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Endothelial adhesion molecules for nasal-homing T cells in allergy

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Abstract During the allergic reaction mucosal T cells are activated and a local increase in numbers occurs. In peripheral blood, a concomitant T cell activation and switch towards memory phenotype appears. E-selectin, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 were studied in nasal mucosal biopsies taken during a time-course provocation study, including patients with seasonal allergic rhinitis and healthy controls. Allergic patients were also studied during the natural pollen season with particular attention to the influence of local corticosteroid treatment. Before provocation allergic patients and controls did not differ concerning the expression of endothelial adhesion molecules. However, the epithelial ICAM-1 expression was increased among allergics ($P<0.05$). Repetitive allergen provocation induces an increased endothelial expression of VCAM-1 in allergic patients ($P<0.01$). Similarly, VCAM-1 expression was increased during the natural pollen season ($P<0.05$). Interestingly, the increased VCAM-1 expression was inhibited by the use of local corticosteroids. The present data demonstrate a putative integrin-VCAM-1 mechanism for selective homing of T memory cells to the allergic nasal mucosa and new in vivo effects of local corticosteroid treatment are demonstrated.

Key words Endothelial adhesion molecules · Allergic rhinitis · ICAM-1 · VCAM-1

Introduction

Cell adhesion molecules have been found to be important in several inflammatory conditions, particularly in the mechanisms controlling the accumulation of inflammatory effector cells. The endothelial expression of adhesion molecules of the selectin and immunoglobulin superfamilies function in a close relationship with lymphocyte adhesion molecules, mainly integrins, in a three-step process [31, 39]. This begins with increased transcription and expression of E-selectin on activated endothelial cells and their adhesion to certain glycoprotein ligands on the T lymphocytes [12, 25, 38]. After this initial step local secretion of chemokines will increase the adhesiveness of the T lymphocytes [42]. The third event is the intraction of integrins with certain members of the immunoglobulin superfamily on endothelial cells, some of which are increased by cytokines, for example intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 [31, 39].

CD45RO+ memory T lymphocytes have recently been shown to be the dominant inflammatory cell population in normal nasal mucosa as well as in the mucosa of patients with allergic rhinitis [19]. Memory T cells are also known to express several adhesion molecules [15, 39] including the integrins $\alpha_L\beta_2$ (lymphocyte function associated antigen; LFA-1), which is the ligand for ICAM1- [7] and ICAM-2 [11] and the $\alpha_4\beta_1$ (very late antigen; VLA-4) integrin, which is a VCAM-1 ligand [9]. Moreover, allergen induced peripheral T cell activation and a phentoype switch towards memory [18] is accompanied by a mucosal increase in T cells (CD_4^+ memory cells; see [19, 46]). These findings urged us to extend our time-course allergen provocation study by investigating the possible role of endothelial adhesion molecules in the intranasal accumulation of T cells.

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Materials and methods

Study groups

There were 17 patients with seasonal allergic rhinitis (birch pollen) and 10 healthy, non-allergic, controls. All controls had a negative skin prick test. During non-pollen season a provocation study with intra-nasal administration of allergen in a aqueous solution

was performed. Initially, a nasal mucosal biopsy using 2 mm Geritzma forceps was taken from the inferior turbinate 10 days before the actual time-course provocation. Local anaesthesia (Lidocaine 40 mg/ml without adrenaline) was applied by a cotton swab which was applied into the nostril without touching the site of the biopsy. On every occasion the patients were examined by an ear, nose and throat specialist and were without signs of any recent infection. At day 0 of provocation all participants were provoked with a single dose of 10,000 IE of birch pollen. Thereafter each study group, allergic patients and controls, was divided into three subgroups with different lengths of provocation. The short time groups were provoked at day 2 (1,000 IE) and a contra lateral biopsy was performed on day 3. The groups with intermediate length of provocation were provoked at days 2 and 5 and the biopsy was performed on day 8. The final groups were provoked at days 2, 5 and 8 with sampling of the biopsy an day 13. Furthermore, allergic patients were biopsied once during the natural pollen season. At that time they used their usual medication. Seven patients used nasal steroids, four in combination with oral anti-histamines. Another three patients used oral antihistamines only while the remaining seven patients used no medication. Data concerning participants, study design and medications have been reported in detail elsewhere [3, 20]. The study was approved by the local ethical committee at the Medical Center Hospital, Örebro, Sweden. All participants, allergic patients and controls, were volunteers and written and informed consent had been obtained.

Immunohistochemistry

The nasal biopsy specimens were embedded in Tissue-Tek 4583 OCT compound (Miles Scientific, Elkart, Ind.) and immediately snap-frozen. Specimens were stored at −70° C until sectioned. After cryostat sectioning, air-drying and fixation in acetone, slides were stored wrapped in aluminium foil at −20° C until immunostained. Tissue handling has been described in detail earlier [3, 18, 19].

Monoclonal antibodies (mAb) against endothelial leucocyte adhesion molecule (E-selectin; clone BBIG-E6; dilution 1:2,000), intercellular adhesion molecule-1, (ICAM-1; clone BBIG-I1; dilution 1:5,000), vascular cell adhesion molecule-1 (VCAM-1; clone BBIG-V1; dilution 1:1,000; British Biotechnology, Oxford, U.K.) and factor VIII-related antigen (FVIII; clone F8/86; dilution 1:250; Dakopatts, Sweden) were used. Primary incubation was performed for 30 min at room temperature. After washing, bound antibodies were labelled with a biotinylated goat anti mouse/rabbit antibody (DakoDuet, Dakopatts) for 30 min, washed and detected using the streptavidin-peroxidase system with aminoethylcarbazole applied as the chromogen. Doublestaining with mAb ant-Cd49d (Immunotech) with alkaline phosphatase-anti alkaline phosphatase complex (Dakopatts) and fast-blue as chromogen was performed on the slides initially stained against VCAM-1.

Sections were counterstained with Mayers haematoxylin. Human tonsillar tissue and nasal polyps were used as positive controls. Omission of the primary antibody served as negative control.

Scoring

Immunohistochemical staining was evaluated by a scoring system shown in Table 1. The extent of immunopositivity compared with the adjacent FVIII-stained section and intensity was not evaluated. All slides were coded and scoring was performed on two different occasions by one of us (MGK). The mean of these two observations were used for further statistical analyses.

Intraobserver variation was evaluated by calculating mean differences±SD for the two scorings. For E-selectin staining this was 0.19±0.39, for epithelial ICAM-1 0.20±0.40, for endothelial ICAM-1 0.25±0.44 and for VCAM-1 0.24±0.42. Furthermore, reproducibility of the scoring concerning non-random agreement was evaluated by kappa statistics [40]. Kappa statistics for the different parameters were E-selectin K=0.78, for epithelial ICAM-1 K=0.74, for endothelial ICAM-1 K=0.70 and for VCAM-1 K=0.71, which indicate a substantial non-random agreement [23].

Table 1 Scoring of immunohistochemistry for intercellular adhesion molecule (ICAM)-1 antibody

Epithelial expression

0=No staining
1=Scattered basal staining
2=Continuous basal staining
3=Extensive epithelial staining

Endothelial staining

0=No staining
1=Scattered immunoreactivity within less than three vessels per section or continuous staining of one vessel per section
2=Scattered immunoreactivity within more than three vessels per sections or continuous staining more than one vessel per section
3=>20%, <50% immunopositive vessels
4=50–80% immunopositive vessels
5=>80% immunopositive vessels

Table 2 Endothelial expression of E-selectin, mean±SD

	Allergic patients (n=17)	Controls (n=10)
Before season	3.53 (0.57)	3.65 (0.67)
During provocation	3.50 (1.03)	3.90 (0.46)
In season	3.97 (0.62)*	

* P<0.05 vs before season

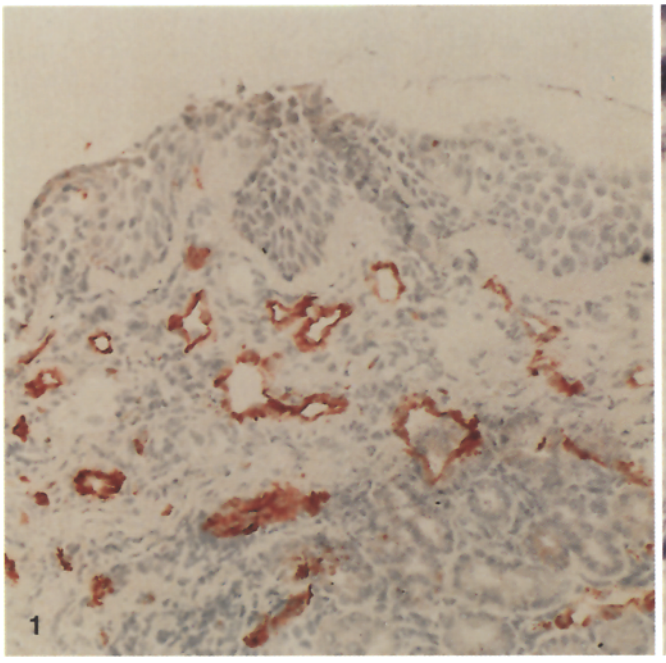
Statistics

Non-parametric tests were applied for statistical analysis where differences between groups were analysed by Mann-Whitney U-test and by Wilcoxons signed rank test for paired samples within groups. Analysis were performed by Statview 4.0 (Abacus Concept, Berkeley, Calif.) software package.

Results

Basal levels of E-selection in endothelial cells did not differ between allergic patients and controls, neither before, nor during the repetitive provocation during non-pollen season. However, a significant increase in expression was observed in allergic patients in specimens taken during the pollen season (P<0.05, Table 2). A representative staining pattern is shown in Fig. 1.

No significant differences were observed for endothelial ICAM-1. The range of the means (3.45–3.74 ICAM-1) was equally expressed when compared with E-selectin at all times studied, except among allergic patients during season (ICAM-1=3.53) when the E-selectin was significantly more expressed (P<0.05). However, allergic patients had increased epithelial expression of ICAM-1 (P<0.05) during non-pollen season compared with controls. During allergen provocation no differences in expression were observed, numerical values in the groups of allergic patients and controls were almost the same as before provocation, but the difference failed to reach significance (P<0.10). In natural pollen season decreased



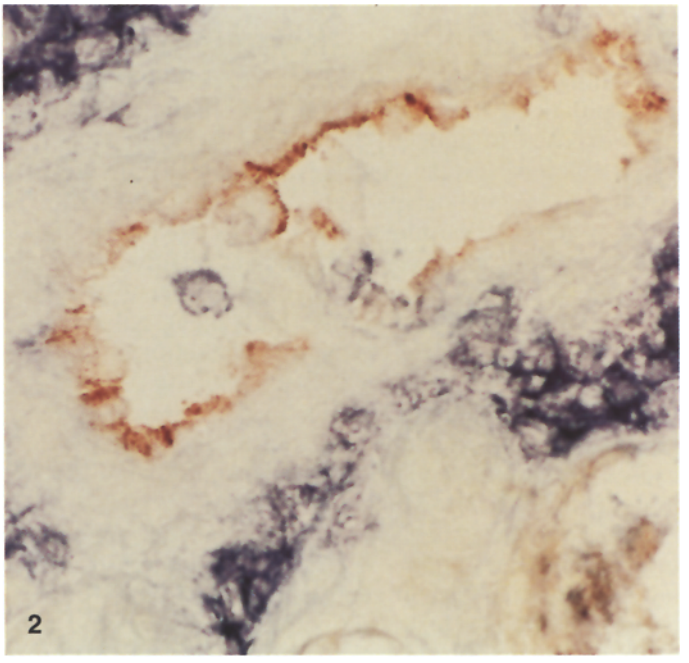


Fig. 1 Nasal biopsy from an allergic patient immunostained with E-selectin monoclonal antibody (mAb) with aminoethylcarbazole (AEC; brown) as chromogen (magnification ×50)

Fig. 2 Nasal biopsy from an allergic patient not using local cortocosteroids during the pollen season. This section shows double-staining with VCAM-1 mAb and AEC (brown), and a mAb (Cd49d, Immunotech; alkaline phosphatase – antialkaline phosphatase, fast-blue) against the α_4 integrin subunit of a lymphocytic VCAM-1 ligand (magnification ×200)

Table 3 Epithelial expression of ICAM-1, mean±SD

	Allergic patients	Controls
Before season	1.76 (0.66)*	1.15 (0.91)
During provocation	1.74 (0.81)	1.10 (0.74)
In season	1.16 (0.40)**	
No corticosteroids	1.22 (0.36)	
Corticosteroids	1.07 (0.45)**	

* $P<0.05$ vs controls
** $P<0.05$ vs before season

Table 4 Endothelial expression of vascular cell adhesion molecule (VCAM)-1, mean±SD

	Allergic patients	Controls
Before season	1.15 (0.63)	0.95 (0.64)
During provocation	2.18 (0.58)*,**	1.10 (0.70)
Day 3	1.92 (0.74)	
Day 8	2.30 (0.45)***	
Day 13	2.33 (0.52)***	
In season	1.41 (0.71)	
No corticosteroids	1.78 (0.51)***	
Corticosteroids	1.21 (0.81)	

* $P<0.01$ vs before season
** $P<0.01$ vs control
*** $P<0.05$ vs before season

expression of epithelial ICAM-1 was seen among those allergic patients using local corticosteroids when compared with the non-pollen season ($P<0.05$, Table 3).

The expression of VCAM-1 before provocation during non-pollen season, did not differ between allergic patients and controls, but was significantly lower than that of E-selectin and ICAM-1 during non-pollen season ($P<0.001$), as well as during provocation and natural season ($P<0.001$). During allergen provocation all patients as a group, regardless of duration of provocation, had a significant increase in the expression of VCAM-1 compared with controls ($P<0.01$), as well as levels before provocation ($P<0.01$). After only 3 days of provocation a marked increase in VCAM-1 was observed among the allergic patients, however, it failed to reach significance. Later during the provocation (after 8 and 13 days) the increase was significant, even when taking into account analysis of subgroups.

Corticosteroid treatment induced differences in VCAM-1 expression, as was the case of epithelial ICAM-1. Allergic patients not using local corticosteroids had, as during provocation, increased expression of VCAM-1 during natural pollen season ($P<0.05$; Table 4; Fig. 2). However, patients using corticosteroids did not have any increase in VCAM-1 during pollen season compared with non-pollen season.

The data included in Tables 2–4 includes subgroup analysis concerning length of provocation and medication during season only when significant differences were obtained.

Discussion

Circulating T-lymphocytes can be separated into two major populations, naive and memory where the latter have been exposed to antigen. These two pools of lymphocytes differ in several aspects, including their route of circulation, immunophenotype including different expression of adhesion molecules and in cytokine production on activation. Naive T-lymphocytes mainly circulate between blood and secondary lymphoid organs (lymph nodes, Peyer's patch and tonsils) while memory lymphocytes will also circulate into non-lymphoid or so called tertiary lymphoid tissues (skin and mucosa [31, 39]). Differences in adhesion molecule expression between lymphocyte subpopulations as well as the target-tissue expression of endothelial ligands constitute the molecular basis for differences in lymphocyte trafficking.

The regulatory mechanisms of endothelial adhesion molecule expression have been extensively studied in in vitro systems. E-selectin expression on endothelial cells is upregulated due to transcriptional events induced by cytokine stimulation. In vitro, human umbilical vein endothelial cells (HUVEC) express E-selectin 30 min after tumour necrosis factor (TNF)-α or interleukin-1 (IL-1) stimulation. The E-selectin expression declines within 24 h in spite of continuous stimulation [35, 36]. However, the expression on endothelium refractory to IL-1 can be reinduced by stimulation by TNF-α and vice versa [35]. Furthermore, TNF-α induced E-selectin expression in vitro can be sustained by costimulation by interferon (IFN)-γ [5]. Cytokines will also increase HUVEC expression of ICAM-1 [36] and induce VCAM-1 [37, 45], endothelial adhesion molecules involved in the third step of the adhesion. This process is slower, compared with E-selectin, but the expression of ICAM-1 and VCAM-1 is more persistent [21].

Endothelial E-selectins will interact with ligands related to sialyl Lewis^x and Lewis^a tetrasaccharaides expressed by certain T cells. There are conflicting data in vitro concerning whether adhesion of memory T lymphocytes to E-selectin is subset restricted [34] or an activation independent event [12, 25, 38]. Adhesion of activated lymphocytes to activated endothelial cells in vitro is increased by some chemokines, RANTES, macrophage inflammatory protein (MIP)-1α and MIP-1β [42]. The expression of integrins, ligands to endothelial adhesion molecules of the superimmunoglobulin gene family, differs between subpopulations of lymphocytes and is also dependent upon the activation state. T memory cells express increased levels of both $\alpha_L\beta_2$ (LFA-1), the ICAMs ligand, as well as $\alpha_4\beta_1$ (VLA-4), the VCAM-1 ligand compared to naive cells [39]. Furthermore, the avidity of integrins is activation-dependent [10, 27].

These in vitro data suggest a number of different regulatory mechanisms in the selective circulation and recruitment of lymphocyte in vivo, an event which could be regulated at any of the steps in the adhesion process [31]. We found rather extensive expression of E-selectin in the nasal mucosa which together with similar findings in bronchi indicate a constitutively in vivo expression of E-selectin in airway mucosa [1]. In our provocation study, repetitive short time allergen exposure did not induce increased E-selectin expression in allergic nasal mucosa, or in asthmatic bronchial mucosa [1]. Only during continuous allergen provocation was E-selectin expression increased as demonstrated in Fig. 1. This has also been indicated in a study comprising symptomatic perennial allergic patients [29]. Although similar in character, the extent of the expression in that study differed from our own and others [1] which are probably due to differences in methodology.

In contrast to respiratory mucosa, E-selectin expression in uninflamed skin is low, while in allergic dermatitis there is increased expression of E-selectin [22, 24]. Although all memory T helper cells seems to adhere to E-selectin [12, 25, 38], the expression of an E-selectin ligand, HECA 452, is restricted to skin homing T memory lymphocytes [32, 33].

Differences in the expression of homing associated antigens such as HECA 452 seem to be dependent upon the actual site of T cell activation and phenotype switch. Peripheral lymph node memory T cells express HECA 452 epitope to a significantly higher extent than tonsils or Peyer's patches [30]. The mechanisms behind these differences are unknown but the local expression of cytokines could certainly be important [30].

We found no differences in ICAM-1 expression of endothelial cells during either provocation or the pollen season. Similar results have been shown in extrinsic (allergic) asthmatic patients [1]. However, in perennial allergics immunohistochemical data from nasal biopsies show low but increased, despite the use of plastic embedded material, should reach ICAM-1 positivity [29]. ICAM-1 expression is not only limited to endothelial cells, but is also expressed by lymphocytes and epithelial cells [8]. Our study thus confirms earlier findings of increased epithelial ICAM-1 in the allergic state [1] and demonstrates decreased expression by treatment with local corticosteriod.

In our opinion, the most interesting result of this study was the increased VCAM-1 expression during the allergen provocation study among allergic patients and in non-corticosteroid treated patients during the pollen season. We have confirmed earlier indications [1, 29] of increased VCAM-1 expression during allergen challenge to the respiratory mucosa. This pronounced increase in VCAM-1 expression is likely to be due to an effect upon endothelial cells of an interaction between locally synthesised cytokines. In vitro data reveals differences in the effect of costimulation by cytokines upon endothelial adhesion molecule expression. IL-1 as well as TNF-α induce VCAM-1 expression on HUVEC [37, 45]. However, IL-4 enhances the effect of these cytokines upon VCAM-1 expression whilst IFN-γ does not [26, 43, 44]. Interestingly, local IL-4 synthesis, of which T cells are the main source [46], is increased during the allergic reaction [6, 20], whilst IFN-γ is not [6], a cytokine pattern associated with memory T helper cells [4]. Furthermore,

VCAM-1 expression on dermal endothelial cell in vitro is not affected by IL-4 which implies differences in cytokine responsiveness between endothelial cells from different sites [41]. This indicates that VCAM-1 interactions with integrins could be a putative selective homing mechanism for T-memory lymphocytes to the mucosal tissues. Interactions between $\alpha_4\beta_1$ (VLA-4) may thus constitute an important homing mechanism for T memory lymphocytes, as demonstrated in Fig. 2. Interestingly, $\alpha_4\beta_1$ (VLA-4) is also expressed on other cells including eosinophils, which are increased within the mucosa during the allergic reaction. The scope of the study was to determine mechanisms for lymphocyte homing, thus an evaluation of α_4 subunit reactivity was not performed. Furthermore, an additional lymphocyte ligand for endothelial VCAM-1 has been reported, the $\alpha_4\beta_7$ integrin [16]. The mucosal lymphocyte associated antigen (MLA) HML-1 [2] is related to β7 integrin subunit expression [28], and MLA+ memory T cells express the $\alpha_4\beta_7$ integrin at high density whilst the $\alpha_4\beta_1$ (VLA-4) expression is low [33]. MLA+ T cells of the memory phenotype are present at mucosal sites [39] whilst their role during the allergic reaction remains to be elucidated. However, we have recently demonstrated the presence of MLA+ cells in the normal and allergic nasal mucosa [14, 17].

We have shown that the allergic reaction is accompanied by T cell activation and phenotype switch towards CD4+ memory T cells in peripheral blood [18]. Furthermore there is an accumulation of memory T cells in the allergic nasal mucosa [13, 19] and a local, allergen induced, synthesis of IL-4 during the allergic reaction [20]. The pronounced increase in VCAM-1 expression reported in the present study is likely an effect of the local cytokine panorama. VCAM-1 interaction with the appropriate lymphocyte integrins may constitute the molecular basis for this T lymphocyte mucosal homing. Furthermore, and importantly in terms of therapeutic implications, we have now been able to demonstrate further effects of local corticosteroid treatment. Steroids not only decrease IL-4 mRNA expression per se [20] but also affect homing process events [19] by inhibiting an IL-4 induced amplification of the expression of VCAM-1.

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