Allergy to pine processionary caterpillar (*Thaumetopoea pityocampa*) in children

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**ABSTRACT**
Contact with pine processionary caterpillar (*Thaumetopoea pityocampa*) induces dermatitis usually located in exposed areas through a toxic-irritative mechanism. Over the last few years an immediate hypersensitivity mechanism have mainly been demonstrated in adult patients. However, there are few studies carried out in children.

Objective: To evaluate a group of 16 children who experienced allergic reactions after exposure to pine processionary caterpillar.

Patients and methods: All patients underwent allergy testing through skin prick test. Serum specific IgE determination was performed by EAST technique. The molecular mass of the IgE–binding bands was studied by SDS-PAGE Immunoblotting.

Results: Skin prick test with the caterpillar extract was positive in all patients. Specific IgE was positive (higher than 0.35 kU/L) in 15 patients’ sera. Western blotting showed several IgE-binding bands with molecular mass values ranging from 17.5 to 168 kDa. Electrophoretic mobility of some of the relevant allergens was related to the conditions of sample preparation (reduced or non-reduced).

Conclusions: The results of this study demonstrate the existence of an allergic IgE-mediated mechanism caused by pine processionary caterpillar proteins. Airborne urticating hairs of this animal should be considered as seasonal inhalant allergen, which is able to induce allergic pathologies in children who frequent pine areas.

**Key words:** Pine processionary caterpillar. Allergy. Children. Immunoblotting.

**INTRODUCTION**

Pine processionary caterpillar (*Thaumetopoea pityocampa*), belonging to the Thaumetopoeidae family, can induce skin eruptions, generally located in exposed areas, and less frequently ocular lesions. Usually these reactions are produced by a toxic-irritative mechanism1,2 motivated by airborne urticating hairs of this animal3, which, upon entering and breaking inside the skin produce a basophil degranulation with histamine release4,5.

This caterpillar represents a real pest in Europe, mainly in the Mediterranean area3. The urticating capacity of its hairs is well known from antiquity, however the first descriptions were made by Reaumur in 1736 and by Fabre in 19003. Since then different studies have provided new advances on the etiopathogeny of these reactions, which involve mechanical and chemical factors1,2,4,5. Although this is the main pathogenic mechanism, they are more and more frequent bibliographical references of cases in those a
mechanism of immediate hypersensitivity is implied, generally due to an occupational exposition.

This study emphasizes the appearance of IgE-mediated symptomatology produced by this Lepidoptera in children more frequently than previously observed.

PATIENTS AND METHODS

Patients

We evaluated sixteen children from 6 to 15 years old, who came to our clinical setting with different allergic symptoms probably related to pine processionary caterpillar exposure. Clinical and demographic data of patients are shown in Table I.

Complementary clinical tests

All patients underwent a basic physical examination and a blood sample analysis which included hemogram with leukocyte formula, erythrocyte sedimentation rate (ESR) and biochemical blood analysis, as well as a fecal parasitologic examination when urticaria was the main clinical manifestation, and spirometry in presence of asthma.

Skin test

Skin prick tests (SPTs) were carried out with extracts of common aeroallergens (pollens, animal epithelias, moulds, mites and cockroaches), from Anisakis simplex and from caterpillars at the last larval stage (L5), provided by Laboratorios Bial-Arístegui. The caterpillar extract was also tested in 30 control subjects (atopic and non-atopic). Besides, SPT with mosquito (Aedes sp) extract was carried out in three patients with prurigo disease (patients n.º 2, 6 and 7) and in patient n.º 12.

Preparation of pine processionary caterpillar extract

Some specimens of Thaumetopoea pityocampa in L5 larval stages were ground in a pool of liquid nitrogen into a course “powder” of frozen fragments in a mortar and extracted by magnetic stirring in agitation in 50 mM phosphate-buffered saline (PBS) at pH

<table>
<thead>
<tr>
<th>Patient n.º</th>
<th>Age</th>
<th>Sex</th>
<th>Atopy</th>
<th>Symptomatology</th>
<th>Specific IgE (kU/L)</th>
<th>Class</th>
<th>Prick wheal (mm)</th>
<th>Month of symptoms of skin lesions</th>
<th>Localization of skin lesions</th>
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<tbody>
<tr>
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<td>14</td>
<td>Female</td>
<td>Yes</td>
<td>Urticaria</td>
<td>0.9</td>
<td>2</td>
<td>3 x 3</td>
<td>March-June</td>
<td>IIIE/SSEE*</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>Male</td>
<td>Non</td>
<td>Prurigo</td>
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<td>2</td>
<td>4 x 4</td>
<td>June</td>
<td>IIIE/SSEE</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>Female</td>
<td>Non</td>
<td>Anafilays</td>
<td>1.3</td>
<td>2</td>
<td>6 x 5</td>
<td>February-March</td>
<td>Face/Hands</td>
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<td>4</td>
<td>10</td>
<td>Male</td>
<td>Non</td>
<td>Urticaria-asthma</td>
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<td>3</td>
<td>7 x 5</td>
<td>March-April</td>
<td>SSEE/IEEE</td>
</tr>
<tr>
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<td>6</td>
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<td>Non</td>
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<td>3 x 3</td>
<td>Springtime</td>
<td>NON</td>
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<td>Female</td>
<td>Yes</td>
<td>Prurigo-angioedema palpebral</td>
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<td>3</td>
<td>5 x 4</td>
<td>Springtime</td>
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<td>7 x 9</td>
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<td>4 x 5</td>
<td>Springtime</td>
<td>Generalised</td>
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<td>Springtime</td>
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<td>4 x 3</td>
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<td>Female</td>
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<td>Rhinocconjunctivitis-asthma</td>
<td>&lt; 0.35</td>
<td>0-1**</td>
<td>4 x 3</td>
<td>May-June</td>
<td>NON</td>
</tr>
</tbody>
</table>

*IIIE/SSEE: superior and inferior extremities.
**This value was less than 0.35 kU/L, but greater than the value obtained with the negative control serum (pool from non-atopic subjects’ sera).
75 during 4 h at room temperature. After centrifuga-
tion, supernatant was dialyzed against water. The di-
alyzed extract was filtered through a 0.22 μm-pore di-
ameter membrane and freeze-dried.

**Determination of specific IgE**

The level of serum specific IgE to common aeroal-
lergens and *Anisakis simplex* was measured by CAP
(Pharmacia Diagnostics, Uppsala, Sweden). Mea-
surement of specific IgE to pine processionary cater-
pillar was performed by EAST method using Bial-Aris-
tegui discs with the allergen coupled (10 mg/ml).
Cellulose discs were activated with BrCN following
the method of Ceska et al. and measurement was carried
out with the HY-TEC EIA Kit for specific IgE (HYCOR
Biomedical Ltd. UK) following the manufacturer in-
structions.

**SDS-PAGE Immunoblotting**

SDS-PAGE was carried out according to the
method of Laemmli, 12.5 % and 4 % of acrylamide
were used for separating and stacking gel respective-
ly. Samples were studied in two conditions: reduced
(with β-mercaptoethanol) and non-reduced conditions
(without β-mercaptoethanol). Separated proteins
bands were electrophoretically transferred to
polyvinylene difluoride (PVDF) essentially described
by Towbin et al. and after incubation with patients’
sera detection was performed by a chemilumines-
cence method as recommended by the manufacturer
(ECL-Plus; Amersham Pharmacia Biotech).

**RESULTS**

**Complementary clinical tests**

Physical examination and complementary analy-
ses performed were normal.

**Skin test**

Skin tests against pine processionary caterpillar
eXtract were positive in all patients and negative in
controls.

SPT against common aeroallergens was positive
to pollens in 68.75 % of patients, to mites in 6.25 %,
to moulds in 6.25 % and to epithelia in 25 %.

SPT to mosquito extract was negative in patients
n° 2, 6 and 7, and positive in patient n° 12 who suffers
from allergy to shellfish and showed skin sensitiza-
tion to *Anisakis simplex*. His positive reactivity could
be related to a crossed reactivity. Also, positive SPT
to *A. simplex* extract was also observed in patient
n° 6.

**Measurement of specific IgE**

Results of specific IgE determination are shown in
table I. Serum specific IgE against pine processionary
caterpillar was positive in all cases, except in patient
n° 16. Significant high levels of specific IgE (class = 2)
were detected in 81 % of the sera (13/16).

**Immunoblotting**

Results of SDS-PAGE Immunoblotting with caterpil-
lar extract were different depending on the conditions
in which the sample was prepared: in non-reduced
condition (without β-mercaptoethanol), IgE-binding
bands of 168, 70, 60, 64, 57, 44, 37 and 17.5 kDa
were detected whereas in reduced ones (with
β-mercaptoethanol) the molecular mass of the bands
were 70, 55, 50, 40, 33, 21, 18 and 17/15 kDa (fig. 1).
It is highlighted the presence of a band of approxi-
mately 37 kDa which was only detected in absence
of β-mercaptoethanol, in with 80 % of the studied pa-
tients’ sera detection was performed by a chemilumines-
cence method as recommended by the manufacturer
(ECL-Plus; Amersham Pharmacia Biotech).

**DISCUSSION**

Pine processionary is one of the main European
forest pests. Approximately 150 species of lepi-
doptera have been described which are able to be
harmful on human skin. Several species of cater-
pillars in larvae phase are equipped with an urticat-
ing mechanism provided with chitinous spines able
to penetrate the dermis and to induce contact der-
matitis. The effects of these urticating hairs in peo-
ple are mainly skin injuries (dermatitis and contact ur-
ticaria), conjunctivitis, and, occasionally, bronchial
effects and anaphylactic shock. These spicules are detectable in air by aerobiological methods and
because of its size, (approximate length of
150-200 μm and diameter of 5 μm), its may pene-
trate in the human respiratory systems as far as the
trachea and zones of the primary bronchi, inducing
respiratory pathology. Over the last years and generally in adult popula-
tion, several studies reported the existence
of a hypersensitivity by an 
IgE-mediated mechanism in a high percentage of the reactions to this caterpillar.

Lamy et al in 1986, and Werno et al in 1993 described a specific IgE-binding band of 28 kDa by western blot composed by two polypeptides of 13 and 15 kDa, and they identified it as Thaumetopoein. More recently, Moneo et al described an IgE-binding protein of 15 kDa as the major allergen of pine processionary (Tha p 1). This latter author underlined that this protein showed the same molecular mass regardless of the electrophoretic conditions (reduced or non-reduced) and demonstrated the monomeric nature of the Tha p 1 protein.

In our study, Immunoblotting in absence of β-mercaptoethanol showed an IgE-binding band of 37 kDa in 80% of the patients' sera. In a previous study we detected an IgE-binding band by western blot of similar molecular mass with two of the patient sera (patient 2 and 4). These results indicate the importance of carry out immunoblotting studies in presence and absence of β-mercaptoethanol: if the assay is only performed in standard conditions (with β-mercaptoethanol), the presence of certain relevant allergens as the 37 kDa one here indicated, could be not detected. In reduced conditions, serum of patient n° 12 revealed a pair of IgE-binding bands of approximately 21 kDa/18 kDa, similar to those appeared with the sera of the two patients reported in our previous series. With the patient's serum 3, under reduced conditions, the fixation of IgE is detected in proteins of 15 and 17 kDa whose molecular mass would coincide initially with the most relevant allergen found in a study performed with sera from 16 patients who suffer from contact urticaria described by Vega et al. This allergen was later also described by Moneo et al when they characterized Tha p 1 as the major allergen from Thaumetopoea pityocampa.

In geographical areas with plenty of pine trees, outpatient pediatric consultations for symptoms related to pine processionary are frequent. Nevertheless, studies carried out in children are scarce, and epidemiological studies have not been underwent. As far as we know only in three previous studies an IgE-mediated mechanism has been pointed out as the cause of hypersensitivity to this caterpillar during the childhood. One of them, which included 653 patients aged from 3 to 17 years, demonstrated that reactions to pine processionary affects 9.2% of children and teenagers who frequent pine areas. Our study results agree with Vega et al findings, demonstrating that reactions to pine processionary affects 9.2% of children and teenagers who frequent pine areas. Our study results agree with Vega et al findings, demonstrating that the most common clinical manifestations were the dermatological ones, with lesions generally located in exposed areas. This latter author also described some severe symptomatology like asthma and anaphylactic reactions, in patients exposed to high levels of allergen due to occupational exposure. In our study, in spite of the absence of occupationally exposure, six patients showed associated respiratory pathology, one of them reported asthma as the only clinical manifestation symptoms, and another one suffered an anaphylactic reaction.

In all of the cases here described symptoms appeared mainly between February and April (larvae phase L5), period of the year with the highest presence of caterpillar hairs in the air. Symptoms always appeared several hours after patients have been in pines areas infected with pine processionary caterpillar. Only in one case (patient n° 14) symptomatology occurs in autumn, period of the year when pine processionary is in larvae phase L3-L4, an urticating but
not procession state, and period when anaphylactic reactions are more frequent. Vega et al. noted that 37% of the occupationally exposed patients present symptomatology from October to December, whereas in non-occupationally exposed patients symptoms generally appeared in springtime. Finally, the atopic status of the patients here studied is in accordance with the high percentage of atopic patients found by other authors among non-occupationally patients.

Therefore, the airborne urticating hairs of *T. pityocampa* should be considered, also in children, as seasonal inhalant allergens. In areas where the presence of this caterpillar is endemic, reactions to pine processionary caterpillar proteins should be taken into account in the diagnosis of urticaria, dermatitis and other allergic pathologies in children.

REFERENCES