

Original article

Interleukin-4-positive mast cells are highly associated with the extent of immediate allergic wheal reaction in the skin

Background: In addition to histamine, mast cells contain other potent mediators which can contribute to the allergic wheal reaction in the skin.

Methods: To study the association of tryptase-, chymase-, and interleukin-4 (IL-4)-positive mast cells with the size of the prick-test wheal reaction, 50 sensitive atopic subjects were prick-tested with the cow-dander allergen on the forearm skin, and the wheal area was measured. A corresponding site of intact healthy-looking skin was biopsied and examined enzyme-histochemically for tryptase and chymase. A double-staining method was used to demonstrate the immunoreactivity of IL-4 and chymase inhibitors (α_1 -proteinase inhibitor and α_1 -antichymotrypsin) in mast cells. The levels of total and cow-specific immunoglobulin E (IgE) were measured in serum.

Results: The number of tryptase- and chymase-positive mast cells or those containing chymase inhibitors revealed no correlation with the wheal reaction. In contrast, both the percentage and the number of IL-4-positive mast cells showed significant positive correlation with the wheal size per se ($P < 0.0001$), as well as with the ratio of the wheal size by cow allergen to that by histamine control ($P < 0.003$). In addition, tryptase-, chymase-, and IL-4-positive mast cells correlated with total IgE, but not with specific IgE, levels, and they showed no relation to the clinical manifestation of atopic disease, asthma or atopic dermatitis.

Conclusions: The novel finding was that IL-4-positive, but not tryptase- and chymase-positive, mast cells are intimately associated with the extent of the prick-test wheal.

**J. V. Saarinen¹, R. J. Harvima¹,
M. Horsmanheimo¹, I. T. Harvima¹,
A. Naukkarinen²**

¹Department of Dermatology, Kuopio University Hospital, Kuopio; ²Department of Clinical Pathology, Kuopio University Hospital, Kuopio, Finland

Key words: cytokine; mediator; prick test; protease; type I allergy.

Dr Ilkka T. Harvima
Department of Dermatology
Kuopio University Hospital
P.O.B. 1777
70211 Kuopio
Finland

Accepted for publication 21 August 2000

Antihistamines have been used extensively in the treatment of immediate allergic symptoms but occasionally with poor success. Although histamine is the principal mediator in the allergic wheal reaction in the skin, numerous other mediators released by mast cells and other skin cells can also participate in the wheal. Preformed mediators present in mast-cell secretory granules include serine proteinases (tryptase, chymase, and a cathepsin G-like proteinase), carboxypeptidase, heparin proteoglycan, and chondroitin sulfate E. Furthermore, mast cells produce cytokines, such as interleukin-4 (IL-4), IL-5, IL-6, IL-8, IL-13, tumor necrosis factor- α , interferon- γ , and granulocyte-macrophage colony-stimulating factor (1–7). Newly synthesized mediators of mast cells include prostaglandin D₂, leukotriene C₄, and platelet-activating factor (1).

Mast cells in healthy-looking human skin are mainly MC_{TC} cells (tryptase- and chymase-positive) (8). The enzyme activity of chymase varies in different inflammatory conditions of the skin. The activity declines gradually during the development of inflammatory dermatoses, which is associated with the presence of the protease inhibitors, α_1 -proteinase inhibitor (α_1 -PI) and α_1 -antichymotrypsin (α_1 -AC), in the same mast cells (9–11). In contrast to chymase, no physiologic inhibitors are known for tryptase (12). Chymase is assumed to modulate the allergic wheal reaction since it can potentiate histamine-induced wheals in the skin of ragweed-allergic dogs and can degrade the wheal-forming substances bradykinin and the neuropeptides (13–15). Skin tryptase has been thought to participate in the wheal reaction as well, since tryptase is released together with histamine after

allergen challenge (16), and this enzyme can hydrolyze several peptides and proteins involved in vascular dilation and wheal formation (12). Although exogenous tryptase cannot stimulate histamine release from dispersed human skin mast cells, the addition of tryptase inhibitors to the cell suspension results in inhibition of histamine release by secretagogues (17).

The allergic wheal reaction is mediated by IgE molecules on mast cells. The cytokines IL-4 and IL-13 are essential in the induction of IgE synthesis by B cells (18). In addition, both IgE and IL-4 have been reported to function synergistically in inducing the expression of the high-affinity receptor (FcεRI) on, and IgE-dependent mediator release from, the developing human mast cells *in vitro* (19, 20). Mast cells isolated from human intestinal tissue release more histamine, leukotriene C₄, and IL-5 by IgE receptor cross-linking when treated with IL-4 (21). Furthermore, IL-4 mRNA synthesis can be induced in human dermal mast cells by IgE-dependent activation in the presence of stem-cell factor (22). Mast cells are one major source of IL-4 in the upper dermis of lesional atopic dermatitis skin when chymase activity in mast cells is decreased (23, 24). On the other hand, IL-4 has been reported to be susceptible to the degradation by chymotryptic mast-cell proteases, such as chymase (25).

Very few studies have evaluated the association of mast-cell mediators with the clinical allergic wheal, except for histamine. In order to study the association of the number of tryptase-, chymase-, and IL-4-positive mast cells with the allergic wheal reaction, we examined 50 atopic subjects sensitive to the cow-dander allergen. A skin biopsy was taken from the intact healthy-looking skin of each subject for histochemical analysis of tryptase-, chymase-, and IL-4-positive mast cells, and a serum sample was studied for the levels of total and cow-specific IgE. In addition, α₁-PI and α₁-AC in mast cells were studied, since chymase inhibitors have been reported to inhibit IgE-dependent histamine release from human mast cells *in vitro* (26). These parameters were correlated with the wheal area induced by prick testing with the cow-dander allergen. Prick testing was used as the experimental model for the wheal reaction, since it is widely accepted for clinical allergy testing.

Material and methods

Subjects, skin challenge, and biopsy

Fifty atopic subjects with a previously documented positive prick test reaction to the cow allergen volunteered for the study (25 women and 25 men, mean age 39 years, range 18–58), and all of them gave informed written consent. Only subjects without local or systemic antiallergic treatment known to interfere with skin-test responses were accepted. Antihistamines or local antiallergic therapy was withdrawn for at least 1 week to 1 month (in the case of astemizole) and phototherapy for at least 1 month prior to

the study. Nonsteroidal anti-inflammatory medication was finished at least 4 days before entry. Of the subjects, 22 had atopic dermatitis, 45 had allergic rhinitis, and 17 had asthma. The anterior forearm skin of each subject was prick tested with 0.1, 1.0, and 10 mg/ml crude cow-dander extract (dissolved in 0.9% NaCl) (27), and the wheal area was recorded 15 min later by outlining the borders of the wheal through a transparent film with a fine-point pen. The wheal area measured by the cutting-weighing method (28) was $17.1 \pm 11.2 \text{ mm}^2$ (mean $\pm$ SD), $36.4 \pm 21.3 \text{ mm}^2$, and $65.6 \pm 33.7 \text{ mm}^2$ by 0.1 mg/ml, 1.0 mg/ml, and 10 mg/ml cow-allergen, respectively. The wheal induced by the highest cow allergen concentration (10 mg/ml) was large enough and, thus, was used in further analysis (28). In addition, the prick test reactivity was confirmed with a commercial ALK cow antigen (wheal area $31.8 \pm 19.3 \text{ mm}^2$), ALK 10 mg/ml histamine as the positive control, and ALK negative control (Soluprick, ALK Laboratories, Hørsholm, Denmark). To control individual reactivity to histamine, the wheal ratio was calculated by dividing the wheal area induced by 10 mg/ml cow allergen with that induced by 10 mg/ml histamine control. The wheal ratio was 2.0 ± 1.1 .

In each subject, a 4-mm punch biopsy was taken under local anesthesia (1% lidocaine with epinephrine) from the intact healthy-looking skin of the anterior forearm. The skin specimens were immediately embedded in OCT compound (Miles Scientific, Naperville, IL, USA) and frozen in isopentane cooled with a mixture of dry ice and absolute ethanol. The protocol was approved by the ethics committee of the Kuopio University Hospital, Kuopio, Finland.

Chemicals

Carbobenzoxy-Gly-Pro-Arg-4-methoxy-naphthyl-amide (Z-Gly-Pro-Arg-MNA) and Succinyl (Suc)-Val-Pro-Phe-MNA were purchased from Bachem (Bubendorf, Switzerland). Rabbit polyclonal antibodies against α₁-AC and α₁-PI were purchased from Dako (Glostrup, Denmark), and a sheep polyclonal antibody against IL-4 from Sera-Lab (Crawley Down, Sussex, UK). The chemicals for immunohistochemistry were purchased from Vector Laboratories (Vector, Burlingame, CA, USA). All other chemicals were of reagent grade from E. Merck (Darmstadt, Germany).

Enzyme-histochemical staining methods for mast-cell tryptase and chymase

Mast-cell tryptase and chymase were stained enzyme-histochemically by 1 mM Z-Gly-Pro-Arg-MNA and Suc-Val-Pro-Phe-MNA as their sensitive substrates, respectively (9, 10). Mast cells stained for tryptase and chymase were counted in an observer-blind manner in adjacent skin sections under high magnification with an Olympus BH2 microscope equipped with a 0.2 × 0.2 mm ocular grid. The area measured was 1.0–1.2 mm (width) × 0.6 mm (depth) (9, 10).

Immunohistochemical staining methods

Immunohistochemical staining was carried out by first fixing the skin sections in cold acetone for 10 min. IL-4 was stained with a sheep polyclonal antibody (20 µg/ml), and α₁-PI and α₁-AC were stained with rabbit anti-α₁-PI (0.28 µg/ml) and anti-α₁-AC antibodies (0.61 µg/ml), respectively. The bound polyclonal antibodies on skin sections were visualized with a Vectastain-Elite ABC kit together with 0.05% 3,3'-diaminobenzidine tetrahydrochloride, 0.04% nickel chloride, and 0.03% hydrogen peroxide. The method was controlled by replacing the primary antibody with unrelated immunoglobulin (9, 10, 23).

Sequential double-staining method for IL-4 and protease inhibitors in tryptase-positive mast cells

The localization of IL-4 and protease inhibitors in mast cells was studied by the sequential double-staining method, as described previously (7, 9, 10, 23, 24). First, tryptase was stained enzyme-histochemically and photographed. The precipitated azo dye was removed by overnight 15% Tween 20. Thereafter, the same sections were stained immunohistochemically for IL-4, α_1 -PI, or α_1 -AC and rephotographed at exactly the same scenery as the previous pictures. The number and percentage of the positively stained mast cells were counted by comparing the micrographs in an observer-blind manner.

Concentration of total and specific IgE in serum

The concentration of total IgE (U/ml) and cow-specific IgE (U/ml) in serum was measured by the radioimmunoassay (RIA) (Pharmacia IgE RIA and Pharmacia CAP System RAST RIA, Kabi Pharmacia Diagnostics AB, Uppsala, Sweden).

Statistical analysis

Linear associations between variables were tested with the Spearman correlation coefficients.

Results

No significant correlation between tryptase- and chymase-positive mast cells and wheal size

The density of tryptase-positive (Try⁺) mast cells in the intact, healthy-looking skin of 50 subjects, reflecting the total number of mast cells, was 98 ± 23 cells/mm² (mean $\pm$ SD, range 42–148). The number of chymase-positive (Chy⁺) mast cells was 63 ± 21 cells/mm² (range 25–117); therefore, the Chy⁺/Try⁺ ratio was $64.5 \pm 16.2\%$ (range 21.0–95.9). The age of the subjects had no correlation with the number of Try⁺ ($r = -0.214$, $P = 0.136$) and Chy⁺ cells ($r = -0.055$, $P = 0.703$).

The number of mast cells in the skin may well influence the wheal reaction during prick testing. Thus, the relationship between the number of Try⁺ cells and the wheal size induced by 10 mg/ml cow allergen (Fig. 1), or the wheal ratio of cow allergen to histamine control ($r = 0.076$, $P = 0.599$) was studied, but no significant correlation was obtained. Likewise, the number of Chy⁺ cells showed no correlation with the wheal size ($r = -0.065$, $P = 0.652$) or wheal ratio ($r = -0.107$, $P = 0.459$) either. However, the Chy⁺/Try⁺ ratio revealed a weakly negative correlation with the wheal ratio ($r = -0.280$, $P = 0.049$), but no correlation with the wheal size ($r = -0.211$, $P = 0.140$). Neither the wheal size ($r = -0.001$, $P = 0.996$) nor the wheal ratio ($r = -0.208$, $P = 0.148$) were age-related. Further-more, Try⁺ cells, Chy⁺ cells, and the Chy⁺/Try⁺ ratio were not associated with the wheal size and wheal ratio when the lower cow-allergen concentration (1.0 mg/ml) was used for inducing the wheal reaction.

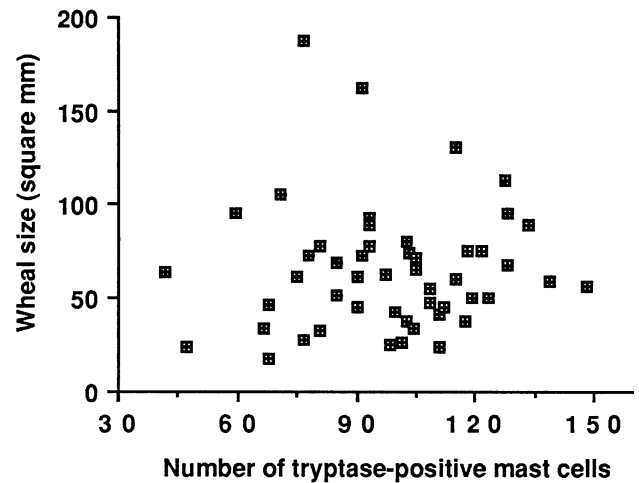


Figure 1. No significant correlation existed between number of tryptase-positive mast cells (cells/mm²) and wheal size induced by 10 mg/ml cow allergen ($r = 0.102$, $P = 0.480$) in healthy-looking forearm skin of 50 sensitive atopic subjects.

IL-4-positive mast cells were correlated significantly with the wheal size and wheal ratio

The sequential double-staining method was employed to stain IL-4 immunoreactivity in the mast cells (for illustrative figures, see ref. 23). Consequently, the percentage of Try⁺ mast cells displaying IL-4 was measured. In the healthy-looking atopic skin, $35.6 \pm 15.4\%$ (range 11.9–71.9) of the Try⁺ cells were positive for IL-4, a finding which parallels our previous study ($37 \pm 18\%$, $n = 20$) (23), indicating that the results are reproducible. The total number of IL-4-positive (IL-4⁺) mast cells was calculated by multiplying this percentage value by the number of Try⁺ cells, resulting in 35 ± 19 cells/mm² (range 5.6–87). There was, however, no correlation between the percentage of IL-4⁺ mast cells and the number of Try⁺ cells ($r = 0.177$, $P = 0.220$). In addition, neither the number ($r = -0.264$, $P = 0.063$) nor the percentage ($r = -0.220$, $P = 0.126$) of IL-4⁺ mast cells revealed any significant association with the age of the subjects.

To test whether the number of IL-4⁺ mast cells could be related to the wheal reaction induced by 10 mg/ml cow allergen, the correlation of the percentage of IL-4⁺ mast cells with the wheal size (Fig. 2a) and wheal ratio (Fig. 2b) was measured, and both correlations proved to be highly significant. Although the number of Try⁺ and Chy⁺ cells showed no relation to the wheal reaction, the number of IL-4⁺ mast cells correlated significantly with the wheal size (Fig. 3) and wheal ratio ($r = 0.418$, $P = 0.003$). The relationship between IL-4⁺ mast cells and the wheal reaction was also confirmed by using 1.0 mg/ml cow allergen – both the number ($P \leq 0.022$) and the percentage ($P \leq 0.030$) of IL-4⁺ mast cells correlated significantly with the wheal size and wheal

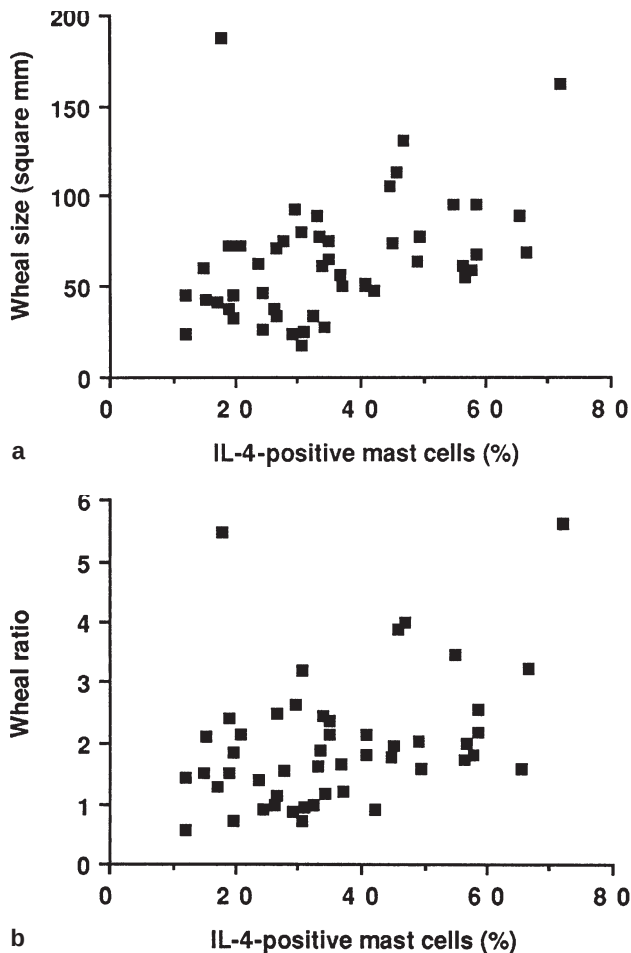


Figure 2. Percentage of IL-4-positive mast cells was significantly associated with (a) wheal size induced by 10 mg/ml cow allergen ($r=0.526$, $P<0.0001$, when single clearly deviating value is omitted), as well as with (b) wheal ratio of cow allergen to histamine control ($r=0.464$, $P=0.001$) in healthy-looking forearm skin of 50 sensitive atopic subjects.

ratio. Regarding the wheal size induced by 10 mg/ml histamine, i.e., the control wheal, no correlation or even any tendency to IL-4 values was noted.

Previously, chymotryptic mast-cell proteases have been reported to degrade IL-4 (25), a finding which prompted us to study the association between Chy^+ and IL-4 $^+$ mast cells. However, no significant correlation was observed between these variables in respect to cell numbers and percentages. Nevertheless, a tendency ($P=0.058$) toward inverse association was noted. Thus, it may be possible that the lower the chymase activity, the higher the IL-4 level.

α_1 -PI- and α_1 -AC-positive mast cells are not associated with the wheal size, IL-4, and chymase

The chymase inhibitors, α_1 -PI and α_1 -AC (11), in mast cells were stained by using the double-staining method (for illustrative figures, see refs. 9 and 10). Consequently, $95.2 \pm 5.5\%$ and $75.5 \pm 11.2\%$ of the

Try $^+$ cells proved to be positive for α_1 -AC and α_1 -PI, respectively, but neither value was dependent on the age of the subjects.

The association of the percentage of these protease inhibitors with the $\text{Chy}^+/\text{Try}^+$ ratio was not significant ($r=0.003$, $P=0.985$ for α_1 -AC; $r=0.139$, $P=0.336$ for α_1 -PI). In addition, neither inhibitor was related to the percentage or number of IL-4 $^+$ mast cells. Furthermore, no significant correlation between α_1 -PI $^+$ and α_1 -AC $^+$ mast cells and the wheal ratio or wheal size induced by 1.0 or 10 mg/ml cow allergen was found. Since both inhibitors were stained by the same double-staining method as IL-4, this statistically nonsignificant association of α_1 -PI and α_1 -AC with the wheal reaction served as a methodological control for the significant association between IL-4 and the wheal size.

The number of mast cells was correlated with serum total IgE

The serum level for total and cow-specific IgE was 285 ± 429 U/ml and 6.5 ± 11.8 U/ml, respectively. Neither value revealed any significant relation to the age of the subjects. An association between the wheal ratio and the total ($r=0.292$, $P=0.040$) or specific IgE ($r=0.285$, $P=0.047$) was observed, but no significant correlation between the wheal size and these IgE values.

In correlation of mast cells with IgE levels, the number of Try $^+$ ($r=0.289$, $P=0.042$), IL-4 $^+$ (Fig. 4), and Chy^+ ($r=0.285$, $P=0.045$) mast cells was associated with the total IgE, but not with the specific IgE. However, the percentage of IL-4 $^+$ mast cells was not related to either total or specific IgE.

Wheal size and mast cells are not related to the clinical manifestation of atopic disease

To determine whether asthma and atopic dermatitis, i.e., the extent of atopic disease, could influence the wheal size and mast-cell numbers in the healthy-looking skin, the subjects were divided either into asthmatic ($n=17$) and nonasthmatic ($n=33$) subjects, or dermatitic ($n=22$) and nondermatitic ($n=28$) subjects. Nevertheless, no significant (t -test) differences in Try $^+$, Chy^+ , and IL-4 $^+$ cells, $\text{Chy}^+/\text{Try}^+$ ratio, IL-4 percentage, and wheal size between the groups were noted.

Discussion

The allergic wheal reaction is induced by several mediators released from mast cells and other sources. Of these mediators, the significance of histamine has been well characterized. However, in our previous microdialysis studies with 10 and 31 subjects, we could not find any significant correlation between the

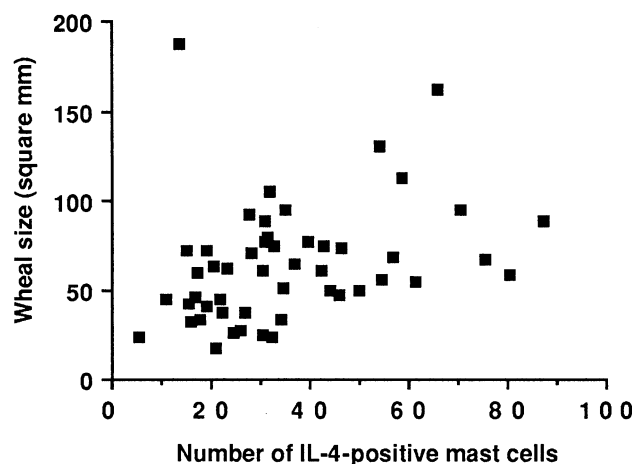


Figure 3. Number of IL-4-positive mast cells (cells/mm²) correlated significantly with wheal size induced by 10 mg/ml cow allergen ($r=0.492$, $P<0.0001$, when single deviating value is omitted) in healthy-looking forearm skin of 50 sensitive atopic subjects.

wheal size and histamine release induced by the cow allergen (27, 29). The major proteins in the secretory granules of the skin mast cells are tryptase and chymase (1, 8), but their contribution to the wheal reaction is unknown. Of the several cytokines in mast cells, IL-4 is essential in IgE synthesis and thus capable of modulating mast-cell activation indirectly, but IL-4 may also augment the mediator release from the mast cells in a more direct manner (18–21).

In this study, the number of Try⁺ cells did not correlate with the wheal ratio or wheal size induced by 1.0 or 10 mg/ml cow allergen, suggesting that tryptase has only a minor role, if any, in the wheal reaction. This assumption is also supported by the recent finding that tryptase can induce histamine release from the human tonsillar mast cells, but not from the skin mast cells (17). Since Try⁺ cells reflect the total number of mast cells in the skin, other mediators than tryptase are more likely to cause the wheal. Nevertheless, although tryptase injected intradermally may induce whealing, the principal function of this enzyme is likely to be in the slower inflammatory responses in the skin (12, 30). Like the number of Try⁺ cells, that of Chy⁺ cells was not associated with the wheal reaction. The Chy⁺/Try⁺ ratio showed a weakly negative correlation with the wheal ratio, but not with the wheal size. Thus, these results suggest that chymase has no crucial role in the wheal reaction. Previously, it has been shown that mast cells release IL-4 upon IgE-dependent stimulation, but this cytokine may be degraded by chymotryptic proteases (25). Recently, we have found, however, that during the wheal reaction in the prick test chymase is inactivated within 30 min, as determined enzyme- and immunohistochemically (Saarinen et al., unpublished results; see also ref.

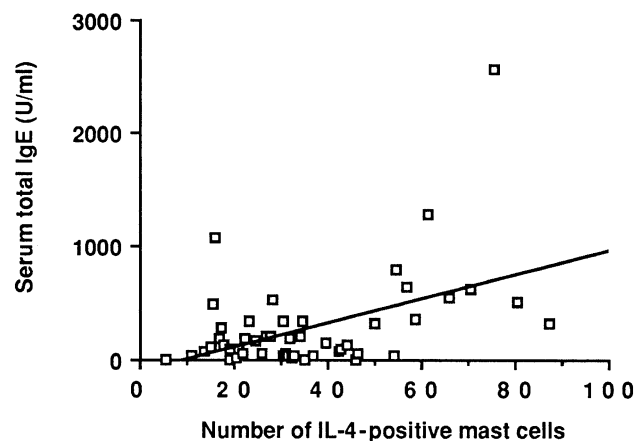


Figure 4. Total IgE levels in serum were associated with the number of IL-4-positive mast cells ($r=0.314$, $P=0.026$) in 50 atopic subjects.

10). The high levels of the chymase inhibitors α_1 -PI and α_1 -AC (11) in the mast cells may protect IL-4 from being degraded.

The involvement of IL-4 in atopic diseases is widely recognized, and Th2 lymphocytes are essential in these disorders (18, 31). However, IL-4 derived from the mast cells can recruit immune cells via the induction of adhesion molecules and promote the development of Th2 cells, an effect which eventually leads to the formation of an atopic dermatitis lesion (23, 31, 32). Despite the extensive characterization of IL-4 in more chronic conditions, evidence hardly exists to show whether IL-4 can be involved in immediate wheal reactions. Induction of IgE synthesis by B cells is an example of the indirect functions of IL-4 (18). On the other hand, IgE-dependent stimulation of the dermal mast cells leads to increased synthesis of IL-4 mRNA (22). Recent studies show that IL-4 and IgE, alone or in combination, can induce FcεRI expression and render the mast cells more reactive to IgE-dependent mediator release (19–21). Moreover, IL-4 can inhibit nitric oxide production by rat peritoneal cells, resulting in increased IgE-dependent secretory function of rat mast cells (33). These experimental results suggest that IL-4 could augment the wheal formation also locally in the skin upon IgE-dependent mast-cell activation. Whether IL-4 can directly induce wheals is unclear. Intravenous administration of recombinant IL-4 to cancer patients has caused toxic symptoms such as nasal congestion, diarrhea, nausea, dyspnea, and edema (34), but subcutaneous administration reportedly is well tolerated at the injection sites (35).

In contrast to the lack of correlation of tryptase, chymase, α_1 -PI, and α_1 -AC with the wheal reaction in this study, both the number and percentage of IL-4⁺ mast cells in the healthy-looking skin before prick testing correlated significantly with the wheal ratio and wheal size induced by 1.0 or 10 mg/ml cow

allergen, but not with the wheal size induced by 10 mg/ml histamine control. The increased number of IL-4⁺ mast cells along with increased wheal size could be a consequence of increased allergy severity. However, the wheal reaction was rather insignificantly related to the levels of specific or total IgE in serum. Moreover, Try⁺, Chy⁺, and IL-4⁺ cells, as well as wheal size, were not significantly related to the manifestation of atopic disease. This suggests that general activation of the immune system and the extent of clinical atopic disease were not the critical factors that simply explain the wheal reaction and its association with IL-4⁺ mast cells in this study. This conclusion is supported by the finding that the percentage of IL-4⁺ mast cells in the healthy-looking skin of atopic dermatitis is equal to that in the healthy-looking skin of nonatopic nummular eczema and close to that in the normal skin (23). Since the number, but not the percentage, of IL-4⁺ mast cells was associated with total IgE in serum, as were also the numbers of Try⁺ and Chy⁺ cells, it is possible that IL-4 from mast cells stimulates local IgE production. However, this is rather unlikely since B cells are rarely present in significant numbers in the healthy-looking skin. Although IL-4 alone seems not to be sufficient to induce wheal reactions (21, 35), IgE and IL-4 may function alone or synergistically in

increasing the reactivity of the skin mast cells to mediator release (19, 20, 33). Furthermore, although the levels of cow-specific IgE did not correlate with the number of Try⁺, Chy⁺, and IL-4⁺ cells, both IgE and IL-4 could induce FcεRI expression on skin mast cells (19, 20); therefore, more IgE and also specific IgE bound to the cells. Nevertheless, the clear linear correlation between the wheal size and IL-4⁺ mast cells suggests that IL-4 is markedly involved in the clinical wheal reaction, but the mechanisms need to be studied in future.

In conclusion, this study shows for the first time that IL-4⁺ mast cells, but not tryptase⁺ and chymase⁺ mast cells, correlate significantly with the extent of clinically relevant allergic wheal reaction under experimentally controlled conditions. It may be possible that IL-4 can exert a direct local effect on the skin, or that the effect is mediated, in part, via elevated IgE levels.

Acknowledgments

We thank the Finnish Allergy Foundation and the North Savo Fund of the Finnish Cultural Foundation for financial support, and Ms Anne Koivisto for expert technical assistance.

References

1. HARVIMA RJ, SCHWARTZ LB. Mast cell derived mediators. In: FOREMAN J, editor. Immunopharmacology of mast cells and basophils. London: Academic Press, 1993:115–138.
2. BRADDDING P, FEATHER IH, HOWARTH PH, et al. Interleukin 4 is localized to and released by human mast cells. *J Exp Med* 1992;**176**:1381–1386.
3. BRESSLER RB, LESKO J, JONES ML, et al. Production of IL-5 and granulocyte-macrophage colony-stimulating factor by naive human mast cells activated by high-affinity IgE receptor ligation. *J Allergy Clin Immunol* 1997;**99**:508–514.
4. KRÜGER-KRASAGAKES S, MÖLLER A, KOLDE G, LIPPERT U, WEBER M, HENZ BM. Production of interleukin-6 by human mast cells and basophilic cells. *J Invest Dermatol* 1996;**106**:75–79.
5. MÖLLER A, LIPPERT U, LESSMANN D, et al. Human mast cells produce IL-8. *J Immunol* 1993;**151**:3261–3266.
6. BURD PR, THOMPSON WC, MAX EE, MILLS FC. Activated mast cells produce interleukin 13. *J Exp Med* 1995;**181**:1373–1380.
7. ACKERMANN L, HARVIMA IT, PELKONEN J, et al. Mast cells in psoriatic skin are strongly positive for interferon-gamma. *Br J Dermatol* 1999;**140**:624–633.
8. IRANI AA, BRADFORD TR, KEPLEY CL, SCHECTER NM, SCHWARTZ LB. Detection of MC_T and MC_{TC} types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. *J Histochem Cytochem* 1989;**37**:1509–1515.
9. HARVIMA IT, HAAPANEN L, ACKERMANN L, NAUKKARINEN A, HARVIMA RJ, HORSMANHEIMO M. Decreased chymase activity is associated with increased levels of protease inhibitors in mast cells of psoriatic lesions. *Acta Derm Venereol (Stockh)* 1999;**79**:98–104.
10. KAMINSKA R, HARVIMA IT, NAUKKARINEN A, NILSSON G, HORSMANHEIMO M. Alterations in mast cell proteinases and protease inhibitors in the progress of cutaneous herpes zoster infection. *J Pathol* 1996;**180**:434–440.
11. SCHECHTER NM, SPROWS JL, SCHOENBERGER OL, LAZARUS GS, COOPERMAN BS, RUBIN H. Reaction of human skin chymotrypsin-like proteinase chymase with plasma proteinase inhibitors. *J Biol Chem* 1989;**264**:21308–21315.
12. HARVIMA IT, HARVIMA RJ, NAUKKARINEN A, HORSMANHEIMO M. Skin tryptase: features and expression in human dermatological disorders. In: CAUGHEY GH, editor. Mast cell proteases in immunology and biology. New York: Marcel Dekker, 1995:25–46.
13. RUBINSTEIN I, NADEL JA, GRAF PD, CAUGHEY GH. Mast cell chymase potentiates histamine-induced wheal formation in the skin of ragweed-allergic dogs. *J Clin Invest* 1990;**86**:555–559.
14. TRAVIS J, REILLY C. The role of leukocyte cathepsin G and chymase in inflammation. In: OGAWA H, LAZARUS GS, HOPU-HAVU VK, editors. The biological role of proteinases and their inhibitors in skin. Tokyo: University of Tokyo Press, 1985:121–125.
15. CAUGHEY GH, LEIDIG F, VIRO NF, NADEL JA. Substance P and vasoactive intestinal peptide degradation by mast cell tryptase and chymase. *J Pharmacol Exp Ther* 1988;**244**:133–137.
16. SCHWARTZ LB, ATKINS PC, BRADFORD TR, FLEEKOP P, SHALIT M, ZWEIMAN B. Release of tryptase together with histamine during the immediate cutaneous response to allergen. *J Allergy Clin Immunol* 1987;**80**:850–855.

17. HE S, GAÇA MDA, WALLS AF. A role for tryptase in the activation of human mast cells: modulation of histamine release by tryptase and inhibitors of tryptase. *J Pharmacol Exp Ther* 1998;**286**:289–297.
18. WORM M, HENZ BM. Molecular regulation of human IgE synthesis. *J Mol Med* 1997;**75**:440–447.
19. XIA HZ, DU Z, CRAIG S, et al. Effect of recombinant human IL-4 on tryptase, chymase, and Fce receptor type I expression in recombinant human stem cell factor-dependent fetal liver-derived human mast cells. *J Immunol* 1997;**159**:2911–2921.
20. YAMAGUCHI M, SAYAMA K, YANO K, et al. IgE enhances Fce receptor I expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: synergistic effect of IL-4 and IgE on human mast cell Fce receptor I expression and mediator release. *J Immunol* 1999;**162**:5455–5465.
21. BISCHOFF SC, SELLGE G, LORENTZ A, SEBALD W, RAAB R, MANNS MP. IL-4 enhances proliferation and mediator release in mature human mast cells. *Proc Natl Acad Sci U S A* 1999;**96**:8080–8085.
22. OKAYAMA Y, ONO Y, NAKAZAWA T, CHURCH MK, MORI M. Human skin mast cells produce TNF-alpha by substance P. *Int Arch Allergy Immunol* 1998;**117 Suppl 1**:48–51.
23. HORSMANHEIMO L, HARVIMA IT, JÄRVIKALLIO A, HARVIMA RJ, NAUKKARINEN A, HORSMANHEIMO M. Mast cells are one major source of interleukin-4 in atopic dermatitis. *Br J Dermatol* 1994;**131**:348–353.
24. JÄRVIKALLIO A, NAUKKARINEN A, HARVIMA IT, AALTO M-L, HORSMANHEIMO M. Quantitative analysis of tryptase-, and chymase-containing mast cells in atopic dermatitis and nummular eczema. *Br J Dermatol* 1997;**136**:871–877.
25. TUNON DE LARA JM, OKAYAMA Y, McEUEEN AR, HEUSSER CH, CHURCH MK, WALLS AF. Release and inactivation of interleukin-4 by mast cells. *Ann N Y Acad Sci* 1994;**725**:50–58.
26. HE S, GAÇA MDA, McEUEEN AR, WALLS AF. Inhibitors of chymase as mast cell-stabilizing agents: contribution of chymase in the activation of human mast cells. *J Pharmacol Exp Ther* 1999;**291**:517–523.
27. HORSMANHEIMO L, HARVIMA IT, HARVIMA RJ, YLÖNEN J, NAUKKARINEN A, HORSMANHEIMO M. Histamine release in skin monitored with the microdialysis technique does not correlate with the weal size induced by cow allergen. *Br J Dermatol* 1996;**134**:94–100.
28. POULSEN LK, LIISBERG C, BINDSLEV-JENSEN C, MALLING H-J. Precise area determination of skin-prick tests: validation of a scanning device and software for a personal computer. *Clin Exp Allergy* 1993;**23**:61–68.
29. SAARINEN JV, HARVIMA RJ, NAUKKARINEN A, HORSMANHEIMO M, HARVIMA IT. Release of histamine and leukotriene C₄ in immediate allergic wheal reaction as measured with the microdialysis technique. *Arch Dermatol Res* 2000;**292**:333–340.
30. HE S, PENG Q, WALLS AF. Potent induction of a neutrophil and eosinophil-rich infiltrate *in vivo* by human mast cell tryptase: selective enhancement of eosinophil recruitment by histamine. *J Immunol* 1997;**159**:6216–6225.
31. RICCI M, MATUCCI A, ROSSI O. Source of IL-4 able to induce the development of TH2-like cells. *Clin Exp Allergy* 1997;**27**:488–500.
32. WANG M, SAXON A, DIAZ-SANCHEZ D. Early IL-4 production driving Th2 differentiation in a human *in vivo* allergic model is mast cell derived. *Clin Immunol* 1999;**90**:47–54.
33. DESCHOOLMEESTER ML, EASTMOND NC, DEARMAN RJ, KIMBER I, BASKETTER DA, COLEMAN JW. Reciprocal effects of interleukin-4 and interferon- γ on immunoglobulin E-mediated mast cell degranulation: a role for nitric oxide but not peroxynitrite or cyclic guanosine monophosphate. *Immunology* 1999;**96**:138–144.
34. ATKINS MB, VACHINO G, TILG HJ, et al. Phase I evaluation of thrice-daily intravenous bolus interleukin-4 in patients with refractory malignancies. *J Clin Oncol* 1992;**10**:1802–1809.
35. GILLEECCE MH, SCARFFE JH, GHOSH A, et al. Recombinant human interleukin 4 (IL-4) given as daily subcutaneous injections – a phase I dose toxicity trial. *Br J Cancer* 1992;**66**:204–210.