

Antigenic, Adjuvant and Permeability Enhancing Properties of Melittin in Mice

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Melittin, a mast cell lytic agent which comprises 50% of the protein content of bee venom, may be responsible for some of the severe reactions produced by bee stings in allergic and non-allergic individuals. We therefore investigated some of the biologic properties of this molecule in mice and found that repeated intradermal injections of melittin without adjuvant induced IgE formation in mice. In addition, melittin as well as another surfactant, saponin, were potent adjuvants for IgE formation in mice when mixed with ovalbumin (Ov). Titres of 1280 and 2560 to ovalbumin were produced in CBA mice after two injections of melittin and Ov or saponin and Ov. Melittin was also shown to have an enhancing effect on the vascular permeability induced by an antigen antibody interaction in mouse skin. Evan's blue dye was injected intravenously into ovalbumin-sensitized mice which had been injected intradermally with ovalbumin alone, melittin alone or a mixture of melittin and ovalbumin. The amount of local extravasation of dye was significantly greater in the area injected with the melittin and ovalbumin mixture than the amount of dye obtained from the skin site injected with melittin alone plus that extracted from the site injected with ovalbumin alone.

Key words: adjuvant; hypersensitivity; IgE; melittin.

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CLINICAL ASPECTS

We have demonstrated that melittin (which makes up 50% of the protein of bee venom) is a potent adjuvant for IgE formation in mice. If melittin has similar adjuvant properties in humans, it might be advisable that melittin be removed from bee venom preparations used for hyposensitization.

Melittin was also shown to have an enhancing effect on vascular permeability induced by antigen-antibody interaction in mouse skin. If melittin were shown to have a synergistic effect on allergic reactions to venom proteins in humans, it might be of value to induce neutralizing IgG anti-melittin antibodies in venom-sensitive patients.

Melittin, the principal constituent of venom of the honeybee *Apis mellifica*, is an amphipathic polypeptide consisting of 26 amino acid residues (10). It is strongly surface active and lytic for membranes (9) and causes mast cell rupture with the release of histamine (11, 22). Melittin is likely responsible for the non-specific inflammatory effects produced by bee venom (13) and has recently been shown to be an allergen in humans (14, 19, 20, 27).

In view of the likely importance of melittin in the response of humans to bee venom, we decided to investigate some properties of this compound in mice. We first studied the antigenicity of melittin in this species.

Reaginic as well as IgG₁ antibodies have been produced in mice by the injection of bee venom and Al(OH)₃ (23) and by the injection of melittin and Freund's complete adjuvant (6). Our aim was to induce IgE anti-melittin antibodies in

mice without the use of adjuvants. Such a procedure would bear a greater resemblance to natural sensitization and would perhaps yield an animal more amenable to hyposensitization. In addition, we wished to know a) if melittin itself had adjuvant properties and b) if melittin could influence allergic reactions.

MATERIAL AND METHODS

Stimulation of IgE without adjuvants

The inbred strains of mice, CBA, SWR, 129, C57B1, and SJL were obtained from Jackson Labs, Bar Harbor, Maine. ICR outbred Swiss mice were purchased from Bio Breeding, Ottawa, Ontario and Wistar rats from CBFL Laboratories, St. Constantine, Quebec.

Mice were injected intradermally with 100 μ g of melittin (Sigma, St. Louis, Missouri) in 0.1 ml of physiologic saline 3 times weekly for 6 weeks. Seven days after the last injection the animals were bled from the orbital plexus and the pooled sera of three mice used for IgE assays. IgE levels were determined by PCA reactions in the skin of 200 g male Wistar rats (18). Rats used for PCA were challenged with 300 μ g of melittin intravenously. Since melittin may contain small amounts of phospholipase, the major allergen of bee venom (17), we challenged rats used for PCA with either melittin or phospholipase. Rats challenged with phospholipase A₂ from bee venom of *Apis mellifica* (Sigma, St. Louis, Missouri) did not give positive PCA reactions (unpublished observations).

Adjuvant effect of melittin

CBA mice were injected intradermally with 0.1 ml of a mixture containing 10 μ g of ovalbumin (Ov) (Sigma, St. Louis, Missouri) and 100 μ g of melittin. The animals were bled on day 14, boosted on day 21 with the same dose of melittin and Ov that they received as a first injection, and were bled again 7 days after the second administration of antigen and adjuvant. IgE antibodies to Ov were assayed as previously described. Mice injected with Ov alone served as controls.

Effect of melittin on antigen-antibody reactions in mouse skin

SWR mice had their backs shaved and were then injected intravenously with 0.4 ml of a mouse anti-Ov antiserum (IgE titre 1:640). Twenty-four hours later the animals were injected in three different sites (in the skin of the back) with either 0.05 of melittin, 0.05 ml of Ov or 0.05 ml of a mixture of Ov and melittin. The intradermal injections were followed by an intravenous injection of 0.3 ml of 0.5% Evan's blue dye 10 min later. The doses of Ov and melittin were 5 μ g and 2.5 μ g respectively whether administered singly or as a mixture. The doses of Ov and melittin were chosen because singly they produced minimal skin blueing (about 5–8 mm) in SWR mice. After the diameter of blueing was measured, the reaction site was excised and extracted with 4 ml of formamide (BDH Chemicals, Toronto, Ontario) for 4 days at 45°C (4, 7). The amount of dye extracted was estimated with a Hitachi spectrophotometer Model No. 139 using 623 nanometers as a wavelength for maximal absorbancy.

RESULTS

Stimulation of IgE to melittin without adjuvants

The data in Table 1 indicate that CBA, SWR, ICR and 129 mice given injections of melittin thrice weekly for 6 weeks produced IgE anti-melittin antibodies with titres varying from 20 (ICR) to 320 (SWR).

The adjuvant effect of melittin

The data in Table 2 indicate that melittin acted as an adjuvant when injected into mice along with ovalbumin. Single injections of ovalbumin alone did not induce IgE antibodies 14 days later and a booster injection of Ov alone resulted in an IgE titre of 5 in mice given 10 μ g Ov. In contrast, CBA mice injected with melittin and 10 μ g of Ov had IgE titres of 5 and 80 after primary and secondary injections. Mice injected with 50 μ g Ov and melittin had IgE titres of 20 and 1280 after primary and secondary injections. Sapo-

Table 1

Induction of IgE to melittin in various strains of mice without the use of adjuvants

Strain of mouse	IgE titre*
CBA	80
SWR	320
ICR	20
129	80
C57B1	0
SJL	0

* Mice were injected intradermally 3 times a week for 6 weeks with 100 µg of melittin. Animals were bled 7 days after the last injection and the sera of three mice pooled for IgE assays via PCA in rat skin.

Table 2

The adjuvant effect of melittin (Mel) and saponin (Sap) on IgE formation in CBA mice

Mice injected s.c.* on day 0 with	Mice boosted s.c. on day 21 with	IgE titre (anti-Ov) day 14	IgE titre (anti-Ov) day 28
10 µg Ov	10 µg Ov	0	5
100 µg Mel + 10 µg Ov	100 µg Mel + 10 µg Ov	5	80
100 µg Mel + 50 µg Ov	100 µg Mel + 50 µg Ov	20	1280
100 µg Sap + 50 µg Ov	100 µg Sap + 50 µg Ov	20	2560

* Subcutaneously.

nin, a surfactant which had previously been shown to stimulate IgE in cattle (3), was also used as an adjuvant. When given to mice with 50 µg of Ov, it produced titres of 20 after primary, and 2560 after secondary injections.

Effect of melittin on antigen-antibody interaction in mouse skin

Fig. 1 demonstrates the markedly enhanced extravasation of Evan's blue dye at a skin site of ovalbumin-sensitized mice injected with a mixture of melittin and ovalbumin as compared with skin sites injected with Ov alone or melittin alone.

When Evan's blue dye was extracted from the skin of 5 ovalbumin-sensitized mice, which had been challenged with melittin alone, Ov alone, or a mixture of the two, results were obtained which are presented in Table 3. It can be seen that the average optical density (OD) of the dye extracted from sites injected with melittin plus Ov was over twice the OD of the dye extracted from sites injected with melittin alone plus the OD of dye from sites injected with Ov alone. The difference was significant ($P < 0.05$) and leads one to the conclusion that melittin has a synergistic effect on the vascular response of skin to antigen-antibody interaction.

Since we found that phospholipase from bee venom also induced increased vascular permeability at doses even less than that of melittin, it was necessary to inject an additional group of ovalbumin-sensitized mice with a mixture of phospholipase and ovalbumin. However, blueing produced by this mixture was only the sum of blueing induced by ovalbumin alone and phospholipase alone (unpublished observation). We thus believe that the enhanced vascular permeability produced by mixing melittin with antigen was due to melittin.

Table 3

Synergistic effect of melittin on antigen-antibody interaction in mouse skin

Ovalbumin sensitized* mice injected in skin with	Dye extracted from skin (OD)
Ovalbumin	0.07 ± 0.02
Melittin	0.08 ± 0.04
Ovalbumin plus Melittin	0.32 ± 0.12**

* Non-sensitized mice did not react to ovalbumin and the amount of dye present at the site of melittin injection was the same as the amount of dye in the site injected with melittin and ovalbumin (unpublished observation).

** The optical density (OD) of the dye extracted from the skin of mice injected with a mixture of ovalbumin + melittin was significantly greater ($P < 0.05$) than the OD of the dye extracted from mice injected with Ov alone plus the extracted from mice injected with melittin alone.

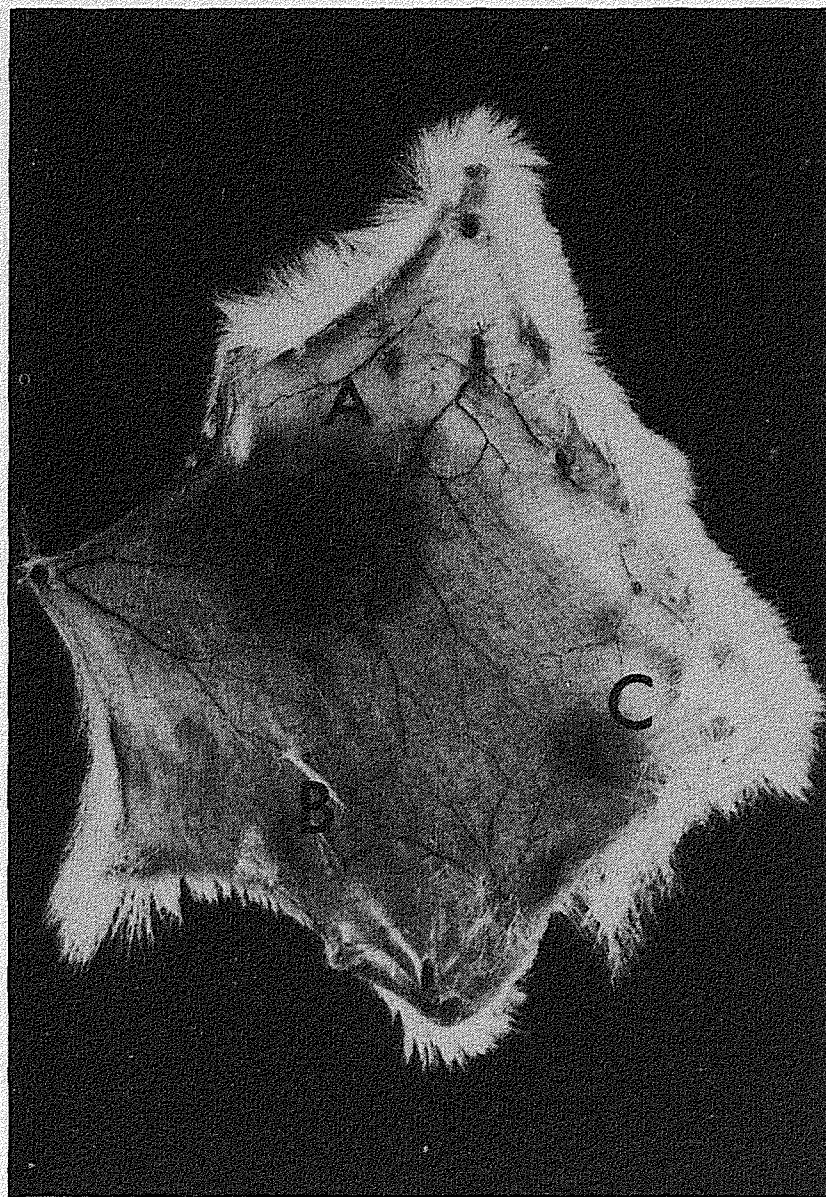


Fig. 1. Effect of melittin on vascular permeability induced by an injection of ovalbumin into the skin of mice passively sensitized with mouse anti-ovalbumin. Dark areas are sites of extravasation of Evan's blue dye. Top (A) = site injected with a mixture of melittin and ovalbumin. Lower left (B) = site injected melittin alone and lower right (C) = site injected with ovalbumin alone.

DISCUSSION

We have found that melittin: a) induces IgE formation in mice without the use of adjuvants, b) acts as an adjuvant for ovalbumin in mice, c) enhances the vascular permeability initiated by antigen-antibody interaction in mouse skin. In addition, it was found that the surfactant saponin is an excellent adjuvant for IgE formation.

Adjuvants have been routinely used for IgE induction in mice. Al(OH)₃ and *B. pertussis* have been most widely employed; Freund's adjuvant

has been used occasionally; and, lately, silica has been shown to be very effective (15).

Recently, Mitchell & Clark demonstrated that a single injection of Con A induced anti-Con A IgE antibodies in mice without the use of adjuvants (16). Repeated subcutaneous injections of grass pollen extract or DNP-ovalbumin without adjuvant stimulated IgE responses to pollen allergens and DNP (19). A soluble product of *Ascaris suum* also induced high IgE levels in guinea pigs without the use of adjuvants (25).

In view of these recent findings, we decided to

inject melittin without adjuvant; such a procedure would more closely approximate natural sensitization to bee venom allergens. One could then attempt to hyposensitize mice (by production of IgG anti-melittin antibodies) without the complication of a previous adjuvant-enhanced priming of the animal for IgG synthesis.

When we found that melittin did stimulate IgE to itself we wondered if it might have adjuvant effects on other antigens. Saponin, a surfactant, has been shown to stimulate haemagglutinins in mice (5) and reagins in cattle (3). The surfactant dimethyldioctadecyl ammonium bromide has been reported to enhance both delayed hypersensitivity response (24) and humoral antibody formation in mice (8). Lysolecithin and some synthetic lysolecithin analogues have similar properties (24). We are currently testing various other surfactants and mast cell degranulating agents to determine if adjuvanticity for IgE is a common property of such molecules.

Also worthy of further study is our finding that melittin enhances the vascular permeability induced in mouse skin by the interaction of Ov and anti-Ov. Melittin has been shown to be lytic for mast cells (11, 22): the blueing induced in mouse skin by melittin alone is likely due to the release of histamine and serotonin from murine mast cells. However, we have thus far been unable to enhance skin reactions produced by Ov in Ov-sensitized mice by injecting mice with mixtures of Ov and histamine or Ov and serotonin. It is conceivable that products of phospholipid metabolism may be important in the permeability enhancing effect of melittin. It has recently been demonstrated that melittin stimulates phospholipase A₂ activity in rat mast cells (2).

A possible role for melittin in human anaphylactic reactions to bee venom should be considered. Melittin might enhance IgE formation to the various allergens in bee venom. In some cases anaphylactic reactions in humans to bee venom cannot be correlated with IgE levels to venom allergens, previous allergic reactions to bee venom or positive skin tests (12, 21, 28). Could melittin enhance the effects of mediators released by interaction of undetectable amounts of IgE and venom allergens?

REFERENCES

1. Arnold, B., Miller, J. A. F. P. & Weltzien, H. N.: Lysolecithin analogues as adjuvants in delayed-type hypersensitivity in mice. *Eur. J. Immunol.* 9, 363-366, 1980.
2. Balentine, L. T., Helmkamp, G. M. & Stechschulte, D. J.: Melittin stimulation of phospholipase A₂ in rat mast cells. *Fed. Proc.* 39, 692, 1980.
3. Black, L.: The adjuvant activity of saponin and aluminium hydroxide for promoting reagins. *J. Immunol. Methods* 15, 193-195, 1977.
4. Blum, J. & Ovary, Z.: Evaluation of methods of measurements of permeability increases. *Int. Arch. Allergy Appl. Immunol.* 46, 121-127, 1974.
5. Bomford, R.: The comparative selectivity of adjuvants for humoral and cell mediated immunity. *Clin. Exp. Immunol.* 39, 426-434, 1980.
6. Charavejasarn, C., Reisman, R. E. & Arbesman, C. E.: Reactions of anti-bee venom mouse reagins and other antibodies with related antigens. *Int. Arch. Allergy Appl. Immunol.* 48, 691-697, 1975.
7. Frimmer, M. & Muller, F. W.: Brauchbarkeit und Grenzen der farbstoff-Methoden zur Bestimmung vermehrter Durchlässigkeit der Hautkapillaren. *Med. Exp.* 6, 327-330, 1962.
8. Gordon, W. C., Prager, M. D. & Carroll, M. C.: The enhancement of humoral and cellular immune responses by dimethyldioctadecyl ammonium bromide. *Cell. Immunol.* 49, 329-340, 1980.
9. Habermann, E.: Bee and wasp venoms. *Science* 177, 314, 1972.
10. Habermann, E. & Jentsch, J.: Sequenzanalyse des Melittins aus den Trypsischen und Peptischen Spaltstücken. *Hoppe-Seyler's Z. Physiol. Chem.* 348, 37, 1967.
11. Jajani, B. & Stanworth, D. R.: Studies on the mast cell triggering action of certain artificial histamine liberators. *Int. Arch. Allergy Appl. Immunol.* 45, 74-81, 1979.
12. Lichtenstein, L. M., Valentine, M. D. & Sobotka, A. K.: Insect allergy: the state of the art. *J. Allergy Clin. Immunol.* 64, 5-12, 1979.
13. Mackler, B. F. & Krell, G.: Honey bee venom melittin: Correlation of nonspecific inflammatory activities with amino acid sequences. *Inflammation* 2, 55-65, 1977.
14. Mackler, B. F., Russell, A. S. & Krell, G.: Allergenic and biological activities of melittin from honey bee venom. *Clin. Allergy* 2, 317-323, 1972.
15. Mancino, D. & Ovary, Z.: Adjuvant effects of amorphous silica and aluminum hydroxide on IgE and IgG₁ antibody production in different inbred mouse strains. *Int. Arch. Allergy Appl. Immunol.* 61, 253-258, 1980.
16. Mitchell, G. F. & Clarke, A. E.: Allergenicity of concanavalin A in mice. *Int. Arch. Allergy Appl. Immunol.* 58, 391-401, 1979.
17. Mollay, C., Kreil, G. & Burger, H.: Action of phospholipases on the cytoplasmic membrane of *Escherichia coli*. *Biochim. Biophys. Acta* 426, 317-364, 1976.
18. Mota, J. & Wong, D.: Homologous and heterologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. *Life Sci.* 8, 813-820, 1969.

19. Mueller, U., Elliot, W., Reisman, R., Wypych, J., Wicher, J. & Arbesman, C.: Preparation and analysis of chemically modified bee venom (venomoid). *J. Allergy Clin. Immunol.* 65, 202, 1980.
20. Paull, B. R., Yuniger, J. W. & Gleich, G. J.: Melittin: An allergen of honeybee venom. *J. Allergy Clin. Immunol.* 59, 334-338, 1977.
21. Reisman, R. G.: Stinging insect allergy. *J. Allergy Clin. Immunol.* 64, 3-4, 1979.
22. Rothschild, A. M.: Histamine release by bee venom phospholipase A and melittin in the rat. *Br. J. Pharmacol.* 25, 59-66, 1965.
23. Saelinger, C. B. & Higginbotham, R. D.: Hypersensitivity responses to bee venom and melittin. *Int. Arch. Allergy Appl. Immunol.* 42, 28-37, 1974.
24. Snippe, H., Belder, M. & Willers, J. M. N.: Dimethyldioctadecyl ammonium bromide as adjuvant for delayed hypersensitivity in mice. *Immunology* 33, 931-936, 1977.
25. Stromberg, B. E.: IgE and IgG₁ antibody production by a soluble product of *Ascaris suum* in the guinea pig. *Immunology* 38, 489-495, 1979.
26. Taylor, W. A., Sheldon, D. & Francis, D. H.: IgE antibody formation in BALB/c mice without adjuvant: Induction of responses to grass pollen extract and to a hapten-carrier conjugate. *Immunology* 39, 583-588, 1980.
27. Wypych, J., Reisman, R. E., Lazell, M., Arbesman, C. E., Steger, R. & Elliott, W.: Studies in allergic individuals of serum IgE and IgG antibodies to purified bee venom. *J. Allergy Clin. Immunol.* 65, 201, 1980.
28. Yuninger, J. W.: The sting-revisited. *J. Allergy Clin. Immunol.* 64, 1-2, 1979.

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