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Effects of *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae) Larvae on the Degranulation of Dermal Mast Cells in Mice; an Electron Microscopic Study

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The pine caterpillar *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae) is found in pine woods. Hairs of the *T. pityocampa* caterpillar cause a cutaneous reaction in humans and animals. Mast cells are responsible for allergic reactions in mammals. In this study male swiss albino mice were divided into two groups: 5 mice in the control group and 25 mice in the experimental group. The dorsal skin of mice was shaved. The mice in the experimental group and *T. pityocampa* larvae (fifth instar, approximately n=100) were put in the same cage. Dermal mast cells of mice exposed to *T. pityocampa* were examined with a transmission electron microscope and compared to the control group 1, 3, 6, 12 and 24 hours after exposure. Dermal mast cell degranulation in mice was observed 12 and 24 hours after exposure.

Key words: *Thaumetopoea pityocampa*, allergy, mast cell, degranulation, caterpillar, ultrastructure.

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Over 40 genera and more than 200 species of moths and butterflies are known to produce inflammatory reactions in humans (KAWAMOTO & KUMADA 1984; KOZER *et al.* 1999). The most common effects related to caterpillar exposure are local cutaneous reactions such as rash, edema, pruritus and pain. In a prospective study including 112 caterpillar envenomations, more severe responses, such as muscle spasms, paresthasia and radiating pain to the limbs were found in 26% of the cases (EVERSON *et al.* 1990; KOZER *et al.* 1999). Dyspnea, bronchitis, pharyngitis and keratoconjunctivitis may occur after exposure to a caterpillar (SHAMA *et al.* 1982; KAWAMOTO & KUMADA 1984) and in rare cases, an anaphylactic shock-like syndrome and seizures may occur (EVERSON *et al.* 1990; VEGA *et al.* 1997).

Thaumetopoea pityocampa, the pine processionary caterpillar, is the most important endemic pine pest in the Mediterranean area, not only because of its high defoliating power, but also due to the human health problems caused by the urticating hairs of the larvae (RAUSELL *et al.* 1999). Thaumetopoein, a protein localized on the hairs of *T. pityocampa* larvae produces allergic reactions in humans and animals. (NOVAK *et al.* 1987; REBOLLO *et al*. 2002).

Mast cells are widely distributed in connective tissues but tend to occur in small groups in relation to blood vessels. They are particularly common in connective tissues of rodents. The release of the chemical mediators from mast cells promotes the allergic reactions known as immediate hypersensitivity reactions, including such phenomena as edema, shock, pain, hypercoagulation, and fever. Mediator release initially involves the invasion of the body by antigens (LEESON *et al.* 1988).

The aim of the present study was to investigate the effects of *T. pityocampa* larvae on the degranulation of dermal mast cells in mice.

Material and Methods

Fourty 5-week-old male swiss albino mice, *Mus musculus,* (weighing approximately 35-40 g) were

obtained from the Refik Saydam Central Hygiene Institute, Ankara, Turkey. The animals were housed in 75x75x75 cm plastic cages at standard conditions of temperature (23±2°C); relative humidity (53±2%) and photocycle of 12/12 hours. Mice were fed a standard laboratory diet and water ad libitum, and treated in accordance with the guidelines of the Animal Care Committee of the Hıfzıssıhha Institute, Ministry of Health, Turkey. Animals were quarantined for 10 days before experiments.

Animals were divided into two groups: 5 mice in the control group and 25 mice in the experimental group. In the experimental group, a 20x20x20 cm wire cage carrying approximately 100 *T. pityocampa* larvae (fifth instar) was placed in the plastic cage carrying mice and direct contact of mice with larvae was eliminated. The dorsal skin of mice of both the control and experimental groups was shaved. Dorsal skin samples from mice were taken 1, 3, 6, 12 and 24 hours after exposure to *T. pityocampa* larvae. Because the larvae are nocturnal, experiments were initiated at night.

For electron microscopic examinations of skin, primer fixation was made in 3% glutaraldehyde (Agar Sci. Ltd., Essex, England) in sodium phosphate buffer (200 mM, pH 7.4) (Merck, Alfred Paluka Co., Turkey) for 3 hr at 4°C. Materials were washed with the same buffer and postfixed in 1% osmium tetroxide (Agar Sci. Ltd., Essex, England) and in sodium phosphate buffer pH 7.4 for 1hr at 4°C. Tissue samples were washed with the same buffer for 3 hours at 4°C, and were dehydrated in a graded ethanol series (Agar Sci. Ltd., Essex, England) and embedded in Araldite (Agar Sci. Ltd., Essex, England). Thin sections were cut with a Reichert OM U3 (Leica Co., Austria) ultramicrotome. Samples were stained with 2% uranyl acetate and lead citrate. The sections were viewed and photographed on a Jeol 100 CX II transmission electron microscope (TEM) (Jeol Ltd, Japan) at 80 kV.

Results

The dermal mast cells of the control mice are easily distinguished from other connective tissue cells by the villus-like protrusions (filopodia) of the plasma membrane and specific cytoplasmic granules. Specific granules were abundant throughout the cytoplasm (Fig. 1). Almost all of these granules were electron-dense and homogenous. The nuclei appear to have acquired the shape of the cells. Heterochromatin was in the peripheral and euchromatin was in the central regions of the nucleus (Fig. 1).

No dermal mast cell degranulation in mice was observed 1 and 3 hours after exposure to *T. pityocampa* larvae. However, partial degranulation was observed in some granules (Figs 2 & 3). Partial degranulation in dermal mast cells was observed 6 hours after to exposure *T. pityocampa* larvae (Fig. 4). After 12 hours some granules in dermal mast cells created clear vacuoles following degranulation. In this stage, the swelling of mitochondria in mast cells was detected (Fig. 5). An increase in dermal mast cell degranulation occurred 24 hours after exposure to *T. pityocampa* larvae. Having lost their electron-dense homogenous structure, granules of mast cells changed into a structure with fine granules (Fig. 6).

Discussion

Dermatitis caused by caterpillars may occur as an epidemic. In 1959 a group of 600 Israeli soldiers camping in a pine grove, in the same area as the patients described above, developed an itchy eruption due to *T. wilkinsoni* (ZIPRKOWSKI *et al.* 1959; KOZER *et al.* 1999).

There are several possible explanations for the mechanism of the inflammatory reactions caused by the caterpillar hair. SHAMA *et al.* (1982) demonstrated the presence of histamine in an extract of gypsy moth caterpillar. They also showed that the gypsy moth setae have sharp ends and suggested that a mechanical irritation similar to that produced by fiberglass may contribute to the dermatitis. LAMY *et al.* (1986) studied the *Thaumetopoea pityocampa* caterpillar which is very similar to *T. wilkinsoni*. They found a 28 kilodalton protein formed of two subunits, specific to the caterpillar hair. This protein caused a reaction in pig skin identical to that produced by hair extract. They suggested the name thaumetopoein for this urticaria-producing protein. Thaumetopoein has a direct effect on mast cells leading to their degranulation. An IgE mediated mechanism and delayed hypersensitivity reaction were also suggested as responsible for some of the more severe reactions (ALLEN *et al*. 1991; WERNO *et al.* 1993; KOZER *et al.* 1999; MONEO *et al.* 2003).

In both humans and animals *T. pityocampa* larvae cause symptoms such as atopy, urticarial dermatitis, oedema, conjunctivitis, dyspnea and anaphylactic reactions (ETKIND *et al.* 1982; EVERSON *et al.* 1990; VEGA *et al.* 1999; VEGA *et al.* 2003). Such symptoms are especially severe in people who already have allergic reactions. But so far, fatalities caused by caterpillars have not been recorded (BURNETT *et al.* 1986). Two people in our team developed allergic reactions during the course of the study. Blisters were observed espe-

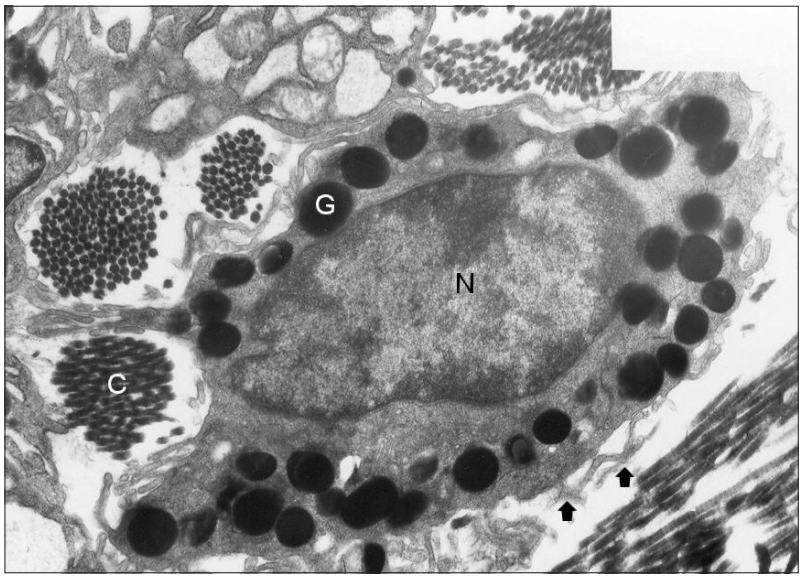

Fig. 1. Dermal mast cell of control group mice. N: Nucleus, G: Granules, C: Collagen, x 12500.

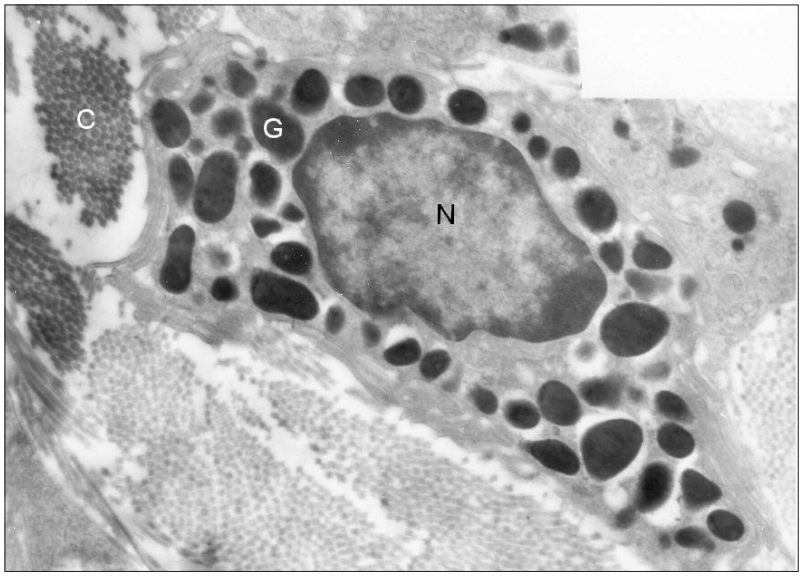

Fig. 2. Dermal mast cell of mice 1 hour after exposure to *T. pityocampa.* N: Nucleus, G: Granules, C: Collagen, x 10000.

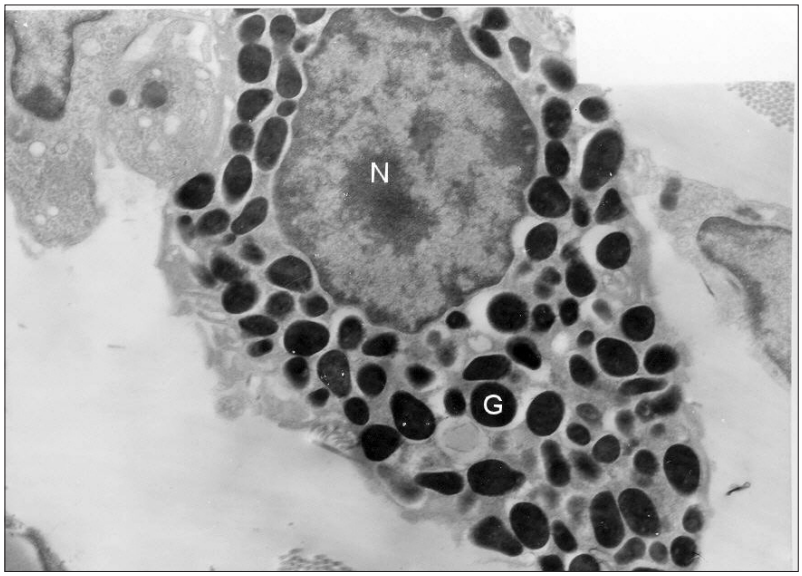

Fig. 3. Dermal mast cell of mice 3 hours after exposure to *T. pityocampa.* N: Nucleus, G: Granules, x 7500.

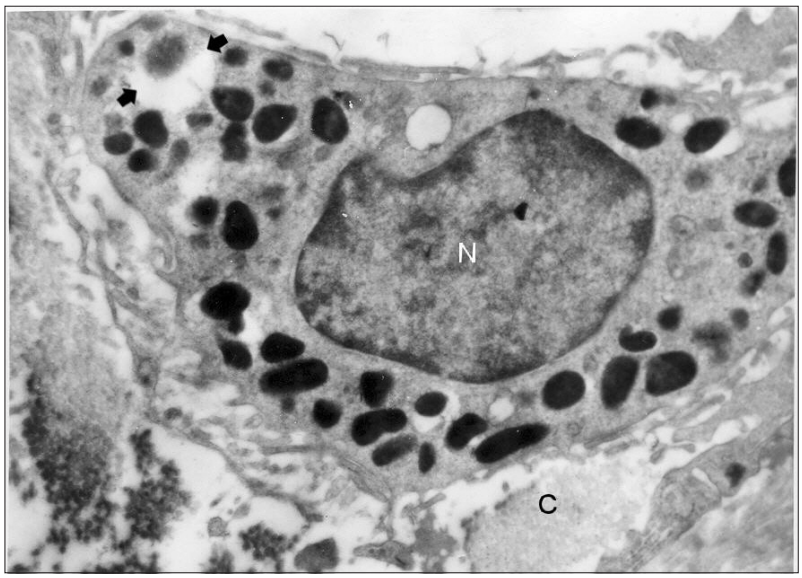

Fig. 4. Partial degranulation (➡), of dermal mast cells of mice 6 hours after exposure to *T. pityocampa*. N: Nucleus, C: Collagen, x 10000.

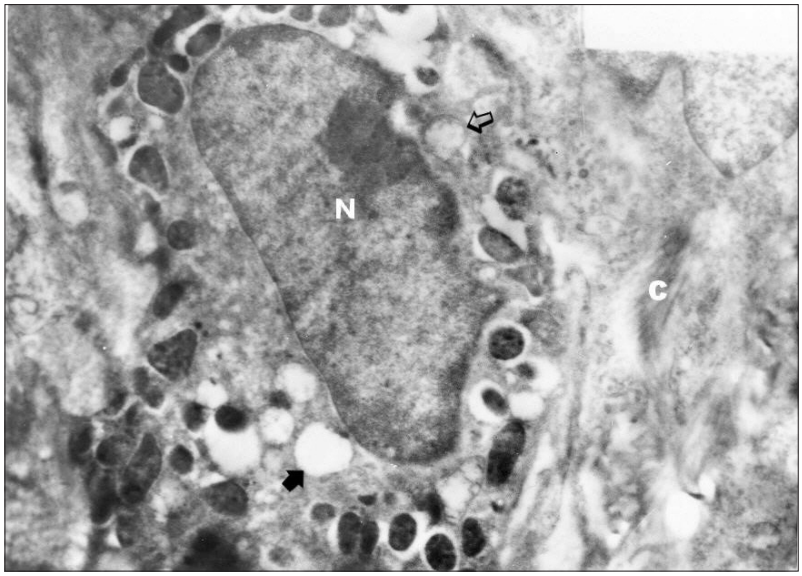

Fig. 5. Partial degranulation (➡) of dermal mast cells of mice and swelling of mitochondria (⇨) 12 hours after exposure to *T. pityocampa*. N: Nucleus, C: Collagen, x 10000.

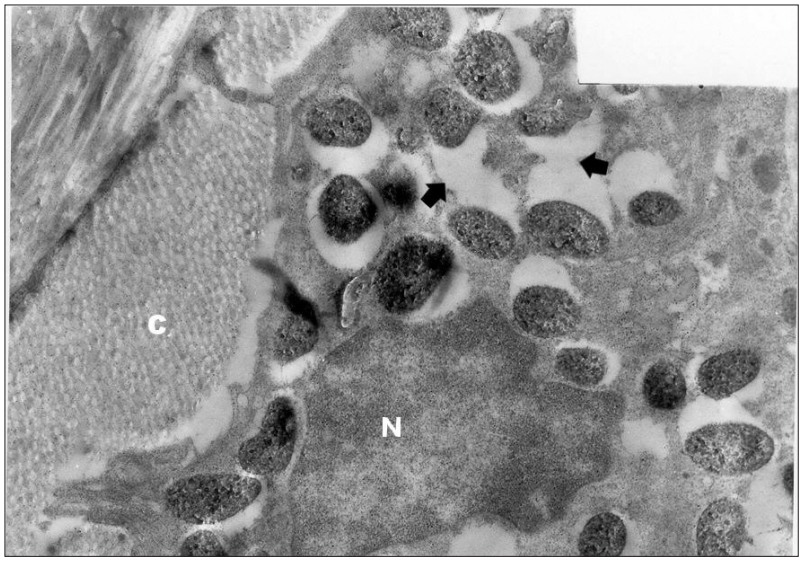

Fig. 6. Dermal mast cell degranulation of mice and fusion (➡) of neighboring granules 24 hours after exposure to *T. pityocampa.* N: Nucleus, C: Collagen, x 12500.

cially on their hands, arms and necks and, hence they were hospitalized for two days. In addition, the same types of allergic reactions were detected even in their family members.

There is no specific antidote for caterpillar envenomation and the recommended treatment for persons exposed to the sting of a caterpillar is mainly symptomatic (GREEN & SIEGEL 1983; KOZER *et al.* 1999). Washing with running water, removing the hairs from the skin with fine forceps or by tape stripping, antihistamines and corticosteroids have all been proposed as therapeutic options (KAWAMOTO & KUMADA 1984; BEAUCHER & FARNHAM 1982; DUNLOP & FREEMAN 1997).

Mast cells contain different kinds of chemical mediators and cytokines, and they release them in allergic and non-allergic reactions together with newly generated mediators from the cell membrane, resulting in inflammatory tissue reactions. Histamine, a major mediator in the allergic reactions, is localized in specific granules. Histamine has many effects such as bronchoconstriction, tissue edema, mucus secretion and fibroblast proliferation. Acute and chronic allergic reactions are triggered by cross-linkage of an antigen-specific IgE antibody with the high affinity IgE receptor on the surface of mast cells, resulting in the aggregation of IgE receptors. This aggregation induces the activation of an intracellular enzyme system which results in the release of preformed chemical mediators such as histamine, chemoattractants, proteoglycans, neutral protease and cytokines, as well as newly generated mediators such as leukotriens and prostaglandins (OKUDA 1999).

Caterpillar hairs cause allergic reactions when they come into contact with skin and through inhalation. The acute effect of caterpillar hairs on dermal mast cells of mice was examined in this study. Dermal mast cell degranulation was observed 12 and 24 hours after exposure to *T. pityocampa* larvae. However, mice were observed scratching approximately 6 hours after exposure to *T. pityocampa* larvae. Actually, no excessive dermal mast cell degranulation emerged as a result of the acute effect. This effect is thought to increase in case of chronic symptoms. It is known that *T. pityocampa* larvae cause allergic reactions in humans and animals. In this study, transmission electron microscopy revealed that *T. pityocampa* larvae cause dermal mast cell degranulation in mice.

Caterpillars on pines in forests, parks and gardens may cause severe allergic reactions in people living or camping in these areas. For this reason warning signs should be placed there and caterpillars should be kept under control.

References

ALLEN V. T., MILLER O. F., TYLER W. B. 1991. Gypsy moth caterpillar dermatitis revisited. J. Am. Acad. Dermatol. **24**: 979-981.

BEAUCHER W. N., FARNHAM J. E. 1982. Gypsy moth caterpillar dermatitis. N. Engl. J. Med. **306**: 1301-1302.

BURNETT J. W., CALTON G. J., MORGAN R. J. 1986. Caterpillar and moth dermatitis. Cutis **37**: 320.

DUNLOP K., FREEMAN S. 1997. Caterpillar dermatitis. Australas. J. Dermatol. **38**: 193-195.

ETKIND P. H., ODELL T. M., CANADA A. T., SHAMA S. K., FINN A. M., TUTHILL R. 1982. The gypsy moth caterpillar: A significant new occupational and public health problem. J. Occup. Med. **24**: 659-662.

EVERSON G. W., CHAPIN J. B., NORMANN S. A. 1990. Caterpillar envenomations: a prospective study of 112 cases. Vet. Hum. Toxicol. **32**: 114-119.

GREEN V. A., SIEGEL C. J. 1983. Bites and stings of himenoptera, caterpillar and beetle. J. Toxicol. Clin. Toxicol. **21**: 491-502.

KAWAMOTO F., KUMADA N. 1984. Biology and venoms of Lepidoptera. (In: Handbook of Natural Toxins. A. T. Tu ed. Marcel Dekker Inc., New York): 291-330.

KOZER E., LAHAT E., BERKOVITCH M. 1999. Hypertension and abdominal pain: uncommon presentation after exposure to a pine caterpillar. Toxicon **37**: 1797-1801.

LAMY M., PASTUREAUD M. H., NOVAK F., DUCOMBS G., VINCENDEAU P., MALEVILLE J., TEXIER L. 1986. Thaumetopoein: an urticating protein from the hairs and integument of the pine processionary caterpillar (*Thaumetopoea pityocampa* Schiff., Lepidoptera, Thaumetopoeidae). Toxicon **24**: 347-356.

LEESON T. S., LEESON C. R., PAPARO A. A. 1988. Text Atlas of Histology. W. B. Saunders Company, Philadelphia.

MONEO I., VEGA J. M., CABALLERO M. L., VEGA J., ALDAY E. 2003. Isolation and characterization of Tha p 1, a major allergen from the pine processionary caterpillar *Thaumetopoea pityocampa*. Allergy **58**: 34-37.

NOVAK F., PELISSOU V., LAMY M. 1987. Comparative morphological, anatomical and biochemical studies of the urticating apparatus and urticating hairs of some Lepidoptera: *Thaumetopoea pityocampa* Schiff., *Th. Processionea* L. (Lepidoptera, Thaumetopoeidae) and *Hylesia metabus* Cramer (Lepidoptera, Saturniidae). Comp. Biochem. Physiol. **88A**: 141-146.

OKUDA M. 1999. Functional heterogeneity of airway mast cells. Allergy **54**: 50-62.

RAUSELI C., MARTINEZ-RAMIREZ A. C., GARCIA-ROBLES I., REAL M. D. 1999. The toxicity and physiological effects of *Bacillus thuringiensis* toxins and formulations on *Thaumetopoea pityocampa*, the pine processionary caterpillar. Pest. Biochem. Physiol. **65**: 44-54.

REBOLLO S., MONEO I., VEGA J. M., HERRERA I., CABALLERO M. L. 2002. Pine processionary caterpillar allergenicity increases during larval development. Int. Arch. Allergy Immunol. **128**: 310-314.

SHAMA S. K., ETKIND P. H., ODELL T. M., CANADA A. T., FINN A. M., SOTER N. A. 1982. A gypsy moth caterpillar dermatitis. N. Engl. J. Med. **306**: 1300-1301.

VEGA J. M., MONEO I., ARMENTIA A., LOPEZ-RICO R., CURIEL G., BARTOLOME B., FERNANDEZ A. 1997. Anaphylaxis to a pine caterpillar. Allergy **52**: 1244-1245.

VEGA J. M., MONEO I., ARMENTIA A., FERNANDEZ A., VEGA J., DE LA FUENTE R., SANCHEZ P., SANCHIS E. 1999. Allergy to the pine processionary caterpillar (*Thaumetopoea pityocampa*). Clin. exp. Allergy **29**: 1418-1423.

VEGA J. M., VEGA M. L., MONEO I., ARMENTIA A. 2003. Skin reactions to pine processionary caterpillar. Allergy **58**: 87-88.

WERNO J., LAMY M., VINCENDEAU P. 1993. Caterpillar hairs as allergens. Lancet **342**: 936-937.

ZIPRKOWSKI L., HOFSHI E., TAHORI A. S. 1959. Contribution to caterpillar dermatitis. Harefuah **56**: 139-141.