

Enhanced IgE and IgG Anti-Melittin Antibody Formation Induced by Heparin-Melittin Complexes in Mice

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It is known that heparin which is strongly anionic will precipitate *in vitro* with cationic proteins such as melittin, the principal constituent of bee venom. Our experiments indicate that mice immunized with complexes of melittin and heparin produced greater amounts of IgE and IgG antibody (to this protein) than did mice immunized with melittin alone. It is possible that heparin released from mast cells following a bee sting could complex with melittin and enhance the allergenic properties of this molecule.

Key words: allergy; bee venom; heparin; IgE; IgG; melittin.

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CLINICAL ASPECTS

We have shown that complexes of heparin and cationic proteins result in an enhanced immune response to the protein present in the complex. The allergenicity of melittin may depend on its ability to release heparin from mast cells and then form complexes with this polysaccharide. In addition, our experiments suggest that reported allergic reactions to protamine (administered to neutralize injected heparin) may be due to heparin-protamine complexes stimulating the immune response to protamine. We cannot at this time recommend a compound other than protamine for neutralizing injected heparin; however, an awareness of possible anaphylactic reactions after repeated protamine administration may be beneficial.

Melittin is the principal constituent of the venom of the honeybee *Apis mellifica* comprising 50% of the protein content of this venom. It is an amphipathic cationic peptide consisting of 26 amino acid residues (5, 6), is strongly surface active and lytic for membranes, and induces mast cell rupture with release of histamine (10, 24). Melittin is probably responsible for the non-specific inflammatory effects produced by bee venom (17) and is also an allergen in humans (21, 22, 29).

In 1970, Higginbotham et al. suggested that the secretion of heparin from mast cells following a bee sting resulted in complex formation between heparin and melittin with neutralization of the cytotoxic effects of melittin (9). The

ability of strongly anionic heparin to form complexes *in vitro* with cationic proteins such as melittin is well established (4, 7, 8) and it seemed to us that complex formation of heparin with melittin *in vivo* might enhance the antigenicity of melittin. The injection of antigen-antibody complexes has been shown to increase the immune response to the antigen present in the complex (13, 28). Complexes of heparin with cationic proteins such as protamine resemble antigen-antibody complexes in that a) they precipitate *in vitro* and b) they interact with the first component of complement to activate the classical complement pathway (19, 23).

The experiments which follow indicate that enhanced antibody formation of the IgE and

IgG class to the cationic protein melittin occurs in mice immunized with complexes of this protein and heparin.

While this paper was being written, a report appeared which demonstrated that complexes of heparin and trichinella antigens induced a greater antibody response to trichinella antigen in mice than did the antigen alone (11).

MATERIAL AND METHODS

CBA inbred mice were purchased from Jackson Laboratories, Bar Harbor, Maine. Outbred CFW mice were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts and Sprague-Dahli rats were purchased from CBFL Laboratories, St. Constantine, Quebec. SWR mice were raised in our own laboratory. Melittin was purchased from Sigma, St. Louis, Missouri and the heparin used was Hepalean® (heparin sodium, 10,000 USP units per ml) prepared by Harris Laboratories, Brantford, Ontario.

Complexes were prepared by adding 1 ml of heparin (diluted to 400 µg/ml) to 1 ml of a protein solution containing 2 mg of melittin. All materials were dissolved or diluted in physiologic saline. Although a precipitate formed immediately upon mixing of the heparin with the cationic protein, the reaction was allowed to proceed overnight in the cold room (4°C). The next day the precipitates were washed twice in cold saline, and resuspended in 1 ml of saline. One tenth of a ml of suspension which contained 100 µg of melittin and 32 µg of heparin was injected subcutaneously into test mice. Control mice were injected with 100 µg of melittin alone. We previously reported that this dose of melittin induced IgE antibody formations in four different inbred strains of mice (12). Precipitates were analysed for protein by the Bio-Rad method 82) and for heparin by the method of Smith et al. (25). Mice were given six weekly injections of complex or antigen alone and were then tested and boosted on day 70. The animals were bled from the orbital plexus on days 21, 28, 35, 70, and 77. IgG (γ_1) antibodies were determined by 2 h passive cutaneous anaphylaxis (PCA) reactions in CFW mice (14) and IgE

titres were measured by PCA reactions in the skin of 200 g male Sprague-Dahli rats (19). Each titre represents the results obtained from the pooled sera of three mice. PCA titres for a particular serum were reproducible by a factor of 2. Thus a four-fold difference in titre was considered significant. We found that mice injected with melittin-heparin complex had PCA titres 4 to 32 times higher than the titres obtained in mice injected with melittin alone.

RESULTS

The data in Fig. 1 indicate that repeated weekly injections of melittin alone or melittin-heparin complex resulted in detectable IgE and IgG anti-melittin antibodies in the sera of CBA and SWR mice by days 21–28. Antibody levels reached a peak by day 35 (at which time injections of antigen were stopped) and fell to zero or low levels by day 70. A booster injection on day 70 resulted in a rise in titre on day 77. Antibody levels were 4 to 32 times higher in mice injected with the heparin-melittin complex than in mice injected with melittin alone. Thus, in CBA mice, the IgE-antibody titre was 320 on day 77 in mice injected with heparin-melittin, as compared with a titre of 20 in mice injected with melittin alone. The IgG titre was 160 in the complex-injected mice as compared with a titre of 5 in the mice injected with melittin alone.

Since we wished to know whether the enhanced antibody formation induced by heparin-melittin complexes was a phenomenon limited to melittin alone, we injected mice with heparin complexed with the cationic proteins, lysozyme or methylated bovine serum albumin (BSA). We found that CBA mice injected with complexes of heparin and lysozyme or heparin and methylated albumin had IgE and IgG antibody levels 4 to 80 times that of animals injected with these proteins alone (unpublished observation).

Although we used 100 µg of melittin to stimulate antibody formation in these experiments, we have in the past obtained IgE formation by injecting mice with 10 µg of melittin repeatedly

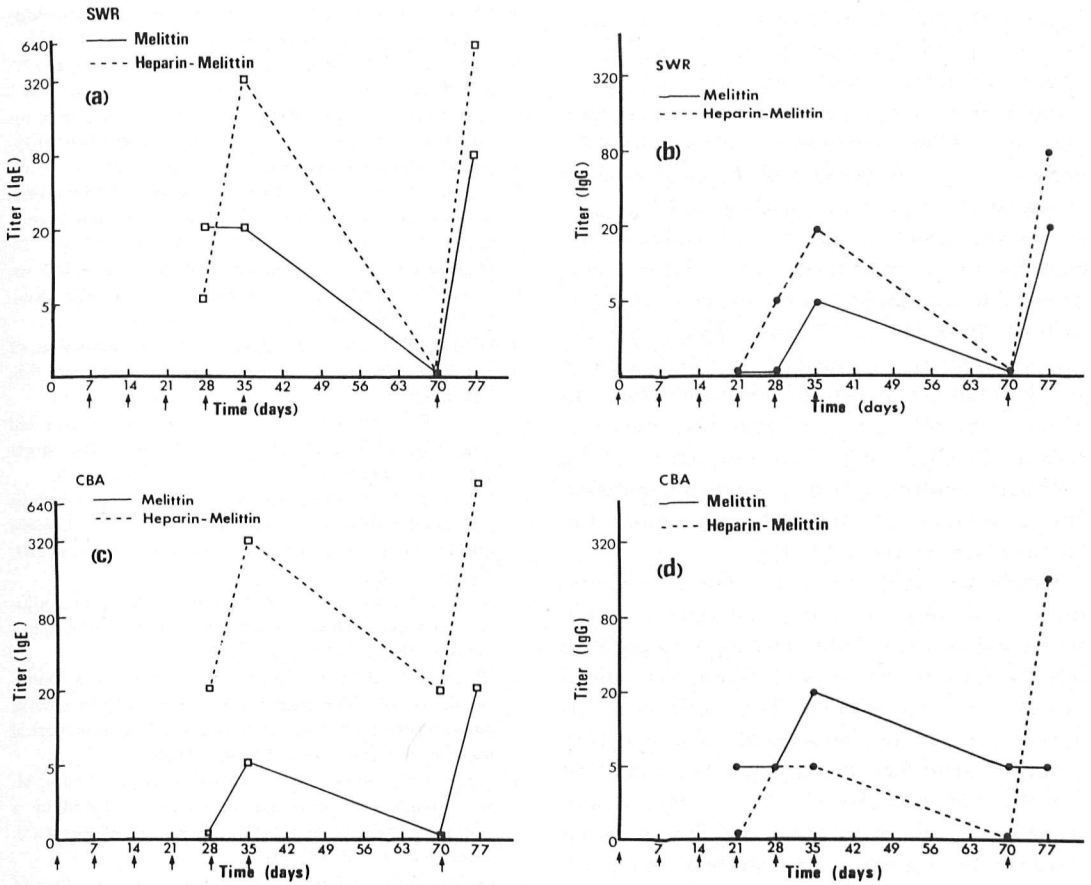


Fig. 1. Comparison of antibody response to melittin alone (100 μ g) with antibody response to melittin-heparin complex (100 μ g melittin, 32 μ g heparin). Arrows (↑↑↑) indicate

- IgE antibodies to melittin alone ($\square$ — $\square$) in SWR mice. IgE antibodies to melittin-heparin complex ($\square$ — $\square$) in SWR mice.
- IgG antibodies to melittin alone ($\bullet$ — $\bullet$) in SWR mice. IgG antibodies to melittin-heparin complex ($\bullet$ — $\bullet$) in SWR mice.
- IgE antibodies to melittin alone ($\square$ — $\square$) in CBA mice. IgE antibodies to melittin-heparin complex ($\square$ — $\square$) in CBA mice.
- IgG antibodies to melittin alone ($\bullet$ — $\bullet$) in CBA mice. IgG antibodies to melittin-heparin complex ($\bullet$ — $\bullet$) in CBA mice.

over a period of several months. It is likely that even less than this amount of melittin would induce antibody formation if it were complexed with heparin.

DISCUSSION

Heparin is a mucopolysaccharide composed of partially sulphated units of α -D-glucuronic acid and 2-amino-2-deoxy- α -D-glucose joined by

1-4 bonds (4). It is primarily known for its anticoagulant activity (4). However, its action ranges from improved triglyceride clearing (25), enhanced passive sensitization of basophils (16) and various anti-complementary activities (23) to non-specific neutralization of many cationic proteins (4) and enzymes released from platelets and neutrophils (1, 3).

Our data suggests an additional activity for heparin. It is possible that *in vivo* complexing of

heparin released from mast cells with exogenous cationic proteins may lead to enhanced antigenicity or allergenicity of the protein. Melittin could be one such protein. *Trichinella* antigen may be another (11). It is conceivable that the enhanced immunogenicity of heparin melittin complexes is due to the binding of C' to such complexes, which results in localization of the complexes in areas of the spleen (dendritic cells) favourable for antibody and memory cell formation. Stark et al. (27) have shown that the immunogenicity of various acylated human serum albumins depended on their ability to activate complement and localize in dendritic cells of the white pulp. The importance of the follicular dendritic cell in antigen presentation and generation of immunologic memory has recently been emphasized (13).

Finally, we should like to mention an interesting clinical case (14) which illustrates the possible consequences of the binding of heparin to cationic proteins *in vivo*. An individual was given heparin prior to donating blood and then was injected with protamine after blood donation in order to neutralize the injected heparin. Five months later, this procedure was repeated and the injection of protamine was followed by an anaphylactic reaction. IgG antibody to protamine was shown to be present in the serum of the patient. It is our belief that the formation of heparin protamine complexes *in vivo* was responsible for the antibody against protamine in the case just cited. We have recently succeeded in inducing anti-protamine antibodies in rabbits by the injection of heparin-protamine complexes (unpublished observations).

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