Original Article

Specific IgG to *Thermoactynomices vulgaris*, *Micropolyspora faeni* and *Aspergillus fumigatus* in building workers exposed to Esparto grass (plasterers) and in patients with esparto-induced hypersensitivity pneumonitis

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Summary. *Background:* Esparto is one the most frequent causes of hypersensitivity pneumonitis in Spain.
Objective: Determination of risk factors in the working environment that could explain the sensitisation process, and assessment of the differences in specific IgG levels to *Aspergillus fumigatus, Saccharopolyspora rectivirgula* and *Thermoactynomices vulgaris* in patients with hypersensitivity pneumonitis induced by esparto, exposed healthy plasterers and control population.
Methods: Determination of precipitins and specific IgG to *Aspergillus fumigatus*, *Saccharopolyspora rectivirgula* and *Thermoactynomices vulgaris* in the three previously mentioned groups. Questionnaire on possible risk occupational and extra-occupational factors.
Results: Both healthy and exposed plasterers have higher levels of specific IgG to *Aspergillus fumigatus*, *Saccharopolyspora rectivirgula* and *Thermoactynomices vulgaris* than the healthy controls. The patients had higher levels of IgG than exposed healthy plasterers only to *Thermoactynomices vulgaris*. Precipitins were detected in only two patients. There were no occupational factors influencing on the sensitisation process.
Conclusions: Specific IgG is an occupational exposure marker among plasterers, but it has not been possible to establish a cut off point that differentiates exposed subjects from affected ones. This determination has a greater sensitivity than precipitins. We did not identify occupational or extra-occupational risk factors that facilitate the sensitisation process.

Key words: Hypersensitivity pneumonitis, precipitins, *Saccharopolyspora rectivirgula*, Specific IgG, *Thermoactynomices vulgaris.*

Introduction

Hypersensitivity pneumonitis (HP) is a low prevalence pulmonary disease in our environment. Except in Japan, in the rest of the countries this is a disease clearly related to occupational exposure, being considered in many cases as an occupational disease [1]. Both organic and inorganic substances, such as animal derivatives, microorganisms, plants, chemical substances, and drugs, have been described as etiological agents.

The esparto grass is a gramineous plant widely distributed in the South of Europe and North of Africa, and used for the manufacturing of clothes, roped-soled sandals, and especially as supporting material for plaster plaques and molds in the building industry.

Exposure to esparto grass, usually among plasterers, induces hypersensitivity pneumonitis described basically in Spain (2-8). In these patients, precipitins have been described to different fungus and thermophilic actinomycetes.

In the present work we describe the increase of specific IgG values to *Thermoactynomices vulgaris*, *Saccharopolyspora rectivirgula* (formerly *Micropolyspora faeni*) and *Aspergillus fumigatus* in five patients with esparto-induced hypersensitivity pneumonitis and in 45 healthy plaster workers exposed to esparto compared to a control population, as well as the occupational characteristics of patients and healthy workers.

Material and methods

All the workers belonging to the four biggest companies in our province were studied, as well as five workers previously diagnosed with stipatosis. Only the workers usually in contact with esparto grass were studied, not the administrative ones.

Fifty plaster workers exposed to esparto grass, 45 healthy workers and 5 patients with allergic alveolitis, were included. All of them were male with a mean age of 38.1 ± 9.9 years. The diagnosis of the patients with stipatosis was reached by bronchial provocation test with esparto grass extract in one of them, and by new working exposure to esparto in the other four. The requirements demanded in previous diagnostic protocols [9] were fulfilled by all of them, abiding by four of the main criteria:

1) Symptoms compatible with hypersensitivity pneumonitis.
2) Evidence of exposure to a suitable antigen reported in the clinical history or detection of serum antibodies and/or bronchoalveolar lavage fluid antibody.
3) Findings compatible with hypersensitivity neumonitis in the thorax x-ray or high resolution scanner.
4) Histological modifications compatible with HP (if a biopsy had been performed).
5) Lymphocytosis in the bronchoalveolar lavage liquid.
6) Positive provocation test.

And at least two of the minor criteria:

1) Bibasal crepitations.
2) Decrease of the diffusion capacity.
3) Arterial hypoxemia at rest or after the exercise.

Dyspnea, hypoxemia, bilateral pulmonary infiltrates, crepitations and fever were observed in all the patients after occupational exposure or bronchial challenge. The symptoms yielded with parenteral corticosteroids.

All the patients and the exposed healthy workers underwent clinical history, physical examination, spirometry, working history including, among other data, the number of years since starting to work with esparto, number of working hours per day, contact with other fibres (sisal), symptoms induced by different lots of esparto, differences during the holidays, smoking habit, any kind of respiratory diseases and chronic obstructive pulmonary disease (COPD) clinic. The following analytical determinations were also performed: precipitins to *Thermoactynomices vulgaris*, *Micropolyspora faeni* and *Aspergillus fumigatus* (Bial-Arístegui, Bilbao, Spain) by means of double immunodiffusion test according to Ouchterlony's method, and specific IgG to *Thermoactynomices vulgaris, Micropolyspora faeni* and *Aspergillus fumigatus* (Pharmacia, Upsala, Sweden) by CAP according to the manufacturer's instructions.

Twenty control subjects were also selected, matched to the patients in age and sex, not exposed to esparto.

The SPSS 10.0 program was used for the statistical analysis of the results. The descriptive data are shown as mean ± standard deviation in the normal distribution variables, and as median and interquartile range (25^{th} – 75^{th} quartiles) in the not normal distribution variables. Comparison of means was done by the Student's t test for variables with normal distribution or the Mann-Whitney test for variables with no normal distribution. The existence of a relation between quantitative variables was done by Spearman rank test. The comparison was bilateral in all cases, accepting $p < 0.05$ as statistically significant.

Results

The 45 healthy workers had a mean age of 38.2 ± 10.1 years. Thirty-four of them were smokers (mean 16.8 ± 10.6 cigarettes/day), 6 former smokers and 5 had never smoked. In the spirometry, 15 showed a slight or moderated obstructive pattern, and none of them had a restrictive pattern. Seventeen of the patients had manifestations compatible with COPD (usual cough and mucous expectoration). The mean time working as plasterer in contact with esparto was 20.6 ± 10.3 years.

Table 1. Specific IgG levels to *Aspergillus fumigatus, Micropolyspora faeni* and *Thermoactynomices vulgaris* (mg/dl) in patients with stipatosis. Values higher than the percentile 97 of the control population are indicated in bold letters.

	Aspergillus fumigatus	***Micropolyspora Faeni***	***Thermoactynomices Vulgaris***
Patient 1	15.6	6.49	**41.3**
Patient 2	**40.3**	9.72	**29**
Patient 3	10.1	7.31	**58.9**
Patient 4	**34.6**	9.27	**32.3**
Patient 5	**152**	7.5	17.4

Four hours were the mean working hours they spent preparing the esparto (when a greater release and inhalation of esparto dust is produced) and a further four hours were used to place the plaster molds and plaques.

The five patients with stipatosis had a mean age of 35.8 ± 8 years. The mean time working as plasterers was 19.3 ± 10.2 years. Four working hours were used to prepare the esparto and a further four to place the plaster. None of these parameters showed significant differences with the healthy workers. Three of the five workers with stipatosis were smokers at the moment of the diagnosis (18.5 ± 6.8 cigarettes/day), one was a former smoker and the other one had never smoked. One of them had COPD (former smoker) and his spirometry showed a slight global obstructive pattern. The spirometry values of the other patients were within normality because the diagnosis was done in early phases (they presented with the acute form of the disease), and none of them had pulmonary fibrosis or restrictive pattern.

Thirty-nine healthy plasterers reported irritant nasal and/or bronchial symptoms (aqueous rhinorrhea, nasal pruritus, cough, etc) depending on the characteristics of the lots of esparto used (greater or lesser amount of powder released at manufacturing, change in smell and humidity of esparto, etc). Four of the patients with stipatosis reported worsening of the symptoms related with some lots of esparto, whereas they did not present symptoms with other lots. There are not significant differences in the different items of the questionnaire between the group of patients and the healthy exposed workers.

The precipitins were positive for *Aspergillus fumigatus* in one of the patients, for *Thermoactynomices vulgaris* and *Mycropolyspora faeni* in another one and only to the esparto extract in the third patient, whereas no precipitins were detected in the other two patients. Precipitins were negative in the healthy plasterers and in the controls.

The specific IgG levels to *Thermophilic actinomycetes* and *Aspergillus* can be seen in Table 1 and 2. The specific IgG levels were higher in patients and in healthy workers than in controls to all the antigens ($p<0.04$ in all the comparisons). The patients with stipatosis had higher levels than the healthy workers only to *Thermoactynomices vulgaris* ($p=0.003$) (Table 2).

Twenty-six healthy workers had specific IgG values over the percentile 97 of the control subjects for some of the antigens studied: seven for *Aspergillus fumigatus* (percentile 97 = 34.4 mg/dl), two for *Micropolyspora faeni* (percentile 97 = 15.1 mg/dl), five for T*hermoactynomices vulgaris* (percentile 97 = 20.7 mg/dl), six for *Aspergillus fumigatus* and any of the actinomycetes, three for both actynomycetes and three for the three antigens.

Three of the five patients with stipatosis had higher levels of specific IgG to *Aspergillus fumigatus* than the percentile 97 of the control population, and four to *Thermoactynomices vulgaris* (two of them had high levels to both antigens).

Specific IgG levels for any of the three antigens studied had no relation with the years of work (Spearman rank test $p > 0.3$ in all the cases) in exposed healthy workers or in affected plasterers.

Discussion

Allergic alveolitis induced by esparto has been associated with different antigens such as *Micropolyspora faeni, Thermoactynomices vulgaris, Penicillium frequentans* and *Aspergillus fumigatus* [3,7,8,10]. By means of a bronchial challenge test with these two last antigens, the clinical pictures could be reproduced in two patients with stipatosis [8, 10]. Nevertheless, challenge with the esparto extract reproduced the clinical picture [2-6] in others. In other patients, techniques such as the enzimoimmunoassay or other less sensitive such as the double immunodiffusion (Ouchterlony) have detected precipitins to several of those antigens or to the esparto extract itself [3,4,6,7,10].

In this work we present the specific IgG levels to

Table 2. Specific IgG values to *Aspergillus fumigatus, Micropolyspora faeni* and *Thermoactynomices vulgaris* (mg/dl) in workers with stipatosis, exposed healthy workers and in the control population.

	Aspergillus fumigatus **(median; percentiles 25^{th} - 75^{th})**	***Micropolyspora Faeni*** **(median; percentiles 25^{th} - 75^{th})**	***Thermoactynomices Vulgaris*** **(median; percentiles 25^{th} - 75^{th})**
Healthy plasterers	18.2; 6.9 - 45.6**	6.3; 3.7 - 15.7**	12.5; 7 - 23.4**
Affected plasterers	34.6; 12.9 - 96.15*	7.5; 6.9 - 9.5*	32.3; 23.2 - 50.1*Ⅱ
Controls	8.4; 5 - 13.4	0.01; 0.01 - 6.1	10.4; 4.9 - 12.4

* $p < 0.001$ between patients and controls
** $p < 0.05$ between healthy plasterers and controls.
Ⅱ $p < 0.005$ between patients and healthy plasterers.

Micropolyspora faeni, *Thermoactynomices vulgaris* and *Aspergillus fumigatus* both in patients and in healthy plasterers usually exposed to esparto. Like with other alveolitis [11], healthy exposed subjects have higher values of serum specific IgG than controls to one or several of these antigens. With our data it is not possible to determine the existence of a cut-off point in these values which allows distinguishing between patient and exposed healthy subjects, since the response of the own patients is not homogeneous to these allergens and furthermore their specific IgG response varies importantly against each one of them.

However, our data do guarantee the superiority of fluoroimmunoassay vs the immunodifussion to detect and quantify more precisely the presence of a humoral response to the different allergens to which these individuals are exposed.

Although the presence of specific IgG to one or several of these antigens is indicative only of exposure to them and not necessarily of an etiological-causal relation, its presence seems to constitute a *sine qua non* condition for the diagnosis of this pathology as well as for the identification of its causal agent. High levels of specific IgG for one or several of the studied antigens are observed in all the patients studied, and in several of them, against several antigens. This fact supports other authors' opinion that at least in some of these patients, this pathology could be due to more than one environmental agent, even though none of them is exclusively responsible for the disease [11]. On the whole, all the data existing up to now support the definition of stipatosis as a syndrome in which there are several causal agents.

These multiple sensitisation data are favoured by the own esparto manufacturing process. This has several phases, and in one of them the esparto is kept in pools of water for two months in order to eliminate the pod and the paraffin layer. Subsequently it is piled up until manipulation. During this process the esparto can be contaminated by different moulds [6-8] and additionally, due to the material degradation and spontaneous heating, it reaches humidity (30-40%) and temperature (50-60ºC) conditions suitable for the growing of moulds such as *Aspergillus fumigatus, Penicillium,* etc., or actynomycetes such as *Micropolyspora faeni* and *Thermoactynomices vulgaris*. These conditions are not repeated in all the lots and therefore most patients reported that their respiratory symptoms varied depending on the characteristics of the different esparto lots. The plasterers' usual exposure to these antigens induces an IgG response. The concomitant existence of factors such as respiratory infections [11] is likely to start in susceptible individuals the immunological response which triggers alveolitis. It is surprising that most of our patients as well as in most publications [4-6] are smokers, very rare in the rest of the allergic alveolitis [12]. Like the rest of the cases published, they are males given the predominance of this sex in this profession. There are no data in the working environment which allow to establish risk criteria for the development of esparto-induced alveolitis, since in all the working items studied there are no differences between patients and exposed healthy workers. This fact supports the idea that, like in other alveolitis, there could exist a genetic-immunologic susceptibility, or infectious processes that could act as a triggering factor in these pathologies [1,11].

In one of the companies where two cases of alveolitis were noticed, substitution of esparto by another natural fibre such as sisal allowed the patients' continuing with their job suffering from no respiratory symptoms, like the other workers in the same company after a two-year follow up.

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