

Influence of H₁-receptor antagonists on adhesion molecules and cellular traffic

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The immunopathologic nature of allergic inflammation has now been well characterized in the skin, nose, and lungs. Studies have been done on allergen challenge and steady-state disease. Allergen challenge evokes an IgE-dependent inflammatory response characterized by an immediate reaction as a result of the release of vasoactive and bronchoconstrictor mediators, probably from mast cells, and a delayed and more prolonged reaction associated with an influx of inflammatory cells. The inflammatory cells are mainly eosinophils and mononuclear cells, both monocytes and T cells being recruited. Neutrophil numbers are also increased, and there is some evidence of basophil and platelet recruitment. Eosinophils are activated and degranulation is evident. T cells are also activated, and they generate mRNA for Th2-type cytokines (interleukin (IL)-3, IL-4, IL-5, and granulocyte/macrophage-colony stimulating factor (GM-CSF)) (1). The three principal mechanisms currently thought to be important in the accumulation of inflammatory cells are adhesion pathways, selective chemoattractants, and accumulation caused by cytokine-induced prolonged survival, particularly in the case of eosinophils.

There is increasing evidence that adhesion is a mechanism of fundamental importance in determining the nature of the cellular infiltrate in an inflammatory reaction. The accumulation of leukocytes in response to an inflammatory stimulus is an orderly event in which the emigration and accumulation of different types of leukocytes are explained, at least in part, by the selective effects of diverse inflammatory mediators and differences in expression or configuration of leukocyte/endothelial cell adhesion molecules and their ligands.

Remarkable progress has been made in recent years in identifying and characterizing the molecules involved in leukocyte adhesion to endothelium. The sequence of events resulting in the extravasation of leukocytes in response to inflammatory stimuli can be summarized as follows.

Inflammatory substances generated at the site of inflammation activate the circulating leukocytes and adjacent endothelium. Circulating leukocytes then migrate to the vessel wall and form a loose association with the endothelium, in effect, "rolling" along the vessel wall. This association occurs under conditions of blood flow or shear stress, and it has been convincingly shown to be mediated by the selectin family of adhesion molecules (E-selectin, L-selectin, and P-selectin, which bind to carbohydrate moieties). They then adhere firmly to the endothelial cells, migrate along the endothelium, and pass by diapedesis between the junctions of endothelial cells. Many different adhesion molecules may participate in this phase of adhesion. Well-documented adhesion molecule interactions include LFA-1 (CD11a/CD18) with intercellular adhesion molecule-1 (ICAM-1; CD54) and ICAM-2; CR3 (CD11b/CD18) with ICAM-1; and VLA-4 (CD41d/CD29), a member of the β_1 family of integrins, with vascular cell adhesion molecule-1 (VCAM-1) (2). Once inflammatory cells have left the vascular compartment, they make their way through the extracellular matrix (ECM) to the site of the inflammatory reaction. The other β_1 integrins play an important role in this process because their ligands are the proteins of the ECM (e.g., collagen, laminin, and fibronectin), and the interaction of inflammatory cells with ECM proteins can profoundly affect their functional potential.

The importance of the adhesion system is emphasized by the finding of a number of patients with a rare, autosomal-recessive immunodeficiency disease, leukocyte adhesion deficiency (LAD), whose neutrophils have a defect in the β_2 chain of the leukocyte integrins that prevents normal adherence to the endothelium. Despite having abundant neutrophils in their circulation, these patients are subject to life-threatening infections, which, in their severe form, cause death in infancy and much disease even in their moderate phenotype (3).

More recently, another form of LAD has been described which also profoundly affects the ability of these patients to mount a pyogenic inflammatory response. This form of the disease results from the absence of sialyl Lewis X (the ligand for E-selectin) on neutrophils (4).

Epithelial damage is often an added feature of atopic dermatitis, and the shedding of the bronchial epithelium after inflammatory attack is thought to represent a major contribution to the pathogenesis of asthma (5). Epithelial expression of adhesion molecules may be an important mechanism by which these cells interact with other proinflammatory cells. Expression of ICAM-1, a member of the immunoglobulin gene superfamily widely expressed by various cell types (6), has been detected on inflamed epithelium. Endothelial cells express low levels of ICAM-1, and stimulation by various substances, including tumor necrosis factor (TNF), IL-1, interferon-gamma (IFN- γ), or LPS, results in increased ICAM-1 expression (7). ICAM-1 expression may also be induced on human airway epithelial cells after cytokine stimulation (8). Physiologic concentrations of eosinophil cationic protein and major basic protein significantly upregulated ICAM-1 expression by human nasal epithelial cells (9). ICAM-1 has been shown to be involved in the adhesion of platelet-activating factor (PAF)-stimulated primate eosinophils to stimulated human umbilical vein endothelial cells (HUVEC). Moreover, intravenous treatment with an anti-ICAM-1 monoclonal antibody (mAb) attenuated primate allergen-induced airway eosinophilia and the associated bronchial hyperresponsiveness (10). In a recent study, Bentley et al. found higher constitutive epithelial cell expression of ICAM-1 in bronchial biopsies in patients with asthma than in controls. Higher expression was also seen after allergen challenge of asthmatic patients than after diluent challenge (11). Furthermore, Vignola et al. (12) reported statistically significantly higher bronchial epithelial cell expression of ICAM-1 in asthmatic patients than in controls. These increases correlated with the Aas clinical score and the FEV₁ of these patients. These findings suggest an important role for ICAM-1 in allergic-type inflammation. However, Montefort et al. (13) could not detect any differences in ICAM-1 expression between epithelial cells from asthmatic patients and those from normal controls.

The eye is often a site of allergic reaction, either as the main focus or as a concomitant symptom of respiratory allergy. Moreover, the eye, like the nose or bronchus, is a convenient model in man for study of the pathophysiologic mechanisms of the allergic reaction. It can be used both in the acute

natural allergic reaction and as a site for allergen provocation. The conjunctival provocation test is safe, simple, reliable, and reproducible. It has been used both in diagnosis and in the study of the allergic reaction. Furthermore, it is very useful for the collection of cells and mediators, and for assessment of the effects of various treatments on the outcome of the allergic process.

We have utilized conjunctival scrapings to harvest epithelial cells from allergic subjects during the relevant season and also after allergen provocation of the eye. Immunohistochemical analysis was then used to assess the expression of ICAM-1 by epithelial cells. Fifteen allergic subjects were compared with 15 healthy controls for ICAM-1 expression on their conjunctival epithelium after conjunctival provocation test (CPT) with *Parietaria judaica* outside the pollen season. At base line, none of the subjects showed detectable ICAM-1 expression. Within 30 min of allergen challenge, all the allergic subjects had marked ICAM-1 expression, unlike the healthy subjects. Moreover, ICAM-1 expression appeared in the sensitized subjects concomitantly with the local inflammatory reaction and cellular influx. Thus, the conjunctival epithelium appears to be more than a bystander in allergic inflammation. Therefore, ICAM-1 may play a central role in the pathogenesis of allergic inflammation (14). ICAM-1 is not only confined to the cell surface, but is also shed in a soluble form. Thus, it was possible that epithelial cells did not synthesize the expressed ICAM-1, but had taken up the soluble form in a nonspecific or passive way. However, we proved that continuously cultured conjunctival epithelial cell lines expressed ICAM-1, and that treatment of these cells with IFN- γ significantly upregulated ICAM-1 expression. Moreover, we performed *in situ* hybridization on epithelial cells to demonstrate mRNA for ICAM-1. Fig. 1 shows the presence of ICAM-1 mRNA in cultured conjunctival epithelial cells. Similar results were obtained from conjunctival scrapings from allergic subjects (Pesce et al., paper in preparation). These findings confirm that these cells synthesize and specifically express ICAM-1. Activated epithelial cells might therefore directly interact with other proinflammatory cells as a result of enhanced adhesion molecule expression. Furthermore, upregulated ICAM-1 expression might be an important facet of eosinophil-mediated epithelial cell damage. Therefore, new methods of therapeutic intervention in allergic disease might focus on the selective blockade of adhesion pathways.

As well as being an important adhesion molecule, ICAM-1 is the receptor for the major group of rhinoviruses. Although the relationship between

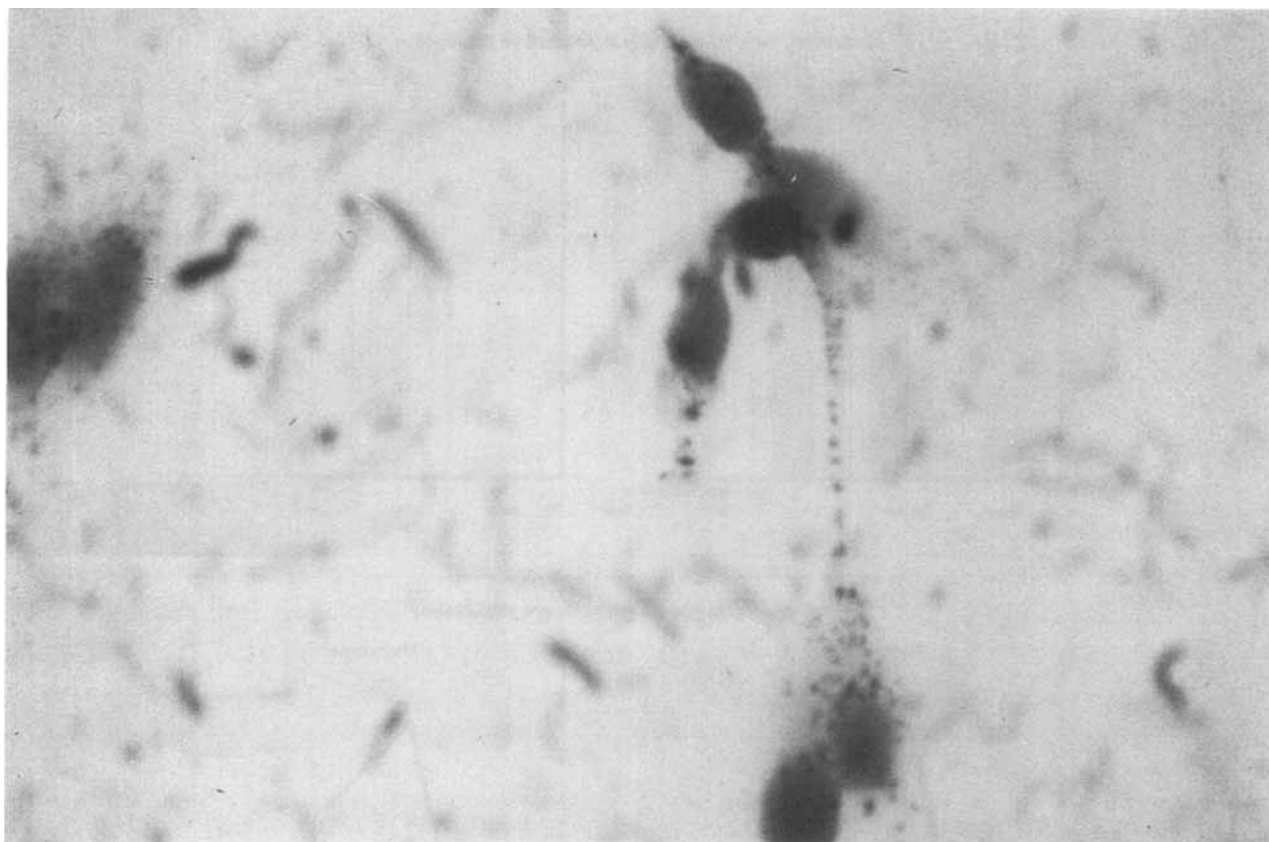


Fig. 1. *In situ* hybridization for ICAM-1 mRNA in continuously cultured conjunctival cell line WK. Digoxigenin-labeled oligonucleotide DNA protein for ICAM-1 was used. Cytoplasmic dark granules indicate positive staining for specific mRNA.

viral infections of the respiratory tract and bronchospasm has been known for some time, the underlying mechanism or mechanisms are still the subject of much debate (15). What is clear, is that viral infections are involved in 50% of asthma attacks in childhood and that asthmatic children do suffer from viral infections more than normal controls (16). Therefore, low-grade exposure to allergen in some allergic subjects may be insufficient to cause any obvious symptoms, but may lead to enhanced epithelial cell expression of ICAM-1. This in turn might result in an increased susceptibility to infection by the rhinoviruses and lead to an asthma attack.

Cetirizine has been shown to have a number of antiallergic effects, in both *in vitro* (it inhibits eosinophil activation and chemotaxis and platelet activation, and also affects platelets and neutrophils) and *in vivo* studies (protective effect against the changes induced by skin prick test with histamine or allergen, or specific nasal provocation test, and also limited protection against the specific bronchial provocation test) (17). In a recent study, the effect of cetirizine on the early and late responses induced by CPT was assessed. The trial was double blind, randomized, and placebo con-

trolled, and it was done outside the pollen season with six patients assigned to each parallel group. CPT was done before and after administration of cetirizine (20 mg/day) or placebo over a 3-day period. As mentioned, challenge of the allergic subjects in both groups resulted in an increase in ICAM-1 expression within 30 min which paralleled the increase in symptom scores. There was also a large increase in the total number of inflammatory cells at the challenge site at 30 min. All three parameter increases persisted for up to 24 h. After treatment with cetirizine, there were significantly lower total symptom scores, ICAM-1 expression, and inflammatory cell infiltration than after placebo, at all time points tested (Fig. 2). These effects were not due to the ability of cetirizine to inhibit the effects of histamine, because we have shown that histamine cannot cause expression of ICAM-1 on epithelial cells (18). Therefore, the effect of cetirizine is not a classical antihistamine effect and is more likely to be a reflection of the additional anti-inflammatory effects of this drug.

This view is supported by various studies which have found an inhibitory effect by cetirizine on adhesion or adhesion-related events. Cetirizine in-

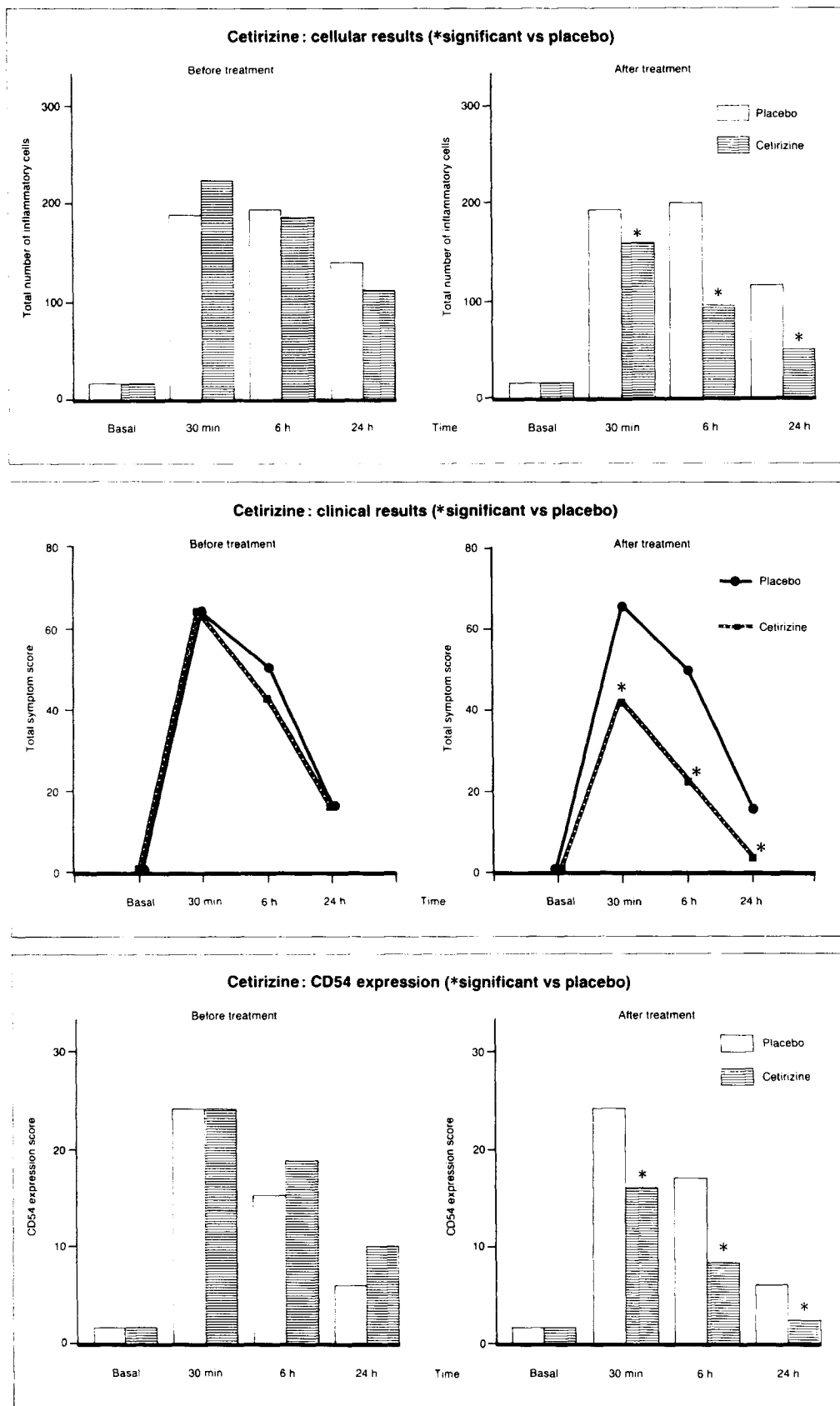


Fig. 2. A) Effect of cetirizine compared with that of placebo on total inflammatory cell numbers in conjunctival scrapings before (top) and after (bottom) treatment. B) Effect of cetirizine compared with that of placebo on total symptom score after CPT before (top) and after (bottom) treatment. C) Effect of cetirizine compared with that of placebo on CD54 expression on conjunctival epithelial cells before (top) and after (bottom) treatment.

hibits eosinophil adhesion to cultured human endothelial cells, although an effect was observed only with high concentrations (10 and 100 µg/ml) (19). Adhesion-dependent functions, such as PAF-induced neutrophil- and eosinophil-enhanced rosette formation and complement-dependent cytotoxicity of eosinophils, were inhibited by pretreatment of the cells with cetirizine. Cetirizine also inhibited eosinophil, but not neutrophil, PAF-induced hyperadherence to plasma-coated glass (20). These data all support the view that cetirizine can inhibit the *in vitro* activation of proinflammatory cells, with particular effect on the eosinophil migration shown *in vivo* in the skin (21). The mechanism by which cetirizine exerts these effects is unknown.

Several studies have looked at the effect of various anti-inflammatory drugs on adhesion molecule expression. Cyclosporin A has been shown to inhibit ICAM-1 synthesis by endothelial cells in healing psoriatic plaques (22), and glucocorticoids inhibit IL-1-induced expression of ICAM-1 on human lung adenocarcinoma cell lines, but not endothelial expression of E-selectin (23). Deflazacort, an effective oxazoline derivative of prednisone, was found to block late-phase reaction events, including ICAM-1 expression on epithelium. Deflazacort also downregulated ICAM-1 expression during the early phase. The latter effect is the first description of activity by a steroid during the early phase of the allergic reaction (24).

In another study, we compared the effects of terfenadine and placebo in 20 patients with allergic rhinitis during the pollen season (no allergen challenges were done in this study). Two groups were treated with either terfenadine or placebo in a double-blind, randomized study. Terfenadine (120 mg/day) and placebo (daily) were given for 1 week. Evaluation of nasal epithelial cell ICAM-1 expression was done at days 0 and 7. Terfenadine was also found to be effective in reducing infiltration by proinflammatory cells, particularly eosinophils, and in the reduction of ICAM-1 expression by epithelial cells.

These studies suggest that the new-generation antihistamines such as cetirizine and terfenadine might exert their additional anti-inflammatory effects via an effect on ICAM-1 expression. However, it must be remembered that the adhesion system is very complex and highly redundant, with many other adhesion proteins and their ligands involved in the accumulation of an inflammatory infiltrate. To this end, we have looked at the expression of other adhesion molecules on conjunctival epithelial cells. Preliminary findings from our group have indicated CD29 (β_1) and LFA-1 ($\alpha\beta_2$) expression by inflamed conjunctival epithelium.

The former observation may be of particular interest, because eosinophils, but not neutrophils, have been shown to adhere to VCAM-1 on cytokine-activated endothelium via its ligand $\alpha 4\beta_1$ (25). Whether the new-generation antihistamines also affect the function or expression of these integrins remains to be elucidated.

Round-table discussion

Simons: When you say the effect of the H₁-antagonists was not classical, do you mean that the effect is not via H₁ blockade?

Bagnasco: It is not an antihistamine effect, because histamine does not induce ICAM-1 expression. The effect is not a peripheral one, but it is impossible to rule out a cascade effect. In this respect, we don't know what mediates the expression of ICAM-1 by epithelial cells. Another point concerns demonstrating ICAM-1 mRNA expression in the epithelial cells. These experiments were absolutely necessary to demonstrate that epithelial cells have the ability to synthesize ICAM-1. If you take conjunctival epithelial cells from a symptomless allergic subject, you cannot detect ICAM-1. Thirty minutes after antigen challenge, ICAM-1 is detectable on the epithelial cells. These kinetics suggest that mRNA is already present. Therefore, the phenomenon acts at different levels, and the mediator effect is post-transcriptional.

Juhlin: Do you have any information on the effect of other antihistamines in your system? You mentioned terfenadine. I know you have been working on loratadine – have you compared this with cetirizine?

Bagnasco: I have no data at this moment regarding loratadine and ICAM-1 expression. In a previous study, we did demonstrate that loratadine could inhibit cellular infiltration and symptoms of allergic conjunctivitis, both in early- and late-phase allergen provocation. As with terfenadine, we have the model of spontaneous ICAM-1 expression, but no challenge data. The studies which require conjunctival challenge are very complex to set up, so it is difficult to get data for every antihistamine.

Broide: Before eosinophils and other inflammatory cells enter an inflammatory site, they must first interact with postcapillary endothelial cells. After they have undergone adhesion and transmigration, they arrive at the epithelial cells. I wonder whether you or any one else has any more information regarding the effect of cetirizine on inflammatory cell/endothelial interactions?

Bagnasco: I think this is a very interesting point, because the interaction of inflammatory cells with the endothelium represents one of the earliest events in the inflammatory response. In our model, it is very easy to get epithelial cells, but endothelial cells would require a biopsy. The nasal model would be best for this, but you would be limited by the number of biopsies you could perform, especially in the challenge situation.

Walsh: We have looked at the effect of cetirizine on eosinophil adhesion using cultured human umbilical vein endothelial cells [HUVEC] as an adhesion substrate. Cetirizine had no effect on resting eosinophil adherence to the unstimulated endothelial cells. Following eosinophil stimulation with PAF, cetirizine inhibited eosinophil hyperadherence to resting HUVEC in a dose-dependent manner. Maximal effect was observed at 10^{-8} M, with an IC_{50} value of 10^{-11} M (26). The most striking feature of these results was the low concentration of cetirizine required to give a significant effect. Tak Lee's group did a similar study using fMLP as the stimulus for eosinophil hyperadhesion (19). They also saw inhibition of enhanced eosinophil adhesion, but only at cetirizine concentrations of 10^{-5} or 10^{-6} M. In both studies, the effect was selective for eosinophils, with little or no inhibition of neutrophil adhesion seen. The reason for the discrepancy in the two groups' results may lie in the stimulus used to enhance eosinophil adhesion to HUVEC. We used PAF; Tak Lee used fMLP. This may be an important clue as to how cetirizine exerts its effect on eosinophils, as these two mediators may exert their stimulatory effects via different intracellular pathways. More work looking at the effect of cetirizine on, for example, signal transduction pathways in eosinophils might yield very interesting data.

Broide: Did you look at the effect of cetirizine on eosinophil adhesion after stimulating your endothelial cells with, for example, IL-1 or TNF with and without PAF stimulation of the eosinophils?

Walsh: We only looked at resting endothelial cells. Again, Tak Lee's group did look at the effect of cetirizine on unstimulated eosinophil adhesion to IL-1-stimulated endothelial cells. They did see some inhibition, but again only at the higher cetirizine concentrations.

Broide: Are there H_1 -receptors on eosinophils?

Walsh: Well, they do respond to histamine stimulation.

De Vos: Yes, eosinophils have H_1 -receptors. They were described by Steve Wasserman, some years ago.

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