

VCAM-1 expression, eosinophil infiltration, and pharmacological modulation in rat allergic airway inflammation¹

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KEY WORDS vascular cell adhesion molecule-1; eosinophils; inflammation; neurokinin-1 receptors; dexamethasone

ABSTRACT

AIM: To determine vascular cell adhesion molecule-1 (VCAM-1) expression and eosinophil infiltration in the lungs of the rats with allergic airway inflammation, and their possible modulation by dexamethasone and neurokinin-1 receptor antagonist SR140333. **METHODS:** Sensitized rats were challenged with inhalation of 1 % ovalbumin aerosol. Protein expression of VCAM-1 in the lungs and eosinophil infiltration around bronchi in different groups were determined 24 h after challenge. SR140333 (0.01 and 0.10 mg/kg) and dexamethasone (0.50 mg/kg) were injected ip, twice a day, for 3 d before challenge. **RESULTS:** The expression of VCAM-1 was increased in the sensitized rat lungs as compared with the non-sensitized rats ($P < 0.05$). The increment was inhibited by pretreatment with dexamethasone ($P < 0.05$), but not with SR140333 ($P > 0.05$). On the other hand, antigen challenge in sensitized rats evoked eosinophil infiltration ($P < 0.05$) but no further increase in VCAM-1 expression ($P > 0.05$). Furthermore, SR140333 inhibited eosinophil infiltration ($P < 0.05$) but had no effect on VCAM-1 expression ($P > 0.05$), whereas dexamethasone inhibited both responses ($P < 0.05$). **CONCLUSION:** The expression of VCAM-1 increases during antigen sensitization in rat lungs, and dexamethasone and SR140333 may inhibit allergic airway inflammation in different mechanisms.

INTRODUCTION

Eosinophils are pivotal effector cells in the development of airway inflammation in allergic bronchial asthma. Migration of eosinophils from the circulation to the lungs is regulated by many factors, including cytokines and adhesion proteins. Vascular cell adhesion molecule-1 (VCAM-1), that is widely expressed in vascular endothelium^[1-4], airway epithelium^[5,6], fibroblasts^[7,8], and airway smooth muscle cells^[11] in the lung, regulates eosinophil migration via interrelating with the ligand termed very late appearing antigen-4 (VLA-4). VCAM-1 expression increases in bronchial asthma^[10,11]. Some anti-asthma drugs, such as glucocorticoids^[8,12], formoterol (a long-acting β_2 receptor agonist)^[8], and sodium cromoglycate^[13], inhibit VCAM-1 expression. These findings suggest that inhibition of VCAM-1 expression might be one of the mechanisms of anti-asthma therapies. Recently, tachykinin NK-1 receptor antagonists were shown to have anti-inflammatory and anti-asthma effects on experimental animals^[14-16], but their effect on VCAM-1 expression has not been investigated. Therefore, this study aims to determine the expression and pharmacological modulation of VCAM-1 in the lungs of rats with allergic airway inflammation, with emphasis on the possible effects of SR140333, (S)-1-(2-[3,4-dichlorophenyl-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl)-4-phenyl-1-azoniabicyclo[2.2.2]octane·chloride, a selective NK-1 receptor antagonist, on VCAM-1 expression and eosinophil infiltration.

MATERIALS AND METHODS

Rats Sprague-Dawley rats of either sex weighing 110 g \pm 10 g ($n = 120$) were obtained from the Laboratory Animal Center of Zhejiang University Medical School (Grade II, certificate No 22-9601018, conferred by Zhejiang Medical Laboratory Animal Administration Committee).

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Reagents Ovalbumin (Grade V, Sigma Chemical Co, USA); dexamethasone sodium phosphate (Xin-Chang Pharmaceutical Factory, Zhejiang); SR140333 (Sanofi Co, France); goat polyclonal antibody against VCAM-1 (Santa Cruz Biotechnology Inc, USA); donkey anti-goat IgG conjugated to horseradish peroxidase (Hua-Mei Biotech Co, Shanghai). Other reagents were commercial products with analytic purity.

Sensitization, challenge, and drug treatments

Aluminum hydroxide gel (10 % in sterile saline, w/v) was freshly prepared, and 1 g/L ovalbumin was added into the gel. In each rat, the mixed gel was injected sc at six sites in the neck, waist, groin, and paws, 0.1 mL for each site. An additional 0.4 mL was injected ip. The non-sensitized control rats were injected with ovalbumin-free aluminum hydroxide gel in the same way. Both sensitized and non-sensitized rats were challenged by inhalation of either aerosolized 1 % ovalbumin in sterile saline or saline alone for 15 min on d 14 after sensitization. Dexamethasone (0.50 mg/kg), SR140333 (0.01 and 0.10 mg/kg) or saline (1.0 mL/kg) were injected intraperitoneally, twice a day, from d 12 to d 14. All experiments were done at room temperature (25 °C ± 1 °C).

Morphological examination Twenty-four hours after challenge, the rats were anesthetized with sodium pentobarbital (30 mg/kg, ip), exsanguinated via the abdominal aorta, and the lungs were isolated. The right lobes were kept at -70 °C for protein extraction, and the left lobes were used to prepare frozen sections. Sections 5-μm thick alongside the main intrapulmonary bronchi were cut on a cryostat (CM 1900, Leica, Germany), placed on slides coated with poly-L-lysine, and stained with hematoxylin-eosin. Eosinophil infiltration around the bronchi was measured using an image analyzer (AnalyPower 2000-1.0, Zhejiang University). Five bronchi with diameters of 0.8 - 1.8 mm were randomly selected from each section and eosinophils around bronchi were counted by an observer who was blind to the source of the samples.

Western blot The right lobes of the lungs were homogenized twice in ice-cold Tris · HCl 10 mmol/L buffer, pH 7.4, containing sucrose 320 mmol/L. The homogenate was centrifuged at 700 × g for 10 min at 4 °C. Then the supernatant was centrifuged at 37 000 × g for 40 min at 4 °C. The precipitation was resuspended in Tris · HCl 10 mmol/L, pH 7.4. After measuring protein concentration, a 15 μg sample of

protein was separated by 7.5 % SDS-polyacrylamide gel electrophoresis, then electrotransferred to a Nitrocellulose membrane. The membranes were reacted with anti-VCAM-1 polyclonal antibody (1:500) overnight at 4 °C, washed twice, then incubated with secondary antibody (1:400) for 2 h at room temperature. An ECL system (Amersham, Buckinghamshire, UK) was used to detect the immunoreaction. Quantitative analysis of gray density of the bands was performed on a laser densitometer (Ultrascan XL, Pharmacia LKB Co, Sweden). A protein sample from normal rat lungs was used as standard, and the relative expression of VCAM-1 was calculated as the ratio of tested/standard sample densities.

Statistical analysis All values were presented as $\bar{x} \pm s$. Significance of differences was determined by student's *t*-test. $P < 0.05$ was considered significant.

RESULTS

VCAM-1 expression Under control conditions (non-sensitized and challenged with saline), a constitutive expression of VCAM-1 was observed (1.1 ± 0.4 , Fig 1). Ovalbumin challenge of non-sensitized rats did not change VCAM-1 expression ($P > 0.05$, Fig 1). Unexpectedly, primary sensitization of rats with ovalbumin enhanced VCAM-1 expression (1.4 ± 0.3) in the lungs compared with that from non-sensitized rats (about a 29 % increment, $P < 0.05$, Fig 1), while ovalbumin challenge, at d 14 after sensitization, did not further increase the VCAM-1 expression ($P > 0.05$) in the lungs of sensitized rats (Fig 1).

Eosinophil infiltration in non-sensitized and sensitized rats In non-sensitized rat lungs, only a few eosinophils were found around the bronchi regardless of challenge with saline or ovalbumin. In contrast, challenge of the sensitized rats with ovalbumin but not saline enhanced eosinophil infiltration around the bronchi (Tab 1, Fig 2).

Effects of dexamethasone and SR140333 on VCAM-1 expression and eosinophil infiltration

Dexamethasone (0.50 mg/kg), but not SR140333 (0.01 and 0.10 mg/kg) inhibited the enhanced VCAM-1 expression in the lungs of ovalbumin-sensitized rats challenged with saline or ovalbumin (Tab 2). In contrast, both dexamethasone and SR140333 attenuated eosinophil infiltration around the bronchi, a marker of airway allergic inflammation induced by ovalbumin sensitization plus ovalbumin challenge (Tab 2).

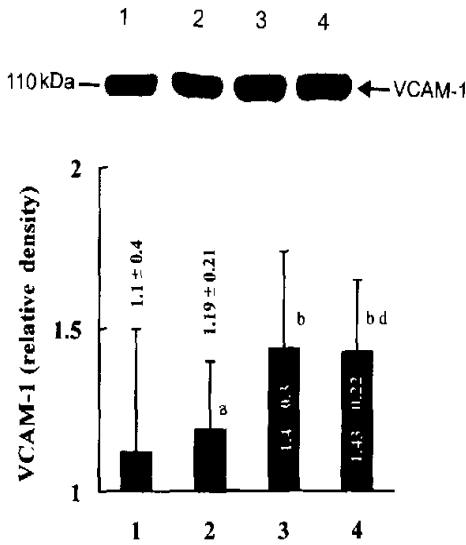


Fig 1. Western blot analysis of the expression of VCAM-1 in rat lungs. 1: non-sensitized and challenged with saline; 2: non-sensitized and challenged with ovalbumin; 3: sensitized and challenged with saline; 4: sensitized and challenged with ovalbumin. $n = 10$. $\bar{x} \pm s$. ^a $P > 0.05$, ^b $P < 0.05$ vs 1. ^d $P > 0.05$ vs 3.

Tab 1. Effects of ovalbumin sensitization and challenge on eosinophil infiltration around the bronchi in rats. $n = 10$. $\bar{x} \pm s$. ^a $P > 0.05$, ^b $P < 0.05$ vs non-sensitized and challenged with saline.

Sensitization	Challenge	$10^{-3} \times$ Eosinophil infiltration/ cells per μm^2
Saline	Saline	0.02 ± 0.06
Saline	Ovalbumin	0 ± 0^a
Ovalbumin	Saline	0.3 ± 0.7^a
Ovalbumin	Ovalbumin	2.6 ± 0.6^b

DISCUSSION

In this study, we unexpectedly found that the expression of VCAM-1 was enhanced in the lungs of ovalbumin-sensitized rats, even in the absence of antigen challenge. Furthermore, antigen challenge did not increase the enhanced VCAM-1 expression. This result suggests that the enhanced VCAM-1 expression is associated not only with antigen-induced airway eosinophilic inflammation as generally believed, but also with the initial antigen sensitization, an early stage required for allergic airway inflammation. Antigen

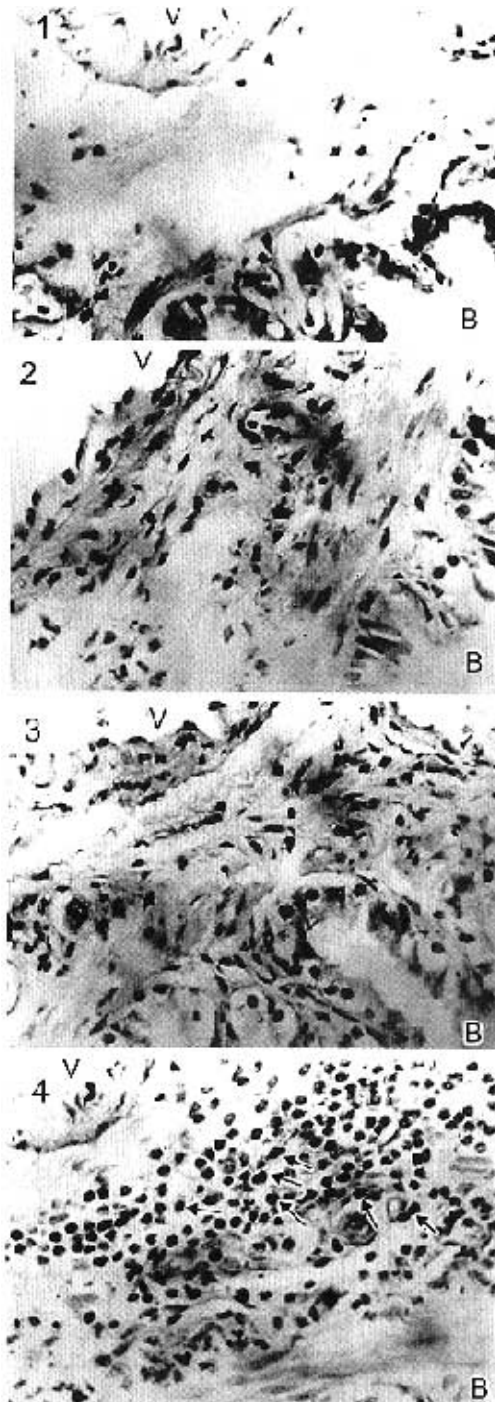


Fig 2. Histological examinations of rat lungs. Lung sections stained with hematoxylin and eosin. 1: non-sensitized and challenged with saline; 2: non-sensitized

and challenged with ovalbumin; 3: sensitized and challenged with saline; 4: sensitized and challenged with ovalbumin. The arrowheads represent some eosinophils. V: vessel, B: bronchi. $\times 400$.

sensitization in airway allergy is generally considered to depend on the production of antigen-specific IgE and its attachment to specific receptors on airway mast cells, which set the stage for inflammatory responses on subsequent antigen exposure^[19]. From our results, we propose that the enhanced expression of VCAM-1 and perhaps other adhesion molecules may play an important role in antigen sensitization states. However, the specific intrapulmonary cells responsible for the enhanced expression of VCAM-1 upon antigen sensitization remain to be defined.

Another interesting result obtained from this study is that SR140333, a selective NK-1 receptor antagonist, inhibited eosinophil infiltration in the airways but did not affect the expression of VCAM-1 in the sensitized rat lungs. In contrast, the glucocorticoid dexamethasone inhibited both responses. This finding suggests that there are at least two different mechanisms through which anti-inflammatory drugs can inhibit eosinophil infiltration in allergic airway inflammation. One is the action of glucocorticoids, long-acting β_2 receptor agonists, and sodium cromoglycate, at least in part, by inhibiting VCAM-1 expression. This notion is supported by our results (Tab 2) and previous reports^[8,12,13]. A new possibility is that the response induced by NK-1 receptor antagonists is mediated by VCAM-1-independent pathways. Hoshino *et al*^[18] reported that SR140333 reduced interleukin-17-induced neutrophil recruitment into the rat airway, but its effect on eosinophil recruitment

(dependent on adhesion molecules and cytokines) is unknown. Since other adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1)^[2,5,8,9,13] are expressed in the lungs, and VLA-4 plays a role in eosinophil infiltration in the airways^[10], the target molecule (s) of SR140333 should be identified.

In summary, this study shows for the first time that antigen sensitization itself increases the expression of VCAM-1 which is not further enhanced by antigen challenge. Our results also suggest that dexamethasone inhibiting airway eosinophil infiltration may be in part mediated by inhibition of VCAM-1 expression, a possible mechanism not shared by NK-1 receptor antagonist.

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Tab 2. Effects of dexamethasone and SR140333 on VCAM-1 expression and eosinophil infiltration around the bronchi in sensitized rats challenged with saline or ovalbumin. $n = 10$. $\bar{x} \pm s$. ^a $P > 0.05$, ^b $P < 0.05$ vs control. ^c $P < 0.05$ vs saline challenge.

Challenge	Drugs/mg·kg ⁻¹	VCAM-1 (relative density)	10 ⁻³ × Eosinophil infiltration/cells per μm^2
Saline	Control (saline 1.0 mL/kg)	1.44 ± 0.25	0.09 ± 0.17
	Dexamethasone 0.50	1.1 ± 0.3 ^b	0 ± 0 ^a
	SR140333 0.01	1.52 ± 0.22 ^a	0.06 ± 0.14 ^a
	SR140333 0.10	1.5 ± 0.3 ^a	0.10 ± 0.10 ^a
Ovalbumin	Control (saline 1.0 mL/kg)	1.56 ± 0.25	2.6 ± 0.7 ^c
	Dexamethasone 0.50	1.2 ± 0.4 ^b	0.09 ± 0.29 ^b
	SR140333 0.01	1.62 ± 0.27 ^a	1.3 ± 0.3 ^b
	SR140333 0.10	1.65 ± 0.28 ^a	0.8 ± 0.4 ^b

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大鼠过敏性气道炎症中 VCAM-1 表达、嗜酸细胞浸润和药理调节¹

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关键词 血管内皮细胞粘附分子-1; 嗜酸细胞; 炎症; 速激肽-1 受体; 地塞米松

目的: 探讨过敏性气道炎症大鼠肺内 VCAM-1 表达和嗜酸细胞浸润, 以及速激肽 NK-1 受体拮抗剂 SR140333 和地塞米松的可能调节方式。 **方法:** 致敏大鼠以 1% 卵白蛋白气雾吸入攻击后 24 小时, 测定 VCAM-1 在不同组别大鼠肺内的表达和支气管周围嗜酸细胞浸润。 抗原攻击前 3 天, 每天 2 次腹腔注射 SR140333 (0.01, 0.10 mg/kg) 或地塞米松 (0.50 mg/kg)。 **结果:** 与未致敏大鼠相比 VCAM-1 在致敏大鼠肺内表达增加 ($P < 0.05$), 预先用地塞米松处理可抑制其增加 ($P < 0.05$), SR140333 无此作用 ($P > 0.05$)。 此外, 抗原攻击致敏大鼠促使支气管周围嗜酸细胞浸润, 但不能进一步增加 VCAM-1 的表达 ($P > 0.05$)。 SR140333 抑制嗜酸细胞浸润 ($P < 0.05$), 但不影响 VCAM-1 表达 ($P > 0.05$); 地塞米松则抑制这两种反应 ($P < 0.05$)。 **结论:** VCAM-1 表达在抗原致敏后增加, 地塞米松和 SR140333 抑制大鼠过敏性气道炎症的机制可能不同。

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