

Pine Processionary Caterpillar Allergenecity Increases during Larval Development

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Key Words

Allergenecity · Larval development · Pine processionary caterpillar · *Thaumetopoea pityocampa*

Abstract

Background: In European countries, pine processionary caterpillar (*Thaumetopoea pityocampa*) is one of the species of Lepidoptera able to affect humans. Its allergenicity has been demonstrated in previous studies. However, in all of them larval extract in the fifth stage of development has been used as antigen. Therefore, allergenicity of other larval stages was unknown. The objective of our study was to detect allergenic changes of pine processionary caterpillar during larval development. **Methods:** Pine processionary caterpillar extracts in all larval stages were analyzed by SDS-PAGE. Immunoblotting was performed using patient sera with suggestive history of allergy and a positive prick test.

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Introduction

In European countries, pine processionary caterpillar (*Thaumetopoea pityocampa*) is one of the species of Lepidoptera with high medical relevance [1]. This caterpillar is able to elicit cutaneous and ocular lesions and more

rarely, respiratory symptoms and anaphylactic reactions [2, 3].

Caterpillars are born in July, approximately 30–40 days after laying (L1). Ten to 12 days later they perform the first molt, proceeding to a second larval stage (L2). The duration of this second stage is about 18 days. After the second molt, they go on to a third larval stage (L3) that develops during 1 month. This is the moment when urticating hairs appear. In October, caterpillars advance to the fourth stage (L4), whose duration is variable, depending on climatic conditions. At the end of winter, they reach the last larval stage (L5) in which the number of urticating hairs is the highest. After a variable period of time, caterpillars leave the trees and move under soil where they remain during spring. Finally, in June, moths are born and perform the laying [4].

During the last larval stage (L5), exposure is often the most likely because caterpillars leave trees at this stage. However, some workers also have symptoms in the months corresponding to larval stages L3–L4 (for instance ‘pinecone collectors’) and a few other workers throughout the whole year; this fact has been explained by the contact with fomites infested with their hairs [3]. Therefore, a direct contact with the caterpillars rather than with fomites infested with caterpillar hairs may be possible; the contact can be also a result of the airborne spread of hairs.

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Nowadays, urticating properties of this caterpillar are explained by its hairs that progressively appear from the third developmental stage. An IgE-mediated mechanism of hypersensitivity to this caterpillar has been demonstrated in previous studies but in all of them caterpillar extract in the last larval stage (L5) was used as an antigen [1, 3, 5, 6]. Our objective was to find the role that possible allergenic changes during the different larval stages of *T. pityocampa* could play among patients with allergy to this caterpillar.

Materials and Methods

Sera

Thirteen patients with suggestive history of allergy to pine processionary caterpillar and a positive prick test were included in the study. The prick test was performed with extract of caterpillar in the last larval stage (L5), with saline as the negative control and histamine phosphate (mg/ml) as the positive control. One subject with a clear history of caterpillar allergy showing no response in the skin tests, even with the positive control, was also included in the study. Two nonexposed patients (atopic and nonatopic) were used as negative controls.

Extracts

Pine processionary caterpillars in all larval stages (L1–L5) were collected on pine trees from Valladolid (Spain) during the year 2000 and stored frozen at -24°C . Extracts of caterpillar in different larval stages were obtained following mincing and diluting in PBS at a dilution of 1/10 w/v as previously described [3, 5, 6]. After centrifugation for 15 min at 4,500 g, supernatants were collected and stored at -24°C until use.

SDS-PAGE and Immunoblotting

SDS-PAGE of different extracts was performed on a 4% acrylamide stacking gel and 16% separating gel. One hundred microliters of each larval allergen extract were loaded per gel, mixed with the same amount of sample buffer. The gels were stained with Coomassie brilliant blue to detect protein components of the five larval stages or transferred to nitrocellulose. Immunoblotting was performed by a diffusion procedure with two nitrocellulose sheets placed at each side of the gel overnight [7]. The following day after blocking in 3% Nonidet P-40 for 30 min, the membranes were placed on a Mini-Protein II Multiscreen (Bio-Rad, Hercules, Calif., USA). The different sera were diluted 1/5 in incubation buffer, and 0.6 ml of the mixture were incubated overnight in each independent chamber. The next day, after washing, the membranes were incubated for 3 h with 10 ml of a 1/1,000 dilution of a monoclonal anti-IgE antiserum (Ingenasa, Madrid, Spain). After further washes, the membranes were incubated with 10 ml of a 1:4,000 dilution of an alkaline phosphatase-labeled goat antimouse IgG antiserum (Biosource, Camarillo, Calif., USA). Finally, the membranes were washed and bands visualized by incubation with the bromochloro-indolylphosphate/nitroblue tetrazolium chloride substrate for 30 min [8].

The absence of unspecificities was demonstrated using negative control sera. The diffusion procedure enables to obtain two mem-

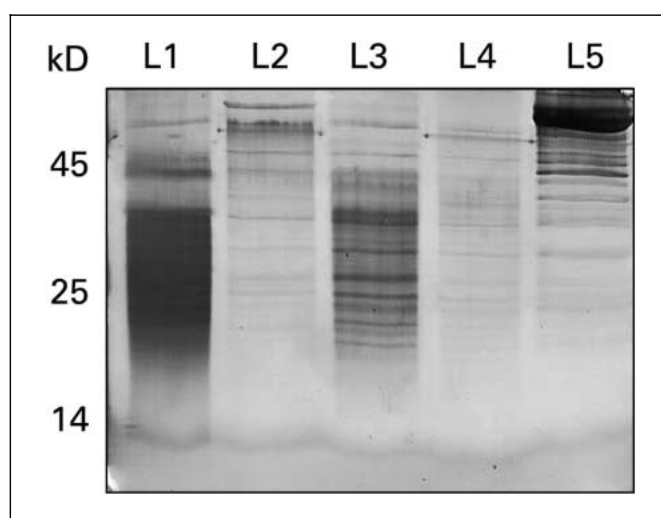


Fig. 1. SDS-PAGE of the extracts of pine processionary caterpillar in all larval stages (L1–L5).

branes from each gel. In this way, the second membrane was always incubated with an irrelevant human serum and the blotting procedure was followed exactly as with the positive sera.

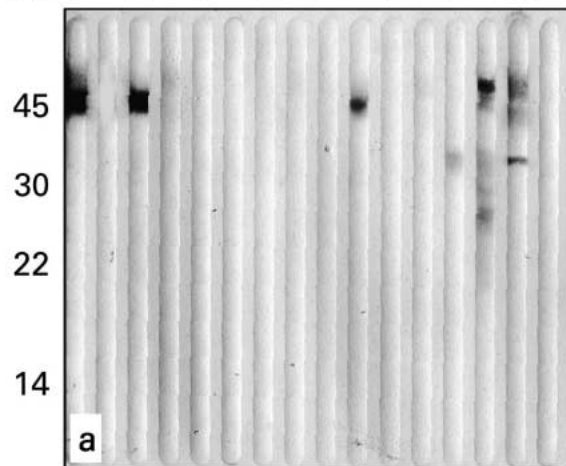
Results

Figure 1 shows the protein pattern of pine processionary caterpillar (*T. pityocampa*) extracts in the five different larval stages (L1–L5). The protein content was similar in the five stages, but less protein was extracted from the fourth larval stage, as shown by SDS-PAGE. Larvae in L1 and L3 had the highest content of proteins with molecular weights <20 kD. On the other hand, in the fifth stage most of the detected proteins had molecular weights >30 kD.

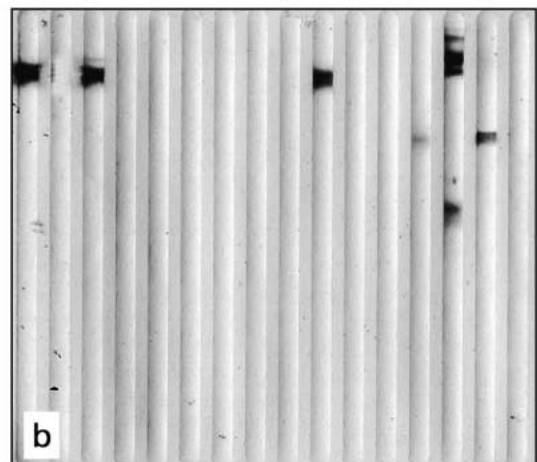
IgE immunoblotting was initially performed with extract of caterpillar larvae in the fifth stage (L5), as previously performed in other studies [3, 5, 6]. Allergens with molecular weights ranging from around 10 to around 45 kD were detected, as described in other studies [3] (fig. 2e). IgE immunoblotting was also performed with extracts of processionary caterpillars in the other four larval stages (L1–L4) using sera from the same patients (fig. 2a–d). When the L1, L2, L3 and L4 extracts were assayed, in none of the patients, even in those highly sensitized, bands with molecular weights <20 kD were detected.

Table 1 shows clinical data of patients whose serum samples were used. Cutaneous lesions with different areas

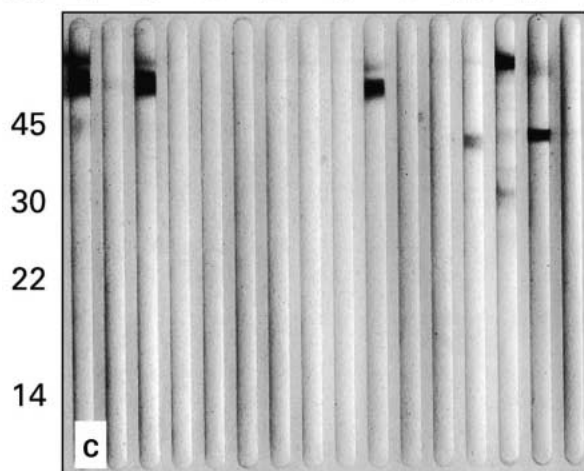
kD 1 3 5 S 9 S 13 15



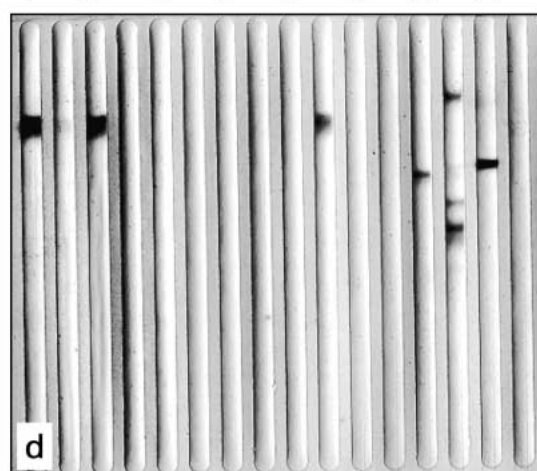
1 3 5 S 9 S 13 15



kD 1 3 5 S 9 S 13 15



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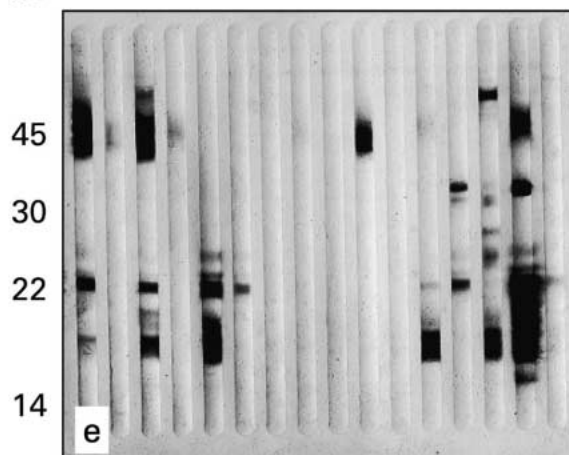


Table 1. Clinical data of the patients and controls

Patient	Occupational exposure	Symptoms	BLOT	Prick area in mm ²
1	yes	CL	L1–L5	130
2	no	CL	L5	25
3	yes	CL	L1–L5	64
4	yes	CL	L5	25
5	no	CL/edema	L5	NE
6	no	CL	L5	49
7	no	control	negative	negative
8	no	CL	negative	49
9	no	CL	negative	25
10	yes	CL	L1–L5	36
11	no	control	negative	negative
12	no	anaphylaxis	L5	130
13	yes	anaphylaxis	L1–L5	49
14	no	CL	L1–L5	32
15	yes	anaphylaxis	L1–L5	100
16	no	anaphylaxis	negative	36

CL = Cutaneous lesions; NE = not evaluable; BLOT = larval stages where specific IgE was detected.

of involvement were the most frequent finding. Nevertheless, 4 patients suffered an anaphylactic reaction. Six of the positive patients (43%) had an occupational exposure, and in all of them positivity was detected by IgE-immunoblotting. In 5 of the 6 patients (83%), IgE binding proteins were detectable when extract of caterpillar larvae from the first four stages of development was used. Eight subjects (57%) had not an occupational exposure; in 5 of them, positive IgE-immunoblotting was noted and in only 1 of the 8 (20%) proteins in the first larval stage were found.

Discussion

Pine processionary caterpillar is one of the most frequent species of Lepidoptera in European countries able to affect humans [1–3]. The allergenicity of this caterpillar has been demonstrated in previous studies [1, 3, 5, 6].

Fig. 2. Immunoblotting of extracts of pine processionary caterpillar in all larval stages. The patient sera are used in the same order in all the immunoblottings. S = Control sera. IgE binding proteins with molecular weights >30 kD were only detected in the sera of some of patients in larval stages 1–5. Allergens with molecular weights <20 kD were only found using caterpillar larvae in the fifth stage.

However, this is the first report studying pine processionary caterpillar (*T. pityocampa*) in different stages of larval development. To our surprise, only in some of the patients, IgE binding proteins with molecular weights >30 kD in larval stages 1–5 were detectable in serum. On the contrary, proteins with molecular weights <20 kD were only detected using caterpillar larvae in the fifth stage. This fact is relevant because it has recently been reported that these low-molecular-weight proteins are the main IgE binding components of crude caterpillar extract in contact urticaria from this caterpillar [5]. Nevertheless, as shown by SDS-PAGE (fig. 1), the initial larval stages (L1–L4) had the highest concentration of proteins with molecular weights <20 kD. In contrast, in the last larval stage most proteins had molecular weights >30 kD. These data suggest that there is no correlation between protein pattern and allergenicity. Probably, the protein profiles differ between the larval stages due to differential gene expression. Hairs are an extension of the cuticle of the caterpillar, possibly explaining the detection of bands since in the first larval stages urticating hairs do not exist. Caterpillars leave trees at the last stage (L5), thus, exposure is more likely, possibly explaining the differences in the protein pattern.

In most of the patients who had an occupational exposure (83%), allergens could be detected when the caterpillar extract from the first larval stage was used. On the contrary, only in 1 of the nonoccupational patients these allergens were found. According to these data, sensitized patients with an occupational exposure should present symptoms in any season of the year. At the same time, this study suggests that a great number of nonoccupational patients may visit pinewoods during the four initial stages of pine processionary caterpillar development.

Perhaps the most important finding of the present work is the dramatic increase in the allergenic potency after molting from the L4 to L5 larval stage. In 5 of the 14 positive patients (36%), only allergens to larvae in the last stage of development could be detected. Therefore, according to our data, this extract should be recommended for in vivo or in vitro diagnosis to avoid false-negative results when testing subjects with suspected caterpillar allergy.

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