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Original scientific paper

# IMMUNE RESPONSES OF A MOUSE TO BEE VENOM

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**Abstract**

The effect of honeybee venom on humoral (gammaglobulin and total protein levels) and cellular immune responses (weight and cellularity of spleen and lymph nodes) was tested in CBA mice. Bee venom was injected subcutaneously into the left footpad at a dose of 0.15, 0.30, or 0.60 mg per mouse, respectively. Mean gammaglobulina and total protein values were significantly elevated ($P < 0.05$) in mice treated with 0.15 mg or 0.30 mg of bee venom, respectively, compared to those recorded in nontreated controls. The weight of the left popliteal lymph node (LPLN) was significantly higher ($P < 0.01$) in mice given 0.30 mg or 0.60 mg of bee venom than that of the controls. The cellularity of LPLN in bee venom-treated mice was much greater ($P < 0.05$; $< 0.01$) when compared to the control values. However, no differences in weight and cellualarity of the popliteal lymph node were noticed in mice treated identically with propolis, used as an antigen. There were no differences in weight or cellularity of spleen between treated and control mice.

## *Introcuction*

Bee venom is known to be a very complex mixture of active peptides, enzymes and amines (Habermann, 1971). The major components of bee venom are histamine, catecholamines, polyamines, melittin and phospholipasde. Melittin, a homolytic peptide (consisting of 26 amino acids) is the main constituent of bee venom which, accounts for 50% of its composition (Orlov, 1979).

Catecholamines of noradrenaline and dopamine, and histamine/apamine are biologically active amines found in the venom causing degranulation of mast cells and/or neurotoxic effects (Nakajima, 1984; Shipolini, 1984). Phospholipase $A_2$ and hyaluronidase play an important role in spreading of the active peptides/amines of bee venom throughout tissues and cells (Inoue and Nakajima, 1985). A mode of action of bee venom is highly dependent on the dose

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of the venom, route of its application, species used in the model and their physiological/immunological status.

The recent interest in the effect of bee venom as an immunostimulator has resulted in research of its intimate relationship with a wide range of degenerative diseases (such as rheumatic diseases), cardio-vascular diseases, and malignant diseases. As a first step in understanding the control of these incurable diseases with immunotherapy, much more research should be done on testing the effect of bee venom in modulation of the immunoresponsiveness (Mraz, 1985).

The increase of specific IgG and IgE antibodies was recorded in patients immunized with purified bee venom proteins or polypeptides (Kemeny et al., 1983). On the other hand, an inhibitory effect of bee venom (doses of 40 $\mu$g or 60 $\mu$g) on the number of murine splenocytes that produced specific antibodies and rosette-forming peripheral blood lymphocytes was observed (Orlov et al., 1981). It is not surprising, therefore, that bee venom presumably acts as a stimulator or inhibitor of the immune response depending on the dose of apitoxin applied.

The objective of the present study was to determine whether different doses of bee venom (0.15, 0.30, and 0.60 mg, respectively) injected into CBA mice affect their humoral and cellular immune responses in the model.

## *Material and methods*

Bee venom was produced using highly efficient technology under industrial beekeeping conditions in the "Medex" (Ljubljana, Slovenia), dissolved in 0.5 ml of destilled water and injected subcutaneously (SC) into the left footpad of 5 or 8-9 CBA mice (of both sexes, 3-month-old, weighing 20 g in average) at different doses (o.15, 0.30, or 0.60 mg/mouse). Propolis (0.15mg/0.05 ml) was identically injected as an antigen. The fifth group of either 5 or 8-9 mice received destilled water only as a placebo. Fourteen days after inoculation mice were killed and spleen and popliteal lymph nodes were taken for weighing, determination of cellularity, and hystology. Cellularity of spleen and lymph nodes was determined by counting in a hemacytometer. Blood samples were obtained by vv. axillaris puncture, and serum proteins were quantified by electrophoresis on cellulosa acetate (Cellogel, Chemetron, Milano, Italy) strips.

## *Results*

As shown in Figure 1 the treatment with either dose of bee venom resulted in elevation of gamma-globulins and total proteins. Mean gamma-globulin values of mice treated with 0.15 mg of bee venom were significantly higher ($P<0.05$) than the respective means of nontreated controls. Total protein levels in mice treated with 0.15 mg or 0.30 mg of bee venom were significantly higher ($P<0.05$) as compared to the values in nontreated mice. The treatments of mice with 0.30/0.60 mg (Experiment 1) or with all three doses of bee venom (Experiment 2) resulted in significantly increased ($P<0.01$; $<0.001$), respectively) weight of the left popliteal lymph node (LPLN) as compared to that in nontreated animals (Table 1). The weight of LPLN was also

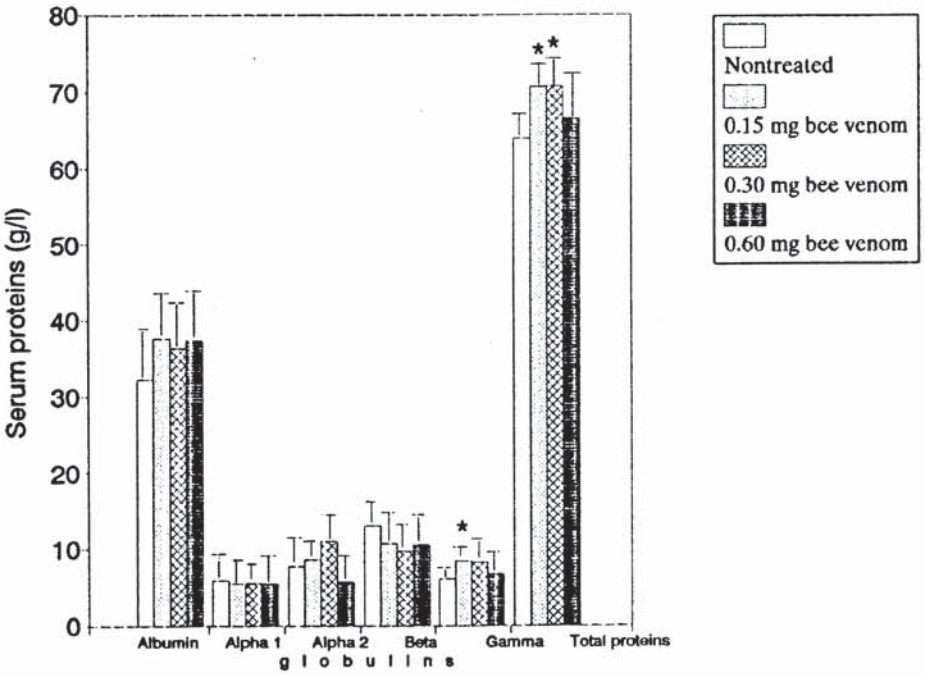


Figure 1 - SERUM PROTEIN LEVELS IN MICE INJECTED SUBCUTANEOUSLY INTO LEFT FOOTPAD WITH THREE DOSES OF BEE VENOM. GROUPS COMPRISED 5 MICE EACH. BLOOD SAMPLES WERE TAKEN 14 DAYS AFTER BEE VENOM INJECTION. ASTERISKS INDICATE THE MEAN VALUES SIGNIFICANTLY HIGHER ($P<0.05$) THAN THE RESPECTIVE MEANS IN NONTREATED MICE.

Table 1 - WEIGHT (MEAN ± SD) OF LEFT AND RIGHT POPLITEAL LYMPH NODE (LPLN AND RPLN), RESPECTIVELY, AND SPLEEN IN MICE INJECTED SUBCUTANEOUSLY IN LEFT FOOTPAD WITH THREE DOSES OF BEE VENOM.
ANIMALS WERE KILLED 14 DAYS AFTER BEE VENOM INJECTION AND THEIR ORGANS WERE WEIGHED.

| TREATMENT | EXPERIMENT 1[a] weight (mg) | | | EXPERIMENT 2[b] weight (mg) | | |
|---|---|---|---|---|---|---|
| | LPLN | RPLN | SPLEEN | LPLN | RPLN | SPLEEN |
| NONE | 1.7 ± 0.3 | 1.9 ± 0.1 | 66.7 ± 10.2 | 1.5 ± 0.2 | 1.7 ± 0.1 | 72.0 ± 4.1 |
| BEE VENOM | | | | | | |
| 0.15 mg | 2.7 ± 1.3 | 1.4 ± 0.5 | 71.2 ± 9.9 | 3.1 ± 0.4[c] | 1.6 ± 0.2[d] | 71.2 ± 3.2 |
| 0.30 mg | 3.6 ± 0.9[c] | 1.8 ± 0.3[d] | 66.4 ± 7.1 | 3.4 ± 0.5[c] | 1.4 ± 0.1[d] | 74.3 ± 4.2 |
| 0.60 mg | 5.3 ± 1.8[c] | 1.8 ± 0.8[d] | 70.2 ± 6.9 | 6.1 ± 0.7[c] | 1.8 ± 0.3[d] | 74.9 ± 3.9 |

a Groups comprised 5 mice each
b Groups comprised 8-9 mice each
c Significantly higher ($P<0.01$ to 0.001) than in nontreated mice
d Weight of LPLN was significantly higher ($P < 0.01$) than that of RPLN

TABLE 2. - CELLULARITY (MEAN ± SD) OF LEFT AND RIGHT POPLITEAL LYMPH NODE (LPLN AND RPLN) RESPECTIVELY, AND SPLEEN IN MICE SUBCUTANEOUSLY INJECTED INTO LEFT FOOT-PAD WITH THREE DOSES OF BEE VENOM. ANIMALS WERE KILLED 14 DAYS AFTER BEE VENOM INJECTION AND CELLULARITY OF THEIR ORGANS WAS DETERMINED.

| TREATMENT[a] | Cellularity (x $10^6$) | | |
|---|---|---|---|
| | LPLN | RPLN | SPLEEN |
| NONE | 1.7 ± 0.4 | 1.7 ± 0.2 | 111 ± 6 |
| BEE VENOM | | | |
| 0.15 mg | 3.1 ± 0.7[b] | 1.9 ± 0.1[d] | 109 ± 7 |
| 0.30 mg | 4.6 ± 0.9[c] | 2.1 ± 0.2[d] | 110 ± 9 |
| 0.60 mg | 7.2 ± 1.3[c] | 1.9 ± 0.2[d] | 111 ± 5 |

a Groups comprised 8-9 animals each (Data from experiment 2-see Table 1)
b Significantly higher ($P<0.01$) than in nontreated mice
c Significantly higher ($P<0.001$) than in nontreated mice
d Cellularity of RPLN was significantly lower ($P<0.05$) than that of LPLN

A

B

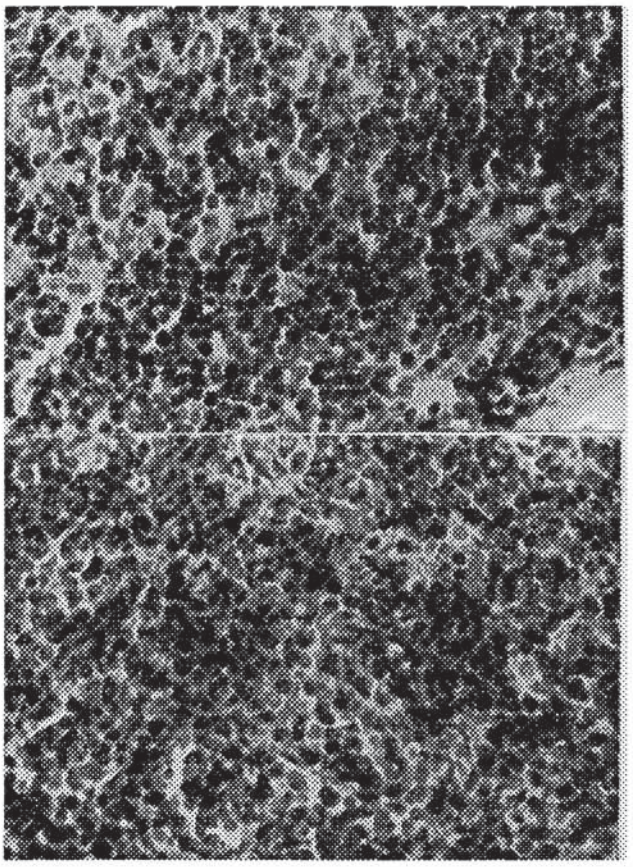

Figure 2. - RIGHT (A) AND LEFT (B) POPLITEAL LYMPH NODE OF A MOUSE INJECTED INTO LEFT FOOTPAD WITH 0.05 ML OF WATER SOLUTION CONTAINING 0.15 MG OF PROPOLIS. HE; MAGNIFICATION X100.

significantly higher ($P<0.01$) than that of the right popliteal lymph node (RPLN), regardless of the dose of bee venom applied. The cellularity of LPLN in bee venom-injected mice was much greater ($P<0.01$; $<0.001$ ) than that of the controls (Table 2). The cellular content of RPLN was much lower ($P<0.05$) than that of LPLN in mice treated with all three doses of bee venom. There were no differences in weight or cellularity of spleen between bee venom-treated and nontreated mice. No differences in weight or cellularity of popliteal lymph node were observed in mice treated identically with another product of bee-propolis, which served as an alternate antigen (data not shown). In these animals, no histological changes in regional lymph node were noticed between either propolis-treated or nontreated mice (Figure 2).

## *Discussion*

Our finding of increased gamma-globulin levels in bee venom- treated mice is in agreement with the observations by others (Hunt et al., 1978; Levine and Lockey, 1981) who reported an increase of IgG during a period of several months after the treatment of patients with honeybee venom. There is also evidence that the bee venom treatment reduced the formation of antibodies by murine spleen cells (Orlov et al., 1981). This evidence appears to be contradicted (at least for lymph node) by our study. Namely, we found increased weight of LPLN in mice injected with bee venom. Moreover, the weight of LPLN (mice received the venom into the left footpad) was significantly elevated as compared to that of RPLN, indicating that the venom induced a local inflammatory reaction of murine immune system similarly as described in humans (Forestier and Palmer, 1984). It is likely that bee venom can stimulate a local tissue reaction through the action of its active amines and release of histamine from degranulated mast cells as suggested earlier (Orlov, 1979). The fact that either of the given doses of bee venom in this study elicitated the cellularity of LPLN, indicates that an intensive differentiation of antibody - producing cells could occur as a local response of immunized mice to the venom's active peptides/amines. However, no differences in weight/cellularity or histology of popliteal lymph node were noticed in mice treated with different doses of propolis-an alternate antigen of bee origin-tested in our recent studies (unpublished).

## *Conclusions*

Before any firm conclusions about immunostimulating properties of bee venom are made, further work using in vivo as well as in vitro models is necessary to confirm its interaction with immunocompetent cells and establish its mode of action. However, the effect of honeybee venom in enhancement of humoral immune responsiveness, i. e. elevated gamma-globulin levels, in CBA mice treated with 0.15 mg or 0.30 mg of the venom was evident. Also, the cellular immune response in bee venom treated mice (doses of 0.30 mg and 0.60 mg) was strongly enhanced as determined by an increase in weight and cellularity of LPLN.

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## IMUNOSNI ODGOVOR MIŠA NA PČELINJI OTROV

### Sažetak

Testirali smo učinak pčelinjeg otrova (Medex. Ljubljana, Slovenija) na humoralni (razine gama globulina i ukupnih proteina) i staničnl imunosn odgovor (težina i staničnost slezene i limfnih čvorova) CBA miševa. Pčelinji smo otrov ubrizgali miševima (po 5 odnosno 8-9 životinja u skupini) potkožno u lijevu šapicu u dozama od 0,15, 0,30 ili 0,60 mg po mišu. Srednje vrijednosti gama globulina i ukupnih proteina bile su značajno povišene ($P<0,05$) u miševa koji su dobili 0,15 ili 0,30 mg pčelinjeg otrova u usporedbi s vrijednostima zabilježenim u neobrađenih kontrola. Težina lijevog poplitealnog limfnog čvora (LPLČ) bila je znatno viša ($P<0,01$) u miševa obrađenih sa 0,30 ili 0,60 mg pčelinjeg otrova nego u kontrolnih životinja. Isto tako je i staničnost LPLČ u obrađenih miševa bila mnogo veća ($P<0,05$; $<0,01$) od one nađene u neobrađenih miševa. Međutim, nismo opazili nikakvu razliku u težini i staničnosti poplitealnog limfnog čvora miševa obrađenih na isti način propolisom - drugim antigenom pčelinjeg porijekla. Nije bilo razlika u težini i staničnosti slezene između obrađenih i neobrađenih miševa.

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