

hypersensitive mice ($n = 8$) and control mice ($n = 8$), respectively, prepared and evaluated as earlier.⁷ All procedures involving animal handling and care were approved by the institutional animal use and care committee. The functional activity of both homologous forms of plasma MBL in rodents, mMBL-A and mMBL-C, was quantified before and after 72 hours of last sensitization (28 days immunization schedule) with *A fumigatus* allergens/PBS in the same mice by using biotinylated mAb anti-mouse MBL (13H6 for MBL-A and 14D12 for MBL-C) and streptavidin horseradish peroxidase.⁸ There was a significant increase of 56% (4.2%) in the mean mMBL-A level of *A fumigatus*-hypersensitive mice 72 hours after the last sensitization with *A fumigatus* allergens-antigens ($P < .05$), indicating that the allergen challenge might be leading to increased functional levels of MBL in the sensitized mice. In agreement with an earlier report, the mean mMBL-C level, however, showed only a marginal increase of 6% (SD, 0.68%) in the sensitized mice.⁹ The mean mMBL-A and mMBL-C levels of the control mice did not show any significant change.

Because polymorphisms in the collagen region of MBL are important determinants of MBL levels and complement activity in the plasma,³ it is interesting to note that the A allele of one of the novel polymorphisms, G1011A in intron 1 of the *MBL* gene, was found to be significantly associated with high plasma levels of functional MBL. Also, allergic patients homozygous for the A allele had significantly higher peripheral blood eosinophilia and lower percent predicted FEV₁ than the patients homozygous for the other G allele (Kaur et al, unpublished results).

Although increased plasma levels of functional MBL in the patient groups and sensitized mice and their significant correlation with hypereosinophilia suggests a possible role of MBL in the pathophysiology of allergy, in view of MBL being documented as a weak acute-phase protein, such an inference necessitates further investigation.³ Furthermore, these observations caution the use of MBL replacement therapy in patients with a history suggestive of asthma. Although MBL levels are undetectable in the bronchoalveolar lavage fluid of normal mice, they are increased significantly during pulmonary infection.¹⁰ Hence MBL levels in the bronchoalveolar lavage fluid of allergic patients might be a sensitive marker of allergic airway diseases than their plasma MBL levels.

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CC chemokine receptors CCR1 and CCR4 are expressed on airway mast cells in allergic asthma

To the Editor:

Mast cells have been implicated as essential inflammatory cells in the asthmatic process. Thus, mast cell numbers are increased in the lungs of both atopic and nonatopic variants of asthma, and the extent of degranulation of the cells is directly related to disease severity.¹ The accumulation of mast cells in the airway is regulated by migration of either progenitor cells circulating in the blood or by relocalization of mature mast cells within the

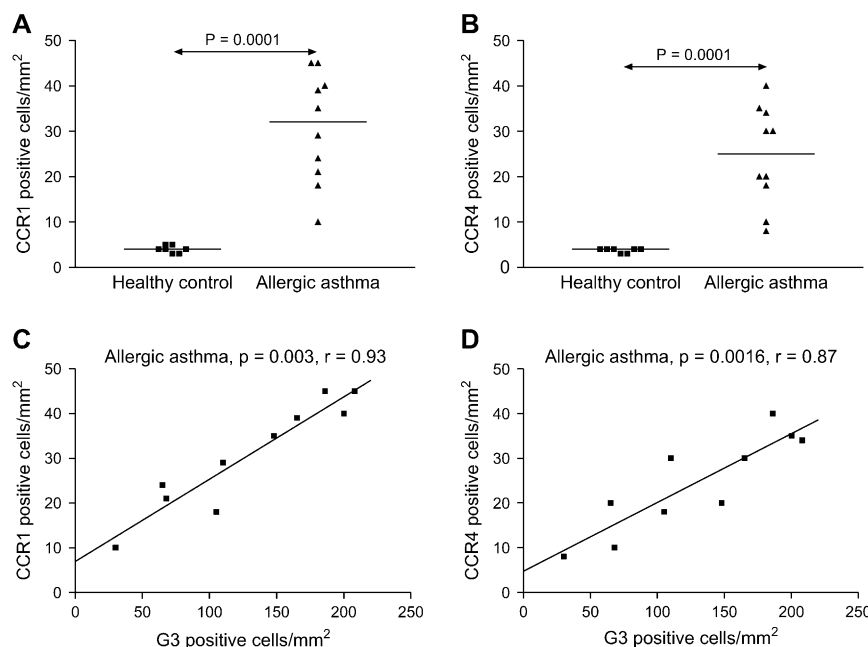


FIG 1. Expression of CCR1 (A) and CCR4 (B) in the airways of patients with allergic asthma. Horizontal bars indicate median values. The numbers of the CCR1 (C) and CCR4 (D) were correlated with tryptase-positive mast cells in patients with allergic asthma. Spearman nonparametric correlation test was used.

tissue. Chemotactic proteins direct this selective recruitment of mast cells, and chemokines have emerged as 1 very important family of chemoattractants. Although the expression of chemokine receptors on human cultured mast cells has been thoroughly examined, the expression pattern of chemokine receptors on airway mast cells is still largely unknown.² The only chemokine receptors described to be expressed on subpopulations of mast cells from normal lung are CCR3, CXCR1, CXCR3, and CXCR4.^{3,4} We have recently found that human cord blood–derived mast cells express CCR1 and CCR4, and that CCL5 acts as a CCR1 and CCR4 agonist.⁵ There has been a special interest on the expression of CCR4 in the lung because this receptor is expressed on polarized T_H2 cells and the number of CCR4-positive T cells increases in the airways after allergen challenge.⁶ We have therefore determined whether airway mast cells express the chemokine receptors CCR1 and CCR4, and whether there is a difference in number of CCR1-positive and CCR4-positive mast cells between healthy individuals and patients with allergic asthma.

Bronchial biopsies were collected from 17 nonsmoking adults: 10 patients with allergic asthma and 7 healthy controls.⁷ All patients with allergy were examined outside the birch and grass pollen season. All but 1 patient with allergy and asthma were on regular treatment with inhaled glucocorticosteroids (budesonide 200–800 µg/d) and inhaled β₂-agonists as needed. Frozen sections of the biopsies were stained with antibodies against tryptase, CCR1, CCR4, laminin, and tenascin. The measurement of the

thickness of the tenascin and laminin layers was processed as previously described.⁷

Very few CCR1-positive or CCR4-positive cells were seen in the biopsies from healthy individuals, whereas patients with asthma exhibited a strong increase in CCR1-positive (Fig 1, A) and CCR4-positive (Fig 1, B) cells. Similarly, and reported previously by several groups, we found a strong increase in mast cell number in patients with asthma compared with healthy individuals. There was a significant correlation between the increase in mast cell number and the increase in CCR1-expressing and CCR4-expressing cells in the lung of patients with asthma (Fig 1, C and D, respectively). Furthermore, the expression of CCR1 and CCR4 also correlated significantly in patients with allergic asthma ($P = .0002$; $r = 0.93$).

Because we previously have shown that human *in vitro*–developed mast cells express CCR1 and CCR4,⁵ we next examined mast cell expression of CCR1 and CCR4 on serial sections of bronchial biopsies from patients with allergic asthma. As shown in Fig 2, both CCR1-positive and CCR4-positive mast cells could be observed within the intraepithelial cell layer and in the lamina propria. CCR1 was more commonly expressed than CCR4 on mast cells. Approximately 24% of the mast cells expressed CCR1 and 19% expressed CCR4 in whole biopsies of asthmatic lung. This is the first description of chemokine receptors expressed on mast cells in the asthmatic lung. In a recent publication from Brightling et al,⁴ most of the chemokine receptors were absent on normal lung mast cells, except for CXCR3 and CXCR4, which are expressed

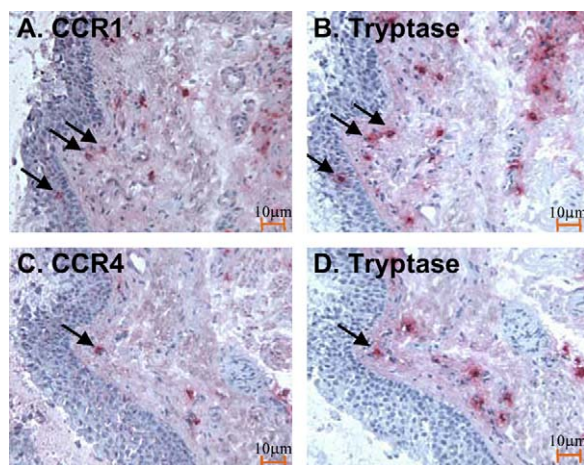


FIG 2. Serial bronchial biopsy specimens from patients with allergic asthma were stained for CCR1 (A) and tryptase G3 (B), or CCR4 (C) and tryptase (D). Original magnification $\times 340$. The scale bars are 10 μ m.

on approximately 25% to 30% of the cells. CCR3 was expressed on 15% of the mast cells, which is similar to what has been reported by Romagnani et al.³ It is apparent that chemokine receptor expression on mast cells is heterogenous, suggesting that several chemokines are involved in the recruitment and relocalization of mast cells. Although CCL5 induces mast cell migration *in vitro*, it remains to be proven that this happens *in vivo* and that CCR1 and CCR4 are expressed on the recruited mast cells. The fact that the expression and secretion of the ligands for CCR1 and CCR4 are increased in the asthmatic lung⁸ makes these ligand-receptor pairs good candidates for mast cell recruitment. Furthermore, the expression of CCR1 and CCR4 on lung mast cells seems to be tissue-specific in that skin mast cells have not been found to express either CCR1 or CCR4 (Harvima, unpublished data, April 2004).

We also found that the number of mast cells in the biopsies correlated positively to the thickness of the laminin and tenascin layer in the group of patients with allergic asthma ($P = .02$; $r = 0.73$; and $P = .02$; $r = 0.72$, respectively). In patients with allergic asthma, total CCR1-positive cells were correlated significantly to the thickness of laminin and tenascin layer. In contrast, there were no significant correlations between the number of CCR4-positive cells and laminin or tenascin in the lung biopsies from allergic asthma. Asthma is characterized by the airway remodeling, in which thickening of the sub-epithelial layers is 1 part of the structural changes. We and others have previously shown that the number of mast cells in the bronchial mucosa of patients with allergic asthma correlates to structural changes such as thickening of the basement membrane, assessed by staining for laminin and tenascin.⁷ CCR1 is expressed on several cell types besides mast cells, including neutrophils, monocytes, lymphocytes, and eosinophils, and binds CCL3, CCL5, and CCL7. All 3 chemokines have been shown to have prominent effects on these leukocytes during allergic airway inflammations. Our

finding that CCR1 correlates to structural changes in the airway suggests that CCR1-positive cells have an active role in tissue remodeling. This conclusion is supported by the diminished airway remodeling in CCR1^{-/-} mice during chronic fungal allergic airway disease.⁹

In summary, the data from the present study show that airway mast cells from asthmatic lung express the chemokine receptors CCR1 and CCR4. These results imply that ligands acting on these receptors—for example, CCL5, CCL17, and CCL22—regulate diverse cellular functions in airway mast cells, including migration. Chemokines and their receptors are potential therapeutical targets for allergic diseases, including asthma.¹⁰ It is conceivable that therapy directed against CCR1 and CCR4 might be clinically useful in the prevention of mast cell infiltration and airway remodeling in allergic asthma.

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Preventive effect of acupuncture on histamine-induced itch: A blinded, randomized, placebo-controlled, crossover trial

To the Editor:

The sensation of itch is the most common symptom and cause of suffering in many dermatologic and some allergic conditions.^{1,2} In its subjective characteristics, it has some psychophysiologic similarity to pain, but recent neurophysiologic research has confirmed the distinctiveness of itch and pain pathways.³ Clinically, itch can be classified by distinguishing on the basis of the peripheral and central origins: pruritoceptive, neuropathic, neurogenic, and psychogenic itch. Pruritoceptive itch plays the greatest role in skin diseases and can be triggered by various itch mediators; it can be elicited experimentally most effectively through a histamine prick test.⁴ Acupuncture was shown to have antipruritic effects in early studies with healthy volunteers in the 1980s.^{5,6} It is unknown, however, whether acupuncture has a preventive effect on itch. Therefore this study aimed to evaluate a possible preventive effect of acupuncture on skin prick test histamine-induced itch and flare and wheal formation in 10 healthy volunteers.

The study design was a blinded, randomized, prospective, 3-arm crossover trial. The acupuncturist and the observer were different individuals. All volunteers provided informed consent, had no acupuncture knowledge, and did not use medication with the potential to influence histamine reactions and normal itch or flare/wheal response to intraepidermal histamine injection.

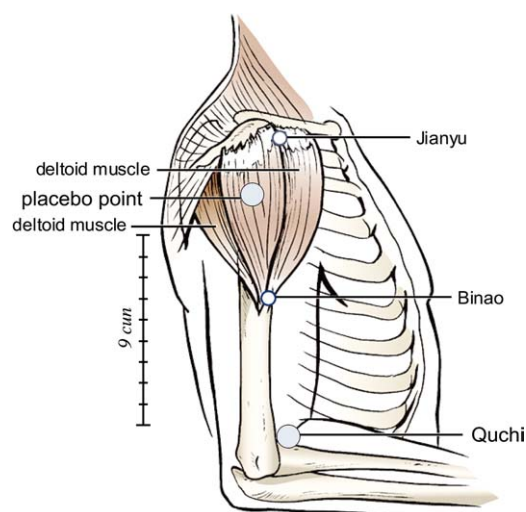


FIG 1. Localization of Quchi (located on the elbow at the midpoint of the line joining the lateral end of the transverse cubital crease and the lateral epicondyle of the humerus) and placebo point (located at the midpoint of the acromial part of the deltoid muscle and therefore being at least 2 cm apart from classical acupuncture points). Reprinted with permission from Hecker U, Steveling A, Peuker E, et al. *Lehrbuch und Repetitorium Akupunktur*. Stuttgart: Karl F. Haug; 2002. Modified with permission from Georg Thieme Verlag, Stuttgart, Germany.

After a 15-minute resting period, the volunteers were randomized into one of 3 groups: “verum-point” acupuncture (A1), “placebo-point” acupuncture (A2) or no acupuncture (NA).⁷ After acupuncture procedures (lasting 15 minutes) or after a corresponding resting period, an evaluated 1% histamine dihydrochloride stimulus was applied on the dorsum of the forearm of the subject’s dominant hand. The technique was performed by the same investigator using conventional blood lancets for skin prick testing as in routine allergy diagnosis. Itch intensity was rated on a computerized visual analog scale (VAS) at 20-second intervals over a period of 10 minutes. At one third of the scale, the intervention point scratch threshold was installed; above this threshold, each individual strongly felt the desire to scratch, which was not permitted.

Itch parameters were quantitatively expressed in percentages of the VAS at 30 different time points. The area under the curve was calculated as VAS (in percentages) multiplied by time (in seconds). After 10 minutes, wheal-and-flare reactions were measured at the stimulus site as averages of 4 perpendicular diameters, and the Eppendorf Itch Questionnaire (EIQ),⁸ a validated instrument for qualitative and quantitative registration of pruritus, was presented to the volunteers. It contains 80 items, including questions concerning painful sensations. Each item was rated from 0 (not applicable) to 4 (very applicable).

Verum-point acupuncture was performed on the arm of the subject’s dominant hand at the point Quchi (Fig 1), which is, according to a Chinese standard acupuncture textbook, most important for treating cutaneous pruritus.⁹