

Original article

The effect of a single inhaled dose of a VLA-4 antagonist on allergen-induced airway responses and airway inflammation in patients with asthma

Adhesion molecule very late antigen-4 (VLA-4) is implicated in the recruitment and activation of inflammatory cells in asthma, including eosinophils, T cells and mast cells. VLA-4 antagonists have been proposed as a new anti-inflammatory treatment modality for asthma. Therefore, we investigated whether a single inhaled dose of VLA-4 antagonist GW559090X could protect against allergen-induced changes in airway responses and airway inflammation in patients with asthma. We performed a randomized, double-blind, three-way crossover study with single inhaled doses of 3 mg of GW559090X, 500 µg of fluticasone propionate (FP) or placebo in 15 patients with mild intermittent asthma, controlled with short-acting β_2 -agonists only. All patients developed a late asthmatic response (LAR) after allergen inhalation during screening. Study medication was administered 30 min prior to allergen challenge. Pre-dose and 24 h post-dose PC₂₀ methacholine and levels of exhaled nitric oxide (eNO) were determined. At the given dose, VLA-4 antagonist GW559090X did not attenuate the early asthmatic response (EAR) when compared with placebo: mean AUC_{0–2h}($\pm$ SEM)(%fall h): 27.2 $\pm$ 3.7 and 21.9 $\pm$ 3.0 respectively (P = 0.33); nor the LAR: mean AUC_{3–8h}($\pm$ SEM)(%fall h): 98.8 $\pm$ 12.9 and 94.8 $\pm$ 6.8 respectively (P = 0.84). However, pretreatment with FP did attenuate both EAR and LAR when compared with placebo: mean AUC_{0–2h} 11.6 $\pm$ 3.3 (P = 0.024) and mean AUC_{3–8h} 6.3 $\pm$ 7.6 (P < 0.001). None of these treatments had an effect on allergen-induced changes in airway hyper-responsiveness or eNO levels. These findings suggest that VLA-4 may not play a major role in allergen-induced airway responses and inflammation in asthma.

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Asthma is a chronic disease, characterized by episodic chest symptoms and variable airways obstruction (1). In many cases asthma is associated with atopy exhibiting IgE-dependent allergic reactions (2). Inhalation of allergens by sensitized patients with asthma, results in acute airway narrowing, the early asthmatic response (EAR) (3). Approximately 50% of these subjects also develop a late asthmatic response (LAR), which occurs 3–12 h after exposure to an allergen (4). It is the LAR that is characterized by increased airway hyperresponsiveness (AHR) (5) and infiltration of inflammatory cells such as eosinophils, mast cells, and Th2 lymphocytes, into the airways (6).

Adhesion molecules are implicated in the events mediating recruitment and activation of leukocytes in inflamed tissue. One subset of adhesion molecules is the integrin family that consists of heterodimeric molecules composed of two noncovalently linked subunits alpha and beta. Key members of this family are the α_4 -integrins, $\alpha_4\beta_1$ [very late

antigen (VLA)-4 or CD49d/CD29] and $\alpha_4\beta_7$ (CD49d/CD7), which are involved in leukocyte-endothelial interactions. They are expressed by monocytes, T lymphocytes, eosinophils, and basophils, and interact with vascular cell adhesion molecule-1 (VCAM-1) located on endothelial cells, and with fibronectin that is present in the tissue interstitium. It has been shown that the expression of VCAM-1 on endothelium is upregulated during allergic inflammatory reactions (7, 8), thereby facilitating migration of α_4 -expressing leukocytes into the tissue.

The importance of VLA-4 for the recruitment of eosinophils has been suggested from several studies in animal models of allergic inflammation, in which pretreatment with anti- α_4 integrin monoclonal antibodies resulted in a reduction of allergen-induced airway eosinophilia and AHR (9–11), expanding the development of drugs targeting this mechanism.

Therefore, the primary objective of this study was to investigate the anti-inflammatory effects of pre-treatment

with a single inhaled dose of a new VLA-4 antagonist GW559090X, on the LAR after allergen challenge in patients with mild asthma. The secondary objectives of this study were to measure its effect on the EAR, AHR and levels of exhaled nitric oxide (eNO).

Material and methods

Subjects

Non-smoking male patients with mild intermittent asthma (1) aged 18–55 years were included in this study if they showed: a baseline forced expiratory volume in 1 second (FEV₁) ≥ 70% of predicted, a provocative concentration of methacholine chloride causing a 20% fall in FEV₁ (PC₂₀) < 8 mg/ml, a positive skin prick test (SPT) for house dust mite (HDM) allergen, an EAR (≥ 20% fall in FEV₁ from baseline, 0–2 h post-allergen) and a LAR (≥ 15% fall in FEV₁ from baseline, 3–8 h post-allergen) following inhaled HDM extract during screening. The patients were clinically stable, only used β₂-agonists on demand, and had no history of recent respiratory tract infection within 4 weeks from the start of the study. Corticosteroid therapy was not allowed within 8 weeks prior to screening, and/or during the study. The Medical Ethics Committees of the Leiden University Medical Center and the University Medical Center Utrecht granted approval for the study. All subjects gave written informed consent prior to the study.

Study design

The study had a two-center randomized, double blind, placebo-controlled, three-way crossover design (Fig. 1) to investigate the effects of pretreatment with a single inhaled dose of GW559090X on allergen-induced airway responses in patients with mild asthma. A treatment period with fluticasone propionate (FP) was included as a positive control.

On screening day 1, baseline measurements of FEV₁, a SPT for nine common airborne allergen extracts including HDM allergen and a methacholine challenge were performed. On screening day 2, a multi-dose SPT and an allergen challenge with HDM were performed. The interval between screening allergen test and the first dose of study medication was 21–35 days.

The three treatment periods consisted of three consecutive days each and were separated by a washout interval of 21–42 days. The PC₂₀ methacholine values determined on day 0 were within 1.5 doubling doses (DD) during each treatment period. On day 1, subjects were supplied with two diskhalers A and B. By taking one inhalation from each diskhaler either 1 × 3 mg of GW559090X plus 1 × placebo, 2 × 250 µg of FP, or 2 × placebo was administered. Thirty minutes after dosing, an allergen challenge was performed starting with diluent inhalation. Subsequently, the three serial doubling concentrations of HDM extract causing an EAR during screening were inhaled. Measurements of eNO were performed pre-dose, 30 min and 8 h post-dose. Blood samples were taken to determine plasma levels of GW559090X 0–8 h post-dose. On day 2 of each period, safety measurements, eNO levels, FEV₁ and PC₂₀ methacholine were determined 24 h post-dose. Following the final dose of study medication, a poststudy safety visit was arranged within 7–10 days.

Study medication and dosing rationale

GW559090X is a high-affinity ligand of the human integrin α₄β₁ (K_i 400 pM) that antagonizes α₄β₁- and α₄β₇-dependent cell adhesion (IC₅₀, 3 and 30 nM respectively). It is highly selective with > 1000-fold selectivity with respect to non-α₄-integrins and > 10 000-fold with respect to β₂-integrin lymphocyte function antigen-1 (LFA-1). In ovalbumin-sensitized guinea pigs, GW559090X is substantially (30- to 100-fold) more potent inhibiting lung eosinophilia and AHR after antigen challenge when dosed intratracheally compared with subcutaneous application. This suggests a site of action in the lung and confirms that topical dosing to the lung is appropriate from a safety perspective to maximize anti-inflammatory activity with minimum systemic exposure. A single intratracheal dose of GW559090X (2 µg/kg) given 0.5 h before antigen challenge substantially attenuated lung eosinophilia (75%) and AHR (75%) measured 24 h post-challenge in guinea pigs (GlaxoSmithKline, unpublished data). These data support the potential for once daily dosing in man. Based on effectivity studies in guinea pigs and rats a dose of 3 mg GW559090X was selected for this study as this was expected to cover the anticipated clinical dose (0.5–3 mg). This dose has been shown to be safe and well tolerated in 16 healthy subjects and 16 patients with mild asthma after inhalation (GlaxoSmithKline, unpublished data).

The dose of 500 µg of FP was selected as a dose that was expected to have a clear effect on the LAR (12).

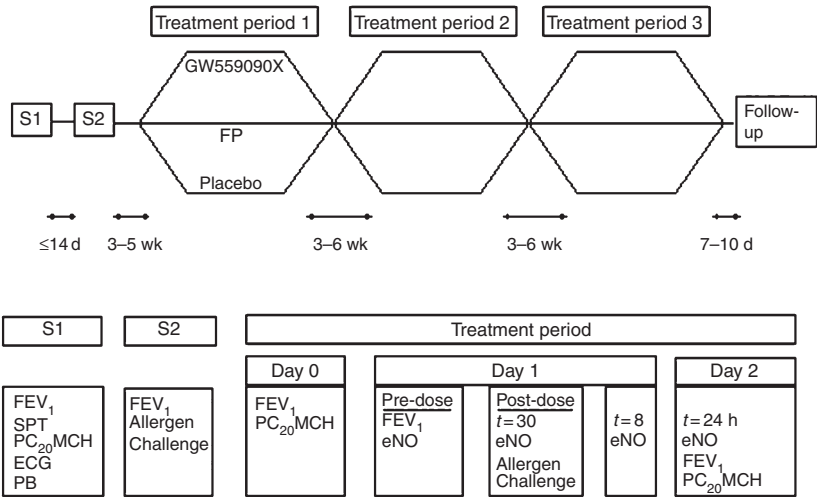


Figure 1. Study design.

Methacholine inhalation test

Maximal flow–volume curves were recorded to determine FEV₁ (13). Methacholine chloride challenges were performed by standardized methods (14), using Provocholine® (Methapharm Inc., Brantford, ON, Canada) in 0.9% sodium chloride containing 0.4% phenol.

Allergen inhalation test

Allergen challenges were performed according to a standardized protocol (14–16). PC₂₀ allergen was predicted from PC₂₀ methacholine and skin sensitivity, derived from a multi-dose SPT, according to Cockcroft's method (15). Purified aqueous allergen extract of *Dermatophagoides pteronyssinus* (SQ 503; Vivodiagnost, ALK Abello, Nieuwegein, The Netherlands) was prepared from the same production batch as the multi-dose SPT.

Exhaled NO measurements

Exhaled NO was measured online with an expiratory flow of 100 ml/s against an expiratory resistance of 5 cm H₂O using a chemiluminescence analyzer (17). Exhaled nitric oxide concentrations were determined at a 10-s plateau and expressed as parts per billion (ppb). The mean value of three successive recordings was used for analysis.

Statistical analysis

It was calculated that 14 completed subjects were needed for at least 90% power to detect a 50% inhibition of the maximum %fall in FEV₁ at the two-sided 5% level of significance (18). Patient characteristics are presented as means with standard deviations (SD). PC₂₀ methacholine was log transformed prior to analysis and is expressed as geometric mean and SD in DD. Pharmacokinetic data are expressed as median and range. Other data are expressed as mean and standard error of the mean (SEM).

The magnitude of both LAR and EAR was expressed as the area under the curve (AUC) (%fall h) or as maximum %fall in FEV₁ from baseline (post-diluent). The AUC was calculated using the linear trapezoidal method. Differences between treatments were analyzed using a randomized block design with treatment as factor, subject as block factor and with baseline value and treatment period as covariates. Tests were performed at the two-sided 5% significance level.

Results

Patient characteristics

Fifteen patients with mild intermittent asthma (1) were included and completed the study. Their baseline characteristics are shown in Table 1.

Safety

Treatment with GW559090X was generally well tolerated. No serious adverse events occurred during this trial. During the period from screening to final visit, 43 adverse events (AE) were recorded of which dyspnea and headache occurred most frequently (20 and 12 respectively). None of the reported AE was thought to be related to treatment. During the treatment periods (day 0 and day

Table 1. Patient characteristics

	Mean	SD
Age (years)	23.73	5.06
Duration of asthma (years)	19.31	5.25
FEV ₁ (% predicted)	90.18	10.44
PC ₂₀ Mch (mg/ml)*	0.56	2.27
EAR (0–2 hours: maximum %fall in FEV ₁ from baseline)	26.18	5.63
LAR (3–8 hours: maximum %fall in FEV ₁ from baseline)	34.12	10.88
EAR (0–2 hours: AUC, %fall h)	20.69	10.97
LAR (3–8 hours: AUC, %fall h)	100.04	46.54

*Geometric mean and SD in doubling doses. FEV₁, forced expiratory volume in 1 second; EAR, early asthmatic response; LAR, late asthmatic response.

1) no AE were reported in the FP group, two AE in the placebo group (dyspnea) and two AE in the GW559090X groups (dyspnea and headache). There were no clinically significant changes in physical examination, ECG, serum hematology, or clinical chemistry.

Pharmacokinetic profile

Pharmacokinetic data were collected on day 1. The median T_{max} was reached 0.73 h (0.12–1.18) post-dose. The median maximum concentration of GW559090X and the area under the plasma concentration–time curve (0–4 h) were 4.16 ng/ml (2.17–8.14) and 9.96 ng h/ml (3.19–32.37) respectively.

Allergen-induced airway responses

At the given dose, the VLA-4 antagonist GW559090X did not attenuate the EAR to allergen in asthmatic patients in terms of maximum %fall FEV₁ from baseline (Fig. 2) or

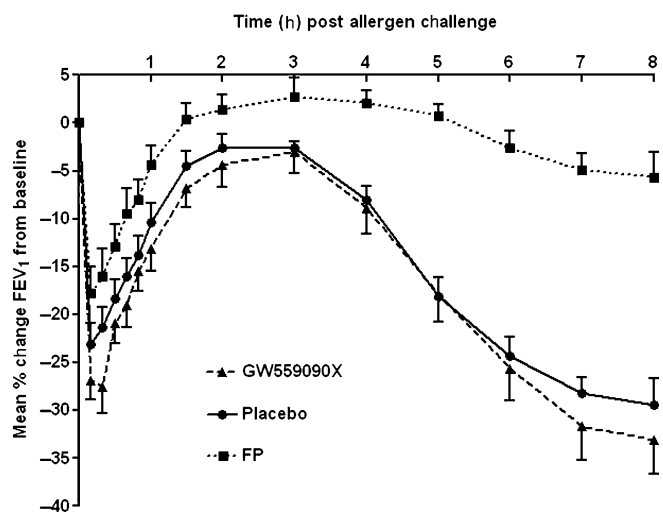


Figure 2. Allergen-induced responses as mean ($\pm$ SEM) from 0 to 8 h post-allergen after pre-treatment with placebo, GW559090X, or FP. The magnitude of the LAR (maximum %fall in FEV₁ from baseline) was significantly attenuated after treatment with FP compared with placebo ($P < 0.01$).

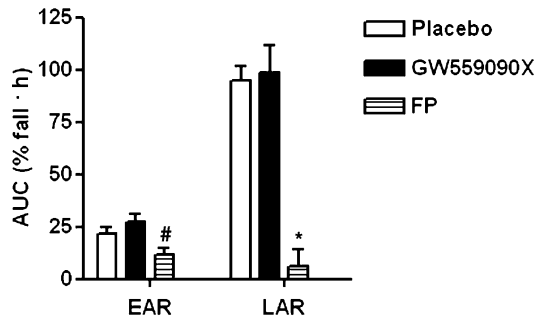


Figure 3. Early and late asthmatic response after pretreatment with placebo, GW559090X or FP expressed as mean AUC (%Fall h) ($\pm$ SEM). There was a significant decrease in EAR and LAR after pretreatment with FP when compared with placebo ($P = 0.024$ and $*P < 0.001$ respectively).

AUC (Fig. 3) when compared with placebo: maximum %fall FEV₁ GW559090X 30.1 ± 2.6 and placebo 24.1 ± 2.3 , $P = 0.06$; AUC_{0–2h} GW559090X 27.2 ± 3.7 %fall h and placebo 21.9 ± 3.0 %fall h, $P = 0.33$. Neither were there any differences between these treatment groups with respect to the magnitude of the LAR: AUC_{3–8h} GW559090X 98.8 ± 12.9 %fall h and placebo 94.8 ± 6.8 %fall h, $P = 0.84$; maximum %fall FEV₁ GW559090X 34.1 ± 3.6 and placebo 32.0 ± 2.4 , $P = 0.70$.

Pre-treatment with FP, however, did attenuate the LAR when compared with placebo (AUC_{3–8h} FP 6.3 ± 7.6 %fall h, $P < 0.001$; maximum %fall FEV₁ FP 7.0 ± 2.4 , $P < 0.001$). In terms of AUC it also reduced the EAR when compared with placebo (AUC_{0–2h} FP 11.6 ± 3.3 %fall h, $P = 0.024$).

Allergen-induced changes in airway responsiveness to methacholine

Airway hyperresponsiveness was significantly increased 24 h after allergen challenge in all groups (Fig. 4). The

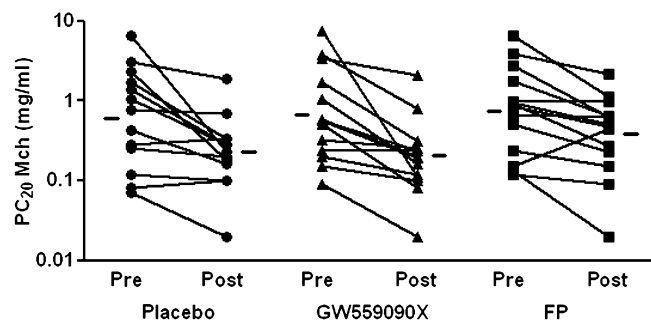


Figure 4. Airway hyperresponsiveness to methacholine predose (day 1) and 24 h post-dose (day 2) with placebo, GW559090X, or FP. The horizontal bars represent the geometric means. Airway responsiveness to methacholine was significantly enhanced after allergen challenge in all treatment groups ($P < 0.05$). However, the change of allergen-induced airway hyperresponsiveness was not significantly different between the treatment groups.

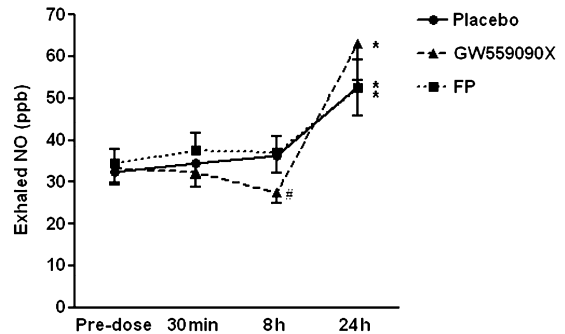


Figure 5. Exhaled nitric oxide before and after treatment ($t = 30$ min, 8, and 24 h) with placebo, GW559090X, or FP expressed as mean ppb ($\pm$ SEM). Levels of eNO were significantly increased in all treatment groups 24 h post-dose when compared with pre-dose ($*P < 0.05$). At 8 h after treatment with GW559090X, eNO was significantly decreased compared with pre-dose ($P = 0.01$). However, there were no significant differences in eNO levels at any time point between the three treatment groups.

PC₂₀ (mg mL⁻¹) decreased from 0.61 ± 2.19 to 0.22 ± 1.49 ($P = 0.01$) after placebo; from 0.66 ± 1.88 to 0.20 ± 1.50 ($P = 0.002$) after GW559090X, and from 0.75 ± 1.81 to 0.38 ± 1.64 ($P = 0.012$) after FP. There were no significant differences in the change of PC₂₀ (24 h post-dose minus pre-dose) between the treatment groups ($P = 0.11$).

Allergen-induced changes in exhaled NO

The allergen challenge induced a significant increase in eNO 24 h post-dose compared with pre-dose in all treatment groups (placebo $P < 0.01$; GW559090X $P < 0.01$; FP $P < 0.05$) (Fig. 5). Eight hours after dosing with GW559090X, the level of eNO was significantly decreased compared with pre-dose ($P = 0.01$). There were no significant differences between the three treatment groups at any of the time points when correcting for pre-dose values ($t = 30$ min $P = 0.27$; $t = 8$ h $P = 0.05$; $t = 24$ h $P = 0.37$).

Discussion

The present study shows that the treatment with a single inhaled dose of VLA-4 antagonist GW559090X could not attenuate allergen-induced airway responses or airway inflammation in a population of atopic asthma patients who were responsive to inhaled allergen. The LAR could, however, be inhibited by a single dose of FP, which confirms the findings of a previous study (12). These results implicate that adhesion molecule VLA-4 may not play a pivotal role in allergen induced airway responses and inflammation in asthma. In addition, we found that a high dose of FP could also attenuate the EAR measured

as AUC, but had no effects on allergen-induced changes in AHR and eNO levels.

Previous studies in a guinea-pig model have demonstrated that treatment with an antibody against VLA-4 effectively reduced ovalbumin-induced eosinophilia in bronchial tissue and BAL as well as infiltration of the bronchial wall by T lymphocytes (9, 19). However, results from similar studies using a rat model are controversial, either showing a partial reduction in eosinophil accumulation in BAL and lung, or no effect on these parameters (20, 21), whereas a significant reduction of early and late responses did occur (21). Although GW559090X effectively inhibited eosinophil recruitment and allergen-induced AHR in rat and guinea pig models of ovalbumin-induced lung inflammation (GSK, unpublished data), pre-treatment with this VLA-4 antagonist could neither attenuate the allergen-induced airway responses nor reduce a marker of airway inflammation in patients with asthma. These results indicate that animal models are of limited value predicting the complex process of leukocyte recruitment in humans. Our findings are in line with those from two recent studies investigating the effect of VLA-4 antagonists HMR 1031 and IVL745 on allergen-induced airway responses and inflammation in patients with mild-to-moderate asthma (22, 23).

Whereas a single dose of inhaled corticosteroid (ICS) could effectively reduce the LAR, no effect on the allergen-induced increase in airway responsiveness was observed in the present study. This interesting finding has also been described previously (24, 25). It has been shown that a temporal relationship exists between the inflammatory events occurring in the airways and the transient increase in AHR after allergen exposure. Both the influx of eosinophils and the increase in AHR precede the development of the LAR (26, 27). When treatment with ICS is continued throughout the allergen challenge and 24 h thereafter, it almost completely abolishes the LAR, airway eosinophilia and allergen-induced AHR (28). However, upon cessation of treatment 12 h prior to allergen challenge, the protective effects of ICS on sputum eosinophilia and allergen-induced AHR are lost rapidly, whereas the LAR is still inhibited (29). Apparently, the various components of the allergen-induced airway responses differ in their sensitivity to ICS. In the present study allergen exposure also caused an increase in eNO levels 24 h postdose, which is in agreement with earlier reports (30, 31). Although it has been shown that a single high dose of nebulized budesonide can decrease eNO levels within 6 h in children with acute asthma (32), a dose of 1000 µg of inhaled FP did not affect eNO levels 2–26 h after administration in mild stable asthma (33). In this study, a single dose of FP was also not sufficient to inhibit the allergen-induced increase of this marker of inflammation.

In our opinion, appropriate methodology and design were used to demonstrate a proof of concept of the mechanism for VLA-4 antagonist GW559090X in asth-

ma. It must be recognized that experimental allergen challenge does not reflect the real-life situation of patients with asthma who are not likely to encounter such high doses of allergen in daily life. However, the allergen challenge model is a validated technique and has been considered as a model system to study the mechanisms of asthmatic airways inflammation (14–16). Bronchial exposure to allergen can induce a LAR that is accompanied by local recruitment and activation of eosinophils (6) and lymphocytes (34). Therefore, this model can be used to assess the efficacy of new anti-inflammatory treatment modalities. In the present study, all subjects displayed a significant airway response to allergen challenge in terms of fall in FEV₁ during the LAR. Inclusion of a single dose of inhaled FP as a positive control established that the study population was indeed responsive to anti-inflammatory therapy. Moreover, it was estimated that the inclusion of 14 subjects would yield at least 90% power to detect a 50% inhibition of the LAR at the two-sided 5% level of significance (18). This is the approximate degree of inhibition observed after treatment with inhaled steroid (28), which is considered to be the gold standard anti-inflammatory therapy for treatment of asthma (1).

We expected that a daily dose of 3 mg of GW559090X would be therapeutically active in asthma. A dose-response study to determine the best dose for GW559090X in humans was not performed prior to this trial. However, it was estimated that lung concentrations over a 24-h period in man following a 3-mg inhaled dose would generally exceed those for a 100 µg/kg intratracheal dose to the rat, whereas it has been shown that a 5 µg/kg intratracheal dose given to a 200-g rat resulted in 70% inhibition of antigen-induced lung eosinophilia. The lack of effect of GW559090X in the present study may still be ascribed to a low bioavailability locally at the receptor site as plasma concentrations may not fully reflect the situation within the airways.

Can we explain the lack of effect of GW559090X in the present study from the general mechanisms that mediate leukocyte recruitment? The migration of leukocytes from the circulation into the lung tissue in response to allergen provocation involves tethering, rolling, and firm adhesion to the endothelial surface. These steps are controlled by cell surface receptor–ligand interactions that include the binding of leukocyte α 4-containing integrins with VCAM-1 and binding of leukocyte-expressed P-selectin glycoprotein ligand-1 (PSGL-1) with endothelial cell P-selectin (35). In allergic patients with asthma, it was shown that granulocytes express more PSGL-1 when compared with granulocytes from normal patients and that blocking PSGL-1 abolished granulocyte binding to immobilized P-selectin *in vitro* (36). These results suggest that the enhanced recruitment of eosinophils in atopic patients with asthma is due to, at least in part, increased PSGL-1 expression. A previous study showed that the administration of a soluble form of PSGL-1 was as effective as an antibody directed against VLA-4 in

reducing eosinophil and lymphocyte accumulation (80% and 50% respectively) in the lungs of ovalbumin-challenged mice (11). In combination, eosinophil recruitment was completely blocked without additional effects on the accumulation of lymphocytes. The incomplete inhibition of pulmonary inflammation observed by blocking either P-selectin binding or VCAM-1 binding suggests that there are other mechanisms for tethering and adhesion and/or overlapping functions between P-selectin and VCAM-1. Indeed, interactions of intercellular adhesion molecule-1 (ICAM-1) with β_2 -integrins (CD18) are also known to mediate adhesion of leukocytes to vascular endothelium. Eosinophils isolated from allergic asthmatics demonstrate increased static adhesion to both VCAM-1 and ICAM-1 when compared with eosinophils from normal donors (37). In addition, it was shown that both an anti-ICAM-1 antibody and an anti-VCAM-1 antibody could suppress the migration of T lymphocytes and eosinophils into the lung following ovalbumin challenge in a mouse model of asthma (38). Furthermore, it was demonstrated that eosinophil recruitment and activation appears to be mediated by a combination of VLA-4 and LFA-1 (CD11a/CD18), whereas lymphocyte recruitment appeared to be mediated by a combination of ICAM-1 receptors LFA-1 and Mac-1 (CD18/CD11b) (39). These additional mechanisms were also suggested by the findings of VLA-4 antagonist WAY103 on isolated human peripheral eosinophils that inhibited eosinophil adhesion, whereas migration across an endothelial cell monolayer was not (40). These studies show that eosinophils and T

lymphocytes can bypass the VLA-4/VCAM-1 adhesion pathway in allergic inflammation via alternate cell-cell interactions (PSGL-1/P-selectin and LFA-1/ICAM-1), which might explain the lack of effect of GW559090X on the LAR in the present study. Furthermore, it must be considered that the recruitment of leukocytes leading to airway inflammation is the result of a complex multi-step process that is orchestrated by allergen-specific Th2 lymphocytes. This implicates that also antagonists of Th2 cytokines and chemokines or their receptors could have the potential to inhibit airway inflammation in asthma.

In conclusion, treatment with a single dose of VLA-4 antagonist GW559090X did not inhibit allergen-induced airway responses or reduce eNO levels in patients with mild asthma. These findings suggest that the VLA-4/VCAM-1 interaction may not play a major role in asthma with respect to the recruitment of inflammatory cells into the airways following allergen challenge. Further investigations are warranted to determine whether targeting adhesion molecules could provide an effective therapy for atopy related diseases.

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