were diluted in the appropriate volume of HDMEC assay medium immediately before use. In selected studies, 1 μM NK-1 receptor antagonist GR82334 (Peninsula Laboratories) was added to cultured endothelial cells 20 min before the addition of SP or TNF-α. Polyclonal rat anti-human SP (American Research Products, Belmont, MA) was diluted to 15 μg/ml in HDMEC medium. The SP-degrading enzyme neutral endopeptidase (NEP) was a gift from Nigel Bunnett (University of California, San Francisco, CA). Lyophilized human recombinant TNF-α and anti-TNF-α Ab were obtained from R&D Systems (Minneapolis, MN). Murine mAb PSC4 directed against human VCAM-1 was a generous gift from Dr. Elizabeth Wayner (University of Minnesota, Minneapolis, MN). Murine mAb 8H10 directed against human ICAM-1, which functions as a blocking Ab was provided by Dr. Stephen Shaw (National Institutes of Health, Bethesda, MD) (20).

In vivo expression of VCAM-1 in human skin microvascular endothelial cells by immunohistochemistry

Capsaicin-high potency (Zostrix, 0.075%, GenDerm, Lincolnshire, IL) was applied topically to the skin of four volunteers to stimulate the release of cutaneous neuropeptides and 4-mm punch biopsies were taken from treated sites at 6, 24, and 48 h as well as from an untreated site on the opposite limb taken immediately before capsaicin application. Tissue was embedded in O.C.T. Tissue Tek; Miles, Elkhart, IN) and frozen at −70°C. Immunohistochemistry was performed on 8-μm sections using mouse anti-human VCAM-1 mAb PSC4 as the primary Ab (diluted 1:400), a biotinylated horse anti-mouse secondary Ab (Zymed, San Francisco, CA), and strept-avidin-biotin complex (Zymed) as a tertiary reagent to detect specific binding of the primary and secondary reagents. Samples were examined using a Nikon (New York, NY) Microphot SA microscope, and photographed at ×120 magnification using a Nikon NFX-35 microscope camera. Controls for staining were performed on tissue sections without the primary Abs. No staining was obtained with either of the controls.

ELISA measurement of HDMEC cell surface VCAM-1 expression

HDMEC were plated onto 96-well plates and upon reaching 80% confluence were either left untreated or stimulated for 16 h with 1, 10, 100, or 1000 nM of neuropeptides; SP, NKA, NKB, NT, CGRP, or Soms. Cell viability was determined at the end of the stimulation period by performing cell counts in separate plates. The addition of 300 U/ml TNF-α for 16 h to cultured HDMEC was used as a positive control for VCAM-1 cell surface induction. HDMEC VCAM-1 expression was assessed using the anti-VCAM-1 mAb PSC4 or isotype-matched control IgG by ELISA as described previously (21). Results represent the mean of three values ± SD for each variable tested, and results are representative of three independent assays.

HDMEC cell supernatant effects on VCAM-1 cell surface expression

To determine whether SP-treated HDMEC-conditioned medium contained neuropeptide-induced factors that could be responsible for the increased expression of VCAM-1, a series of supernatant transfer experiments were performed. HDMEC were plated onto 96-well plates and upon reaching 80% confluence were left untreated or treated with 10 nM SP for 1–24 h. The addition of 300 U/ml TNF-α for 16 h to cultured HDMEC was used as a positive control for VCAM-1 cell surface induction. After the stimulation period (1–24 h), these cell-conditioned HDMEC supernatants (200 μl per sample) were removed and, with or without the presence of 15 μg/ml polyclonal rat anti-human SP Ab, 15 μg/ml polyclonal rat anti-human TNF-α Ab, or 1 μg/ml NEP, transferred to fresh HDMEC 96-well plates from which the medium had been removed. The HDMEC supernatants were then allowed to incubate for 16 h. In selected experiments, supernatants were “spiked” with 300 U/ml recombinant TNF-α. HDMEC were then assessed for VCAM-1 cell surface expression using mAb P3C4 or isotype control IgG by ELISA as described previously (21).

VCAM-1 mRNA expression determination by Northern blot analysis

Cultured HDMEC (1 × 10⁶ cells) were left untreated or treated with 10 nM SP for 1, 3, 6, or 9 h or with 300 U/ml TNF-α for 6 h. Total cellular RNA was isolated and Northern blot analyses were done as described previously (21). A 0.6-kb BglII fragment of the human VCAM-1 cDNA (provided by Dr. T. Venkat Gopal, Otsuka America Pharmaceuticals, Rockville, MD) was used as a VCAM-1 probe. Hybridization with radiolabeled β-actin cDNA was used for determination of lane loading consistency and for normalization of ICAM-1 signal in the various conditions tested. The autoradiograph was scanned on a flat bed scanner (La Cie, Beaverton, OR) utilizing Photoshop software (Adobe Systems, Mountain View, CA). The digitized image was then imported into a Power Point (Microsoft, Redmond, WA) file where it was labeled and printed on a high-resolution laser printer.

Determination of surface VCAM-1 expression on HMEC-1 by FACS

For FACS studies, we used the HDMEC cell line HMEC-1. We have shown previously that VCAM-1 expression in these cells is similar to that of HDMEC. Cultured HMEC-1 were left untreated or stimulated with 10 nM SP for 16 h. In selected experiments, cells were pretreated with 1 μM of the selective NK-1 receptor antagonist GR82334 for 20 min before the addition of 10 nM SP or 300 U/ml TNF-α. HMEC-1 were detached from tissue culture plates with Versene 1:5000 (Life Technologies) and washed with PBS (minus). Aliquots were transferred into tubes for Ab staining. HMEC-1 were incubated with mouse anti-human VCAM-1 mAb P3C4, or isotype control mouse anti-human IgG (H+L) (Jackson ImmunoResearch, West Grove, PA) at a final concentration of 10 μg/ml for 1 h on ice. Cells were washed twice and incubated with FITC-conjugated affinity purified goat F(ab’)2 anti-mouse IgG (H+L) (Jackson ImmunoResearch) at a final concentration of 0.1 μg/ml for 1 h on ice. Cells were washed twice and analyzed by a FACScan flow cytometer (Becton Dickinson, Rallege, NC) equipped with CellQuest software for data acquisition and analysis. The forward scatter threshold was set to permit analysis of viable endothelial cells.

Adherence of MOLT-4 T cells to HDMEC

Binding assays measuring cellular adherence to HDMEC were performed with human MOLT-4 T cells, which preferentially express the VCAM-1 ligand (22), the integrin α₄β₇, also referred to as very late Ag-4 (VLA-4), and consequently adhere to VCAM-1 on target cells (23). HDMEC were plated in 24-well plates overnight, then either left untreated or treated for 16 h with 0.1–100 nM SP or 300 U/ml TNF-α diluted in HDMEC assay medium in triplicate wells, and then plates were washed three times with HBSS . MOLT-4 cells were incubated in the presence of 500 μCi of 31Cr for 1 h at 37°C at 5% CO₂ and then washed twice with HBSS . Blocking studies were performed on untreated or HDMEC treated for 16 h with or without 10 nM SP, by adding either anti-VCAM-1 mAb P3C4 or isotype-matched anti-ICAM-1 mAb 8H10 at a final concentration of 10 μg/ml and allowing HDMEC to incubate for 20 min at 37°C at 5% CO₂ before the addition of MOLT-4 cells. MOLT-4 cells were then added to untreated and variously treated HDMEC wells at a concentration of 70,000 MOLT-4 cells per well and incubated for 20 min at 37°C. The supernatant was removed and the MOLT-4-overlaid HDMEC were washed gently three times with HBSS to remove nonadherent MOLT-4 cells. One percent SDS was
added and allowed to incubate for 15 min at room temperature. Each well was then swabbed with two cotton-tipped swabs that were counted in a gamma counter. Adherence was calculated as % MOLT-4 binding = [(cpm per well − background cpm)/(cpm added counts − background cpm)] × 100, as described previously (24). Photographs of unlabeled MOLT-4 T cells adhering to untreated or SP-treated HDMEC were taken using Kodak 100, as described previously (24). Photographs of unlabeled MOLT-4 T cells incubated for 16 h with 1, 10, 100, or 1000 nM of NKA, NKB, NT, CGRP, or Sms had no effect on HDMEC VCAM-1 induction (data not shown). TNF-α served as a positive control for HDMEC VCAM-1 cell surface induction (Fig. 2).

**Results**

**In vivo induction of VCAM-1 in capsaicin-treated human skin**

The in vivo effect of C-fiber-released neuropeptides on dermal endothelial cell VCAM-1 expression was examined. A capsaicin-containing cream (Zostrix) was applied topically to human volunteers to stimulate the release of neuropeptides including SP from cutaneous sensory nerves (25). Biopsies were obtained at 0, 6, 24, and 48 h, and evaluated by immunohistochemistry for VCAM-1 expression associated with microvascular endothelial cells in the dermis. As shown in representative photomicrographs from one individual, microvascular VCAM-1 staining was present in very low levels in untreated skin (Fig. 1a). VCAM-1 immunoreactivity increased slightly 6 h after capsaicin application (Fig. 1b). A significant increase in dermal endothelial cell VCAM-1 staining was detected 24 h after application of topical capsaicin (Fig. 1c), which was decreased in intensity by 48 h after capsaicin application (Fig. 1d). In other subjects, VCAM-1 expression persisted at 48 h after application of capsaicin (data not shown). Biopsies obtained from symmetrical untreated sites showed no increased VCAM-1 staining (data not shown). Thus, the release of neuropeptides by cutaneous sensory nerves results in increased HDMEC VCAM-1 expression in vivo.

**Specificity of SP-induced HDMEC VCAM-1 cell surface expression**

A number of neuropeptides are detected in normal and inflamed skin (10). The effect of several of these agents known to be released by cutaneous sensory nerves on HDMEC VCAM-1 expression was investigated by ELISA (Fig. 2). Untreated HDMEC (−) have no constitutive VCAM-1 present on their surface. Similarly, treatment with an isotype control IgG in place of the anti-VCAM mAb P3C4 had no effect on HDMEC VCAM-1 surface expression. As indicated, incubation with 1, 10, 100, or 1000 nM SP for 16 h up-regulated cell surface VCAM-1 on HDMEC. The optimal 7-fold induction was observed with the 10 nM SP dose (Fig. 2). In contrast, incubation for 16 h with 1, 10, 100, or 1000 nM of NKA, NKB, NT, CGRP, or Sms had no effect on HDMEC VCAM-1 induction (Fig. 2). Cell viability as determined by cell counts after stimulation was not affected by any of the neuropeptide treatments

**SP directly induces HDMEC VCAM-1**

To test whether SP directly up-regulates the expression of HDMEC VCAM-1 or whether this was in part due to the induction by SP of HDMEC-secreted soluble factors that in turn could be responsible for increased VCAM-1 expression, we performed a series of supernatant transfer experiments (Fig. 3). Initially, we determined that HDMEC treated between 1 and 24 h with 10 nM SP resulted in a maximal 7-fold up-regulation of cell surface VCAM-1 expression at 16 h (Fig. 3A) as measured by ELISA. TNF-α significantly up-regulated VCAM-1 expression compared with untreated cells and served as a positive control (Fig. 3A). Isotype control IgG in place of the anti-VCAM-1 Ab showed no change in VCAM-1 expression as compared with untreated HDMEC (data not shown). The effect of supernatants obtained from SP-treated HDMEC on VCAM-1 expression was measured (Fig. 3, B–D). As indicated in Fig. 3B, conditioned medium from SP-treated HDMEC added to freshly cultured HDMEC for 16 h was capable of augmenting VCAM-1. This was most apparent with supernatants derived from the short SP-HDMEC incubation

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*FIGURE 1.* In vivo induction of HDMEC VCAM-1 expression. Photomicrographs were taken of human skin left untreated (a) or treated with capsaicin cream (0.075%) and biopsied at 6 h (b), 24 h (c), or 48 h (d). Biopsies were immunostained for VCAM-1 expression. Arrows point to dermal microvascular structures. The data shown are representative of studies conducted with four volunteers.

*FIGURE 2.* ELISA measurement of VCAM-1 expression of neuropeptide-treated HDMEC. HDMEC were stimulated with 1, 10, 100, or 1000 nM of either SP, NKA, NKB, NT, CGRP, or Sms. TNF-α (300 U/ml) was used as a positive control. Incubations were for 16 h at 37°C and cell surface VCAM-1 was measured by ELISA. Statistically significant differences in cell surface VCAM-1 in treated samples as compared with untreated control cells (−) were determined by Student’s t test as indicated by asterisks (*, p < 0.005). The data shown are representative of experiments conducted in triplicate.
time points (1–6 h). The results in Fig. 3B are consistent with the high degree of protein latency of the SP peptide in cell culture studies. After 3 h, most of the SP bioactivity is lost in the HDMEC supernatants as measured by SP receptor activation studies (data not shown).

The induction of HDMEC VCAM-1 expression could be completely abrogated by the addition of the anti-SP Ab to these supernatants (Fig. 3C). This complete abrogation of VCAM-1 induction with the Ab to SP strongly suggests that no factor that could induce VCAM-1 (other than SP) is present in the SP-treated supernatants. No change in HDMEC VCAM-1 expression was observed following treatment with anti-TNF-α Ab (data not shown).

In addition, recent transcriptional studies in our laboratory indicate that the addition of neutralizing Abs to both TNFR-I and TNFR-II time points (1–6 h). The results in Fig. 3B are consistent with the high degree of protein latency of the SP peptide in cell culture studies. After 3 h, most of the SP bioactivity is lost in the HDMEC supernatants as measured by SP receptor activation studies (data not shown).

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In addition, recent transcriptional studies in our laboratory indicate that the addition of neutralizing Abs to both TNFR-I and TNFR-II had no effect on the ability of SP to transcriptionally activate either the ICAM-1 or VCAM-1 gene (our unpublished observations). TNF-α added to the supernatants in the studies conducted in Fig. 3C induced high levels of HDMEC VCAM-1 expression, thus ruling out a nonspecific toxic effect of the SP Ab on HDMEC (Fig. 3D). When untreated and SP-treated HDMEC supernatants were incubated with NEP, which selectively degrades SP, and transferred to fresh HDMEC, there was no up-regulation of VCAM-1, although TNF-α-treated/NEP-treated supernatants were still capable of inducing VCAM-1 on HDMEC (data not shown). These data support the direct role of SP in the induction of HDMEC VCAM-1 expression.

**SP induces HDMEC VCAM-1 mRNA**

To determine whether SP is capable of modulating VCAM-1 mRNA in HDMEC, Northern blot analysis was performed (Fig. 4A). HDMEC were treated with 10 nM SP for 1, 3, 6, or 9 h, or for 6 h with 300 U/ml TNF-α. HDMEC VCAM-1 mRNA expression was normalized to β-actin mRNA expression by densitometric analysis for each experimental condition (Fig. 4B). HDMEC do not constitutively express basal levels of VCAM-1 mRNA. HDMEC VCAM-1 mRNA was significantly induced 1–3 h after treatment with SP, which was further augmented after 6 h and 9 h of exposure to SP (Fig. 4). TNF-α induction of HDMEC VCAM-1 mRNA expression served as a positive control for this study. Thus, SP is capable of inducing HDMEC VCAM-1 mRNA.

**SP induces HMEC-1 VCAM-1 cell surface expression**

The effect of SP on immortalized HMEC-1 cell surface VCAM-1 expression was examined by FACS analysis and mean log 10 fluorescence values were compared. Untreated HMEC-1 were calculated as having a mean channel fluorescence of 1.90 for surface VCAM-1 staining (Fig. 5, dotted lined histograms). HMEC-1 treated with 10 nM SP for 16 h resulted in a mean fluorescence of 45.2 and a 6.5-fold increase in mean fluorescence, with a mean channel fluorescence of 12.73 for surface VCAM-1 (Fig. 5B) compared with control untreated cells. Pretreatment of HMEC-1 with an NK-1R antagonist before the addition of SP resulted in a mean channel fluorescence of 2.26, demonstrating the important role of the NK-1R in SP-induced VCAM-1 up-regulation (Fig. 5C). The NK-1R antagonist alone had no effect on VCAM-1 cell surface expression (data not shown). TNF-α-treated HMEC-1 displayed high levels of cell surface VCAM-1 expression, with a mean fluorescence of 45.2 and served as a positive control (Fig. 5D). TNF-α-mediated HMEC-1 VCAM-1 up-regulation was not blocked by pretreatment with an NK-1R antagonist (data not shown). In addition, no increase in fluorescence occurred when an isotype matched control Ab was used on SP-treated HMEC-1 (Fig. 5A). Therefore, these data demonstrate SP up-regulates the expression of cell surface VCAM-1 on HMEC-1, primarily through interaction with the NK-1R.

**Enhanced binding of MOLT-4 T cells to SP-treated HDMEC**

The biological consequence of SP-induced HDMEC VCAM-1 cell surface expression was determined using a quantitative cellular adhesion assay with MOLT-4 T cells (22). MOLT-4 T cells bear
the counter-receptor ligand for VCAM-1, VLA-4 (α4β1) (3). Although 51Cr-labeled MOLT-4 T cells showed minimal binding to untreated cultured HDMEC (Fig. 6), the adhesion of MOLT-4 T cells to SP-treated HDMEC increased following the addition of 0.1 mM SP for 16 h. The enhanced binding of MOLT-4 cells to 1 mM SP-treated HDMEC was significant as well. As indicated, 10 mM SP induced a 4-fold increase in the adhesion of MOLT-4 cells to HDMEC. There was a small inductive increase in MOLT-4 cell adhesion to HDMEC treated with 100 nM SP. The specificity of VCAM-1 in the adherence of MOLT-4 T cells to HDMEC after SP treatment was examined. Leukocyte binding to SP-treated HDMEC could be specifically blocked by pretreatment of cells with an anti-VCAM-1 blocking Ab before the addition of labeled MOLT-4 T cells, whereas HDMEC pretreated with a blocking anti-ICAM-1 Ab (20) showed no decrease in VCAM-1/VLA-4 binding to MOLT-4 cells (Fig. 6). TNF-α treatment of HDMEC served as a positive control for enhanced MOLT-4 binding (Fig. 6).

Discussion

Neuroimmunologic interactions leading to activation of endothelial cells and modulation of downstream leukocyte trafficking events may play a key role in regulating inflammatory responses. The release of neuropeptides such as SP by cutaneous sensory fibers may function as efferent signals to facilitate increased leukocyte trafficking to sites of infection, inflammation, and wound healing. SP has been well characterized as a potent vasodilator that can cause increased microvascular permeability and protein extravasation (26). Additionally, SP can modulate leukocyte effector activities such as lymphocyte proliferation, cytotoxicity, and Ig production (11, 27); mast cell degranulation (28); macrophage and polymorphonuclear leukocyte activation (29, 30); and cytokine production by monocytes (31–33). We have previously demonstrated that SP can directly activate mast cells and keratinocytes to secrete TNF-α and IL-1, respectively (34, 35). SP has been shown to preferentially enhance the migration of eosinophils in asthmatic patients (36). Understanding the role of SP in leukocyte trafficking will aid in elucidating its contribution to the pathogenesis of certain inflammatory diseases.

By the expression of specific cell adhesion molecules, as well as release of cytokines and chemokines, the microvascular endothelium is a key cell in regulating leukocyte trafficking into inflamed tissue. The nature of the inflammatory stimulus determines whether lymphocytes, monocytes, neutrophils, or eosinophils predominate, exercising specificity in molecular signals that control traffic of leukocyte subsets. The main classes of adhesion molecules that play a part in trafficking are the selectins, the integrins, members of the Ig superfamily, and sialoadhesins (37). In skin, P- and E-selectins mediate initial leukocyte adhesion, whereas ICAM-1 and VCAM-1 mediate subsequent, firm adhesion and transendothelial migration. ICAM-1 binds leukocytes via β2 integrins and can therefore recruit both granulocytes and monocytes from the bloodstream to sites of tissue injury, whereas VCAM-1 binds monocytes, eosinophils, and lymphocytes expressing the integrins α4β1 and α5β1, specifically supporting the recruitment of chronic inflammatory cells (38, 39). SP has been suggested as playing a role in the pathogenesis of psoriasis, where possibly VCAM-1/VLA-4-mediated chronic inflammation exists (40).

Our present evaluation of the effect of SP on cutaneous microvascular endothelial cell VCAM-1 expression further supports the role of neuropeptides in the regulation of leukocyte trafficking in the skin. The ability of neuropeptides to influence adhesion molecule expression on microvascular endothelial cells was shown in our recent studies of SP-induction of dermal endothelial ICAM-1 expression (17). Additionally, SP was found to up-regulate adhesins P-selectin and E-selectin in normal human skin (36) when incubated for a limited time period (0–8 h). We demonstrate induction of dermal microvascular endothelial cell VCAM-1 expression in vivo after topical application of the neuropeptide-releasing agent capsaicin. Cutaneous sensory nerve-released neuropeptides NKA, NKB, NT, CGRP, and Sms had no effect on HDMEC VCAM-1 cell surface up-regulation across a broad concentration range, whereas SP significantly increased VCAM-1 cell surface

FIGURE 6. Enhanced binding of 51Cr-labeled MOLT-4 T cells to SP-treated HDMEC. Functional binding of SP-induced VCAM-1 on HDMEC was determined in a quantitative cell adhesion assay utilizing VLA-4 expressing MOLT-4 T cells. HDMEC were treated for 16 h with 0.1–100 nM SP, or 300 U/ml TNF-α followed by the addition of 31Cr-labeled MOLT-4 T cells. Plates were washed and adherent cell binding activity was expressed as % MOLT-4 binding (see Materials and Methods). Specificity of VCAM-1-mediated binding was determined by pretreatment with anti-VCAM-1 Ab to both untreated HDMEC and to SP-treated HDMEC before incubation with MOLT-4 T cells. Preincubation of untreated and SP-treated HDMEC with the isotype control anti-ICAM-1 Ab was performed as well. Statistically significant differences in % MOLT-4 binding in treated samples as compared with untreated control cells (−) were determined by Student’s t tests and indicated by asterisks (*, p < 0.005). The data shown are representative of three independent experiments.
expression. Additionally, SP was capable of directly up-regulating HDMEC surface VCAM-1, without the participation of other HDMEC-derived factors. This direct SP induction resulted in increased levels of VCAM-1 mRNA and NK-1-mediated VCAM-1 cell surface expression. SP treatment resulted in functional VCAM-1 induction as demonstrated by the ability of VCAM-1 on HDMEC to bind its ligand VLA-4 on MOLT-4 T cells.

SP is known to induce the secretion of cytokines from various immune cells (32, 35, 41). We determined whether SP exposure resulted in HDMEC secretion of soluble factors capable of inducing VCAM-1 expression. Our study indicates that SP was capable of directly inducing increased HDMEC VCAM-1 expression without the participation of HDMEC-derived factors. In vivo, cytokine release may play a role in endothelial adhesion molecule up-regulation, but cytokine release is neither necessary nor required for SP-mediated induction of VCAM-1 on cultured microvascular endothelial cells.

In summary, our studies demonstrate that SP is capable of directly regulating dermal microvascular endothelial VCAM-1 expression, primarily through activation of the NK-1 receptor. These findings further support the role of neuropeptide modulation of leukocyte recruitment in the skin during cutaneous inflammatory reactions. The coordination of signals such as neuropeptide release and adhesion molecule up-regulation may play a role in the development of a wide range of inflammatory responses and wound healing. Further investigation into the interactions of various neuropeptides, adhesion molecules, and other inflammatory mediators should provide a rational basis for controlling inflammatory disorders that may have a significant neurogenic component such as rheumatoid arthritis, asthma, psoriasis, and atopic eczema.

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