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Lavender Oil Inhibits Immediate-type Allergic Reaction in Mice and Rats

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Abstract

We studied the effects of lavender oil on mast cell-mediated immediate-type allergic reactions in mice and rats.

Lavender oil (1 : 500, 1 : 100, 1 : 10, 1 : 1, 1 : 0) inhibited concentration-dependently mast cell-dependent ear swelling response induced by compound 48/80 in mice by both topical and intradermal application. Lavender oil (1 : 500, 1 : 100, 1 : 10, 1 : 1, 1 : 0) inhibited concentration-dependently passive cutaneous anaphylaxis induced by anti-dinitrophenyl (DNP) IgE in rats by both topical and intradermal application.

Lavender oil (1 : 500, 1 : 100, 1 : 10, 1 : 1, 1 : 0) also inhibited concentration-dependently the histamine release from the peritoneal mast cells by compound 48/80 or anti-DNP IgE. Moreover, lavender oil (1 : 1000, 1 : 100, 1 : 10, 1 : 0) had a significant inhibitory effect on anti-DNP IgE-induced tumour necrosis factor-α secretion from peritoneal mast cells.

These results indicate that lavender oil inhibits immediate-type allergic reactions by inhibition of mast cell degranulation in-vivo and in-vitro.

Lavender oil is most versatile and is useful in skincare preparations for all skin types. Several oils present in fruits, seeds and flowers are known to exert anti-anaphylactic effects (Kim et al 1997; Shin et al 1997).

It is now well established that mast cells trigger immediate type allergic reactions in response to allergens by releasing chemical mediators (Church & Caulfield 1993). Degranulation of mast cells is caused by non-immunologic secretagogues like substance P (Hua et al 1996), compound 48/80 (Shin et al 1997), and extracellular ATP (Sudo et al 1996), which result in rapid and marked histamine release (Pearce 1989). Histamine induced anaphylactic responses such as vasodilation, increased vascular permeability and contraction of smooth muscles. Thus, mast cells may play some important roles in diverse immunological and pathological processes (Wershil & Galli 1994). Compound 48/80 is best known as a potent inducer of degranulation and of the release, from mast cells, of histamine and other chemical mediators which are responsible for anaphylactic reactions (Allansmith et al 1989). The secretory response of mast cells can also be induced by aggregation of their cell surface-specific receptors for IgE by the corresponding antigen (Segal et al 1977; Metzger et al 1986; Alber et al 1991). The anti-IgE antibody has been established to induce passive cutaneous anaphylaxis (PCA) reaction as a typical model for the immediate type allergic reactions. The skin of rat is a useful site for studying PCA (Saito & Nomura 1989). Given the recent evidence that upon antigen stimulation mast cells are a potential source of various cytokines, including tumour necrosis factor-α (TNF-α) (Burd et al 1989; Plaut et al 1989; Wodnar-Filipowicz et al 1989; Galli et al 1991; Gurish et al 1991), it is likely that they play a crucial role in allergic inflammation. The role of TNF-α is of particular interest because the mast cell is the only known cell to store TNF-α and is thus able to release this mediator immediately upon activation (Zhang et al 1992). Therefore, modulation of TNF-α secretion by mast cells should provide us with a useful therapeutic strategy for allergic diseases.

In this paper, we show that lavender oil inhibits both compound 48/80-induced ear swelling response

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in mice and anti-dinitrophenyl (DNP) IgE-induced PCA in rats. We also investigated the influence of lavender oil on compound 48/80 and anti-DNP IgE-induced histamine release and anti-DNP IgE-induced TNF-α secretion from peritoneal mast cells.

Materials and Methods

Materials

Lavender oil was purchased from The Aromatherapy Products Limited, Brighton, UK. Compound 48/80, anti-DNP IgE, DNP-human serum albumin (HSA), bovine serum albumin, and metrizamide were purchased from Sigma Chemical Co. (St Louis, MO). Murine TNF-α and anti-murine TNF-α were obtained from Genzyme (Cambridge, MA). Tissue culture flask and plates were obtained from Nunc (Naperville, IL). WB−+/+ and C57BL6−+/+ mice were raised in our laboratory, and (WB × C57BL/6) F_1−+/+ and $-W/W^v$ mice (hereafter called $WBB6F_1$−+/+ and $-W/W^v$ mice) were purchased from the Japan SLC (Hamamatsu, Japan). $WBB6F_1$-W/W^v mice are genetically deficient in mast cells (Kitamura et al 1978). Mice were used at two to four months of age. The original stock of Wistar rats was purchased from Dae-Han Experimental Animal Center (Eumsung, Chungbuk, South Korea), and the animals were kept at the College of Pharmacy, Wonkwang University. The animals were housed five to ten per cage in a laminar air flow room maintained under constant temperature ($22 \pm 1°C$) and relative humidity ($55 \pm 10\%$) throughout the study.

Ear swelling response in mice

Compound 48/80 ($20\,mg\,mL^{-1}$) was freshly dissolved in saline and injected intradermally into the dorsal aspect of a mouse ear using a microsyringe with a 28-gauge hypodermic needle. Ear thickness was measured with a digimatic micrometer (Mitutoyo, Japan) under mild anaesthesia. Ear swelling response represented an increment in thickness above baseline control values. Ear swelling response was determined 30 min after compound 48/80 or vehicle injection. The values obtained would appear to represent the effect of compound 48/80 rather than the effect of the vehicle injected (physical swelling), since, within the 30-min interval, the ear-swelling response evoked by physiologic saline returned to almost baseline thickness.

PCA in the rat

An IgE-dependent skin reaction was generated by sensitizing the skin with an intradermal injection of anti-DNP IgE followed 48 h later with an injection of DNP-HSA into the tail vein. The anti-DNP IgE and DNP-HSA were diluted in phosphate-buffered saline (PBS). The rats were injected intradermally with 0·5 μg (50 μL) anti-DNP IgE into each of four dorsal skin sites that had been shaved 48 h earlier. The sites were outlined with a water-insoluble red marker. Forty-eight hours later each rat received an injection of 1 mg DNP-HSA in PBS containing 4% Evans blue (1 : 4) through the tail vein. Lavender oil was administered topically or intradermally 4 h before the challenge. Lavender oil was diluted in 70% ethanol. Thirty minutes after the challenge, the rats were killed by decapitation after ether anaesthesia, and the dorsal skin was removed for measurement of pigment area. The amount of dye was then determined colorimetrically after extraction with 1 mL 1·0 M KOH and 9 mL acetone–phosphoric acid (5 : 13) by the method of Katayama et al (1978). The absorbant intensity of the extraction was measured at 620 nm using a spectrophotometer, and the amount of dye was calculated with the Evans blue measuring line.

Preparation of peritoneal mast cells

Peritoneal mast cells were isolated as previously described (Shin et al 1997). In brief, rats were anaesthetized by ether and injected with 20 mL Tyrode buffer B (in mM: 137 NaCl, 5·6 glucose, 12 $NaHCO_3$, 2·7 KCl, 0·3 NaH_2PO_4) containing 0·1% gelatin (Sigma Chemical Co.), into the peritoneal cavity, and the abdomen was gently massaged for approximately 90 s. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells was aspirated by a Pasteur pipette. Thereafter, the peritoneal cells were sedimented at 150 *g* for 10 min at room temperature and resuspended in Tyrode buffer B. Mast cells were separated from major components of rat peritoneal cells, that is, macrophages and small lymphocytes, according to the method described by Yurt et al (1977). In brief, peritoneal cells suspended in 1 mL Tyrode buffer B were layered on 2 mL 22·5% w/v metrizamide (density, $1{\cdot}120\,g\,mL^{-1}$, Sigma Chemical Co.) and centrifuged at 400 *g* for 15 min at room temperature. The cells remaining at the buffer-metrizamide interface were aspirated and discarded; the cells in the pellet were washed and resuspended in 1 mL Tyrode buffer A (in mM: 10 HEPES, 130 NaCl, 5 KCl, 1·4 $CaCl_2$, 1 $Mg\,Cl_2$, 5·6 glucose). Mast cell preparations were about 95% pure as assessed by toluidine blue staining. More than 97% of cells were viable, as judged by Trypan blue uptake.

Inhibition of histamine release

Mast cell suspensions (10^6 cells mL^{-1}) were preincubated for 10 min at 37°C before the addition of compound 48/80 (5 $\mu g\,mL^{-1}$). The cells were preincubated with the lavender oil preparations, and then incubated (10 min) with compound 48/80. Mast cell suspensions were also sensitized with anti-DNP IgE (10 $\mu g\,mL^{-1}$) for 6 h. The cells were preincubated with the lavender oil at 37°C for 10 min before the challenge with DNP-HSA (1 $\mu g\,mL^{-1}$). The cells were separated from the released histamine by centrifugation at 400 *g* for 5 min at 4°C. Residual histamine in cells was released by disrupting the cells with perchloric acid and centrifugation at 400 *g* for 5 min at 4°C. Histamine content was measured by the *o*-phthalaldehyde spectroflurometric procedure of Shore et al (1959). The fluorescent intensity was measured at 438 nm (excitation at 353 nm) in a spectrofluorometer.

Assay of histamine release

The inhibition percentage of histamine release was calculated by using the following equation:

$$\% \text{ Inhibition} = (a - b) \times 100/a \qquad (1)$$

where a is histamine release without lavender oil and b is histamine release with lavender oil.

Assay of TNF-α secretion

TNF-α secretion was measured by a modified enzyme-linked immunosorbent assay (ELISA) as described by Scuderi et al (1986). The ELISA was sensitive to TNF-α concentrations in the medium above 1 pg mL^{-1}. The ELISA was devised by coating 96-well plates with 6·25 ng/well of murine monoclonal antibody with specificity for murine TNF-α. Before use and between subsequent steps in the assay, the coated plates were washed twice with PBS containing 0·05% Tween-20 and twice with PBS alone. All reagents used in this assay and the coated wells were incubated for 1 h at room temperature. For the standard curve, rTNF-α was added to plasma previously determined to be negative for endogenous TNF-α. After exposure to the medium, the assay plates were sequentially exposed to rabbit anti-TNF-α, phosphatase-conjugated goat anti-rabbit IgG, and *p*-nitro phenyl phosphate. Optical density readings were made within 10 min of the addition of the substrate on a Titertek Multiscan (Flow Laboratories) with a 405 nm filter. Appropriate specificity controls were included.

Statistical analysis

The results obtained were expressed as mean ± s.e. for the number of experiments. Student's *t*-test was used to make a statistical comparison between the groups. Results with $P < 0.05$ were considered statistically significant.

Results

In initial experiments, we confirmed that topical application of compound 48/80 can induce an ear-swelling response in normal ($WBB6F_1-+/+$) mice but not in the congenic mast cell-deficient $WBB6F_1-W/W^v$ mice. Compound 48/80 significantly induced an ear swelling response at concentrations of 50–200 μg/site (data not shown). We chose a concentration of 200 μg/site for compound 48/80 for optimal ear-swelling response in subsequent experiments. As shown in Figure 1, when mice were pretreated with lavender oil for 30 min, the ear-swelling response with compound 48/80 was reduced concentration-dependently. Given intradermally, the effect of lavender oil was slightly weaker than after topical application. Another way to test mast cell-mediated skin allergic reaction is to induce PCA (Wershil et al 1987). As described in Materials and Methods local extravasation is induced by local injection of anti-DNP IgE followed by an intravenous antigenic challenge. Anti-DNP IgE was injected in the right dorsal skin sites of rats. As a control, the left dorsal skin site of these rats was injected with saline

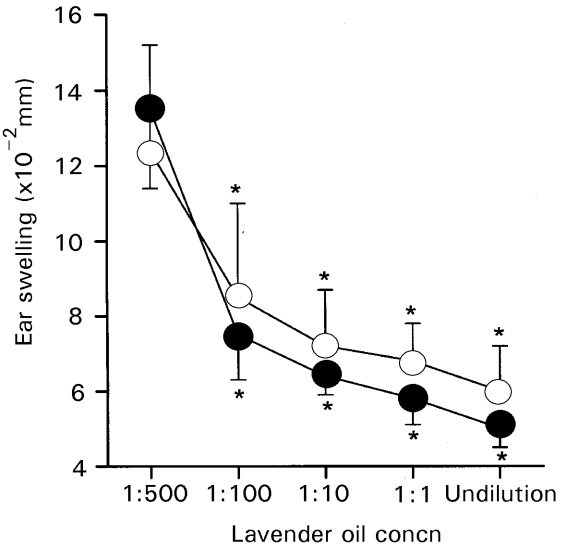

Figure 1. Effect of lavender oil on compound 48/80-induced ear swelling response in $WBB6F_1-+/+$ mice. Ten microlitres of compound 48/80 (200 μg/site) were applied to the ears of mice (●, topically; ○, intradermally). The skins of the ears were treated with the indicated concentrations of lavender oil for 30 min and then stimulated with compound 48/80 (200 μg/site). Each datum represents the mean ± s.e. of seven independent experiments. *$P < 0.05$; significantly different from the saline value.

Table 1. Effect of topical and intradermal application of lavender oil on PCA in rats.

Treatment	Concentration (dilution)	Amount of dye (μg/site)	
		Topical	Intradermal
Saline	–	4·915 ± 0·182	5·673 ± 0·876
Lavender oil	1 : 100	4·662 ± 0·454	5·531 ± 1·004
	1 : 10	4·163 ± 0·501*	5·184 ± 0·833
	1 : 1	1·798 ± 0·324*	4·628 ± 1·720
	1 : 0	0·945 ± 0·157*	2·367 ± 0·462*

Each dose (10 μL) of lavender oil was applied topically 4 h before the challenge with antigen. Each amount of dye represents the mean ± s.e. of three independent experiments. *$P < 0·05$; significantly different from the saline value.

alone. After 48 h, all animals were injected intravenously with DNP-HSA treated with Evans blue dye. The cutaneous allergic reaction was best visualized by the extravasation of the dye. Both the topical and intradermal applications of lavender oil inhibited PCA concentration-dependently (Table 1). We also found that the intradermal application of lavender oil showed slightly less substantial inhibition than that of the topical application.

Lavender oil inhibited concentration-dependently compound 48/80 or anti-DNP IgE-induced histamine release from peritoneal mast cells (Figure 2). The inhibitory effect of lavender oil on histamine release was more significant for compound 48/80 treatment. Lavender oil inhibited concentration-dependently anti-DNP IgE-induced TNF-α secretion from peritoneal mast cells (Table 2). No significant cytotoxicity of the lavender oil on cultures was observed at the concentrations used in the above experiments as assessed by Trypan blue uptake.

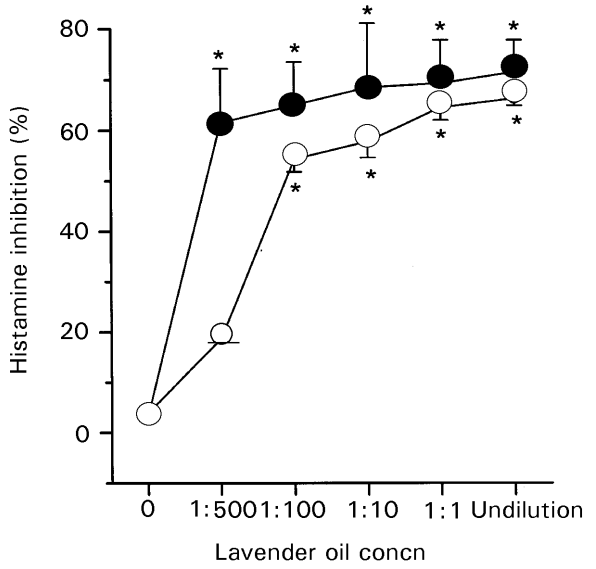

Figure 2. Effect of lavender oil on compound 48/80 (●) or anti-DNP IgE-mediated histamine release (○) from peritoneal mast cells. Peritoneal mast cells (10^6 cells) were preincubated with various concentrations of lavender oil (10 μL) at 37°C for 10 min before incubation with compound 48/80 or before the challenge with DNP-HSA. Each datum represents the mean ± s.e. of three independent experiments. *$P < 0·05$; significantly different from the saline value.

Table 2. Effect of lavender oil on anti-DNP IgE-induced TNF-α secretion.

Lavender oil (dilution)	Anti-DNP IgE plus DNP-HSA	TNF-α secretion (ng mL^{-1})
– (Saline)	– (Saline)	0·014 ± 0·005
– (Saline)	+	0·142 ± 0·007
1 : 1000	+	0·089 ± 0·006*
1 : 100	+	0·083 ± 0·003*
1 : 10	+	0·076 ± 0·009*
1 : 0	+	0·065 ± 0·003*

Peritoneal mast cells (10^6 cells mL^{-1}) were sensitized with anti-DNP IgE (1 μg mL^{-1}) for 16 h and incubated for 20 min with various concentrations of lavender oil (1 μL) before the challenge with DNP-HSA (0·1 μg mL^{-1}) for 4 h. Each datum represents the mean ± s.e. of five independent experiments. *$P < 0·05$; significantly different from the anti-DNP IgE plus DNP-HAS-treated value.

Discussion

The present study showed that lavender oil potently inhibited compound 48/80-induced ear swelling response in mice and anti-DNP IgE-induced PCA reaction in rats by both topical and intradermal application. Topical application of lavender oil tended to be more effective in both reactions than intradermal application. Lavender oil also inhibited the compound 48/80 and anti-DNP IgE-induced histamine release from peritoneal mast cells. There is no doubt that stimulation of mast cells with compound 48/80 initiates the activation of a signal-transduction pathway that leads to histamine release. Some recent studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G proteins (Mousli et al 1990a, b). We observed that topical application of compound 48/80 did not induce a swelling response in ears of WBB6F$_1$-*W*/*W*^{v} mice that had been locally and selectively repaired of their mast cell deficiency (data not shown). Therefore, we speculate that our results indicate that mast cell-mediated immediate-type allergic reactions are inhibited by lavender oil. Tasaka et al (1986) reported that compound 48/80 increased the permeability of the lipid bilayer membrane by causing a perturbation of the membrane. This result indicates that the membrane permeability increase

may be an essential trigger for the release of mediators from mast cells. Lavender oil might act on the lipid bilayer membrane affecting the prevention of the perturbation being induced by compound 48/80.

Rats receiving lavender oil are protected from IgE-mediated allergic reaction. The mechanism of the protection against anti-DNP IgE, while not clear at present, may be suggested only in particular conditions. It is conceivable that lavender oil inhibits the initial phase of immediate type allergic reactions, probably through interference with the degranulation system. TNF-α is a multifunctional cytokine that has a pro-inflammatory role. In the skin, mast cell-derived TNF-α has been shown to be critical for neutrophil migration in allergic inflammation (Wershil et al 1991). Our data showed that lavender oil inhibited anti-DNP IgE-induced TNF-α secretion from mast cells. The effect of lavender oil on mast cell cytokine production in-vivo and the relative importance of mast cells as a source of TNF-α during inflammatory and immune responses are important areas for future studies.

Our results demonstrated that lavender oil inhibited the immediate-type allergic reactions in-vivo and in-vitro in a murine model. To our knowledge, this is the first report of such selective inhibition of mast cell-mediated immediate-type allergic reactions by lavender oil.

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References

Alber, G., Miller, L., Jelsema, C., Varin-Blank, N., Metzger, H. (1991) Structure/function relationships in the mast cell high-affinity receptor for IgE (FcεRI) : role of cytoplasmic domains. J. Biol. Chem. 266: 22613–22620

Allansmith, M. R., Baird, R. S., Ross, R. N., Barney, N. P., Bloch, K. J. (1989) Ocular anaphylaxis induced in the rat by topical application of compound 48/80. Dose response and time course study. Acta Ophthalmol. 67: 145–153

Burd, P. R., Rogers, H. W., Gordon, J. R., Martin, C. A., Jayaraman, S., Wilson, S. D., Dvorak, A. M., Galli, S. J., Dorf, M. E. (1989) Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. J. Exp. Med. 170: 245–257

Church, M. K., Caulfield, J. P. (1993) Mast cell and basophil functions. In: Holgate, S. T., Church, M. K. (eds) Allergy. Gower Medical Publishing, London

Galli, S. J., Gordon, J. R., Wershil, B. K. (1991) Cytokine production by mast cells and basophils. Curr. Opin. Immunol. 3: 865–872

Gurish, M. F., Ghildyal, N., Arm, J., Austen, K. F., Avraham, S., Reynolds, D. S., Stevens, R. L. (1991) Cytokine mRNA are preferentially increased relative to secretory granule protein mRNA in mouse bone marrow-derived mast cells that have undergone IgE-mediated activation and degranulation. J. Immunol. 146: 1527–1533

Hua, X. Y., Back, S. M., Tam, E. K. (1996) Substance P enhances electrical field stimulation-induced mast cell degranulation in rat trachea. Am. J. Physiol. 270: L985–L991

Katayama, S., Shionoya, H., Ohtake, S. (1978) A new method for extraction of extravasated dye in the skin and the influence of fasting stress on passive cutaneous anaphylaxis in guinea pigs and rats. Microbiol. Immunol. 22: 89–101

Kim, H. M., Lee, E. H., Kim, C. Y., Chung, J. G., Kim, S. H., Lim, J. P., Shin, T. Y. (1997) Antianaphylactic properties of eugenol. Pharm. Res. 36: 475–486

Kitamura, Y., Go, S., Hatnaka, K. (1978) Decrease of mast cells in W/W^v mice and their increase by bone marrow transplantation. Blood 52: 447–452

Metzger, H., Alcaraz, G., Hohman, R., Kinet, J. P., Pribluda, V., Quarto, R. (1986) The receptor with high affinity for immunoglobulin E. Annu. Rev. Immunol. 4: 419–470

Mousli, M. C., Bronner, C., Bockaert, J., Rouot, B., Landry, Y. (1990a) Interaction of substance P, compound 48/80 and mastoparan with α-subunit C-terminal of G protein. Immunol. Lett. 25: 355–358

Mousli, M. C., Bronner, C., Landry, Y., Bockaert, J., Rouot, B. (1990b) Direct activation of GTP-binding regulatory proteins (G proteins) by substance P and compound 48/80. FEBS Lett. 259: 260–262

Pearce, F. L. (1989) Mast cells: function, differentiation and activation. Curr. Opin. Immunol. 1: 630–636

Plaut, M., Pierce, J. H., Watson, C. J., Hanley-Hyde, J., Nordon, R. P., Paul, W. E. (1989) Mast cell lines produce lymphokines in response to cross-linkage of FcεRI or to calcium ionophores. Nature 339: 64–67

Saito, H., Nomura, Y. (1989) Screening methods for drug evaluation 3. In: Suzuki, L., Tanaka, H., Yajima, H., Fukuda, H., Sezaki, H., Koga, K., Hirobe, M., Nakajime, T. (eds) Pharmaceutical Research and Development. Hirokawa, Tokyo, p. 22

Scuderi, P., Sterling, R. E., Lam, K. S., Finley, P. R., Ryan, K. J., Ray, C. G., Petersen, E., Slymen, D. J., Salmon, S. E. (1986) Raised serum levels of tumor necrosis factor in parasitic infections. Lancet 2: 1364–1365

Segal, D. M., Taurog, J., Metzger, H. (1977) Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation. Proc. Natl Acad. Sci. USA 74: 2993–2997

Shin, B. K., Lee, E. H., Kim, H. M. (1997) Suppression of L-histidine decarboxylase mRNA expression by methyleugenol. Biochem. Biophysics. Res. Commun. 232: 188–191

Shore, P. A., Burkhalter, A., Cohn, V. H. (1959) A method for fluorometric assay of histamine in tissues. J. Pharmacol. Exp. Ther. 127: 182–186

Sudo, N., Tanaka, K., Koga, Y., Okumura, Y., Kubo, C., Nomoto, K. (1996) Extracellular ATP activates mast cells via a mechanism that is different from the activation induced by the cross-linking of Fc receptors. J. Immunol. 156: 3970–3979

Tasaka, K., Mio, M., Okamoto, M. (1986) Intracellular calcium release induced by histamine releasers and its inhibition by some antiallergic drugs. Ann. Allergy 56: 464–469

Wershil, B. K., Galli, S. J. (1994) The analysis of mast cell function in vivo using mast cell-deficient mice. Adv. Exp. Med. Biol. 347: 39–54

Wershil, B. K., Mekori, Y. A., Murakami, T., Galli, S. J. (1987) ^{125}I-fibrin deposition in IgE-dependent immediate hypersensitivity reactions in mouse skin: demonstration of the role of mast cells using genetically mast cell-deficient mice locally reconstituted with cultured mast cells. J. Immunol. 139: 2605–2614

Wershil, B. K., Wang, Z. S., Gordon, J. R., Galli, S. J. (1991) Recruitment of neutrophils during IgE-dependent cutaneous late phase reactions in the mouse is mast cell-dependent. Partial inhibition of the reaction with antiserum against tumor necrosis factor-alpha. J. Clin. Invest. 87: 446–453

Wodnar-Filipowicz, A., Heusser, C. H., Moroni, C. (1989) Production of the haemopoietic growth factors GM-CSF and interleukin-3 by mast cells in response to IgE receptor-mediated activation. Nature 339: 150–152

Yurt, R. W., Leid, R. W., Austen, K. F. (1977) Native heparin from rat peritoneal mast cells. J. Biol. Chem. 252: 518–521

Zhang, Y., Ramos, B. F., Jakschik, B. K. (1992) Neutrophil recruitment by tumor necrosis factor from mast cells in immune complex peritonitis. Science 258: 1957–1959