

Natural and adaptive immune reactions to inhaled microorganisms in the lungs of farmers

by Per Malmberg, MD, Anna Rask-Andersen, MD¹

The incidence of allergic alveolitis, defined according to the criteria of Terho (15), is relatively low in Finland (16) and in the middle part of Sweden (2–4 cases per 10 000 farmers per year). Symptoms similar to those of an acute attack of allergic alveolitis are however about 30–50 times more common than allergic alveolitis. These symptoms occur in farmers without evidence of hypersensitivity to mold dust after exposure to large amounts of microorganisms. The name “organic dust toxic syndrome” has been suggested (4) for this and similar conditions. Although many differences between the organic dust toxic syndrome and allergic alveolitis exist and are important to recognize in view of their prognostic and therapeutic implications, there are also some similarities which indicate common pathogenic mechanisms. Furthermore, it is possible that transitional states exist between the two diseases. The results presented in this report were derived from four studies, of which one has been published (11) and three have been presented as preliminary reports (7, 9, 10). The purpose of these studies was to investigate the incidence and nature of reactions to inhaled organic dust in the farming community and to study the relation between exposure and symptoms. The presentation is focused on the similarities between the two conditions rather than on the differences, which have been described elsewhere (12).

Subjects and methods

The farmers were all interviewed by trained physicians. Medical records were obtained from those who had sought medical advice. A skin prick test with common allergens, spirometry, and blood tests for the determination of antigen-specific antibodies were performed in association with the interviews. Twenty-six farmers with symptoms of organic dust toxic syndrome underwent extensive physiological investigation, including measurement of arterial blood gases during exercise at a symptom-free interval.

The exposure to microorganisms during farm work was measured on 143 farms. If possible, the exposure conditions associated with the symptoms were re-created, with the use of the same material and work practices as those believed to have caused the symp-

toms. This re-creation reflected an unusually heavy exposure. Therefore, these samples were called “worst case” samples. The adequacy of the samples in this respect was noted on each occasion. In addition, the typical exposure during normal daily activities, excluding “worst case” type of work, was measured as background samples. The microorganisms were collected with filter cassettes and analyzed with an epifluorescence dye method (13). Endotoxin was analyzed with the limulus amebocyte lysate method.

The material was compiled from four studies. Questionnaires were mailed to 550 farmers from Dalecarlia (study A) and to farmers from all 6 267 farms in an area in the middle part of Sweden (study B). The response rate was 70 %. A stratified sample of farmers was invited to attend oral interviews, and a total of 470 farmers were interviewed in studies A and B. In study C 20 dairy farmers from the Stockholm region were randomly selected, and all agreed to have exposure measurements performed (reference farmers). Finally, medical institutions with the possibility of being contacted by patients with attacks of allergic alveolitis or organic dust toxic syndrome were invited to participate in a three-year prospective study (study D). This study allowed immediate notification of new cases and an opportunity to secure material which may have been associated with the acute symptoms in order to reproduce and measure exposure. In addition, a detailed account of the exposure situation was obtained on location by experts in farming practices and microbiology.

Results

Organic dust toxic syndrome

A total of 128 farmers from the compiled material had suffered from at least one febrile reaction following exposure to organic dust, but did not fulfill the diagnostic criteria of allergic alveolitis. They became the “toxic syndrome” group. In the majority of these cases there was no evidence of chronic disease or dyspnea prior to the attack, and recovery to full capacity was usually accomplished within two weeks. Only 14 % had sought medical advice for their condition. Twenty-six farmers who had experienced one or more occurrences of the toxic syndrome were given an extensive physiological examination, including measurement of

¹ National Institute of Occupational Health, Solna, and University Hospital, Uppsala, Sweden.

arterial blood gases during maximal exercise, after recovery from the acute symptoms. These farmers did not differ from 17 reference farmers (11). Chest radiographs were obtained within one week of the occurrence of symptoms in 19 farmers with the toxic syndrome. For five farmers (26 %) the protocols indicated the presence of slight shadowing. Oxygen tension of the arterial blood was measured and was below 10 kPa in four out of the six farmers for whom this parameter was measured at a symptomatic stage. The incidence of a positive skin prick test and the total immunoglobulin E (IgE) levels did not differ from that of the reference farmers. The prevalence of tobacco smoking was lower than among the reference farmers (13 % of the toxic syndrome farmers versus 23 % of the reference farmers).

Allergic alveolitis

Thirty-five subjects fulfilled the major criteria of Terho for allergic alveolitis (15). For 12 of these persons it could not be determined whether they met the additional criteria of Terho due to insufficient examination or documentation in the medical records. In this group a decrease in pulmonary function was found during the acute illness, and for half of them the decrease was evident in measurements performed in connection with the interviews. The same lack of association with atopy and negative association with smoking habits was observed for the allergic alveolitis group (7 % smokers). A positive precipitin reaction (double diffusion in gel) was found for 53 % of those with allergic alveolitis, for 29 % of the toxic syndrome group, and for 11 % of the reference farmers.

Exposure

The exposures associated with illness involved a wide range of microorganisms and were associated with the handling of many types of moldy material. It was not possible to conclude that a particular species was more pathogenic than others except through the indirect association between high exposure levels and disease and the association between high exposure levels and the dominance of thermotolerant microorganisms. Very high spore levels were seen during the handling of material which had been used before the onset of either the toxic syndrome or allergic alveolitis. Such exposures were not observed on the farms of farmers who had not experienced symptoms. As a rule, the background levels were low and did not differ much between symptomatic and reference farmers (table 1).

Very high endotoxin levels were found; however these levels did not differ much between reference farmers and those with the toxic syndrome or allergic alveolitis. About one-third of the values exceeded 50 µg/m³ of inhaled air. Samples were also obtained with a vertical elutriator for the measurement of the respirable fraction (table 2).

Table 1. Spore concentrations during normal farm work (background levels) or while handling moldy materials which have caused allergic alveolitis or toxic reactions (in other words the "worst cases"). In addition the background spore concentration and levels evolved during the handling of the most moldy material available on farms of symptomless farmers.

Group	Number of samples	Mean ^a	SD ^a	Range ^a
Organic dust toxic syndrome				
Worst cases	13	8.4	10.6	0.3—3.6
Background	4	0.01	0.02	0.001—0.03
Allergic alveolitis				
Worst cases	6	2.5	1.4	0.6—4.5
Background	6	0.03	0.03	0.008—0.08
Reference				
Worst cases	17	0.1	0.6	0.001—0.6
Background	8	0.01	0.02	0.003—0.05

^a Microorganisms/ml of air • 1 000 (= • 10⁶/m³).

Table 2. Endotoxin concentrations measured during the handling of moldy material. Values > 50 µg/m³ recorded as 50. See table 1 for further explanations.

Group	Number of samples	Number of samples > 50 µg/m ³	Mean ^a	SD ^a	Minimum ^a
Organic dust toxic syndrome					
Worst cases	6	2	28	24	0.01
Allergic alveolitis					
Worst cases	6	3	20	24	0.01
Reference					
Worst cases	17	6	34	21	0.3
Worst cases with elutriator	17	2	18	18	0.3

^a Endotoxin µg/m³ of air.

Discussion

The results of these studies point at several similarities between the organic dust toxic syndrome and allergic alveolitis. Thus the acute symptoms of the organic toxic syndrome were associated with high exposure levels. High exposure levels may also be necessary for the induction of allergic alveolitis. There was no evidence of hypersensitivity in cases with the toxic syndrome, and in several instances a clustering of cases was observed. In fact, it appeared that all the subjects exposed to an environment causing an acute reaction became ill in proportion to exposure. This observation has been described previously (12). The very high exposure levels observed in several of the environments associated with allergic alveolitis (comparable with the levels causing the toxic syndrome) indicate that high exposure levels may be important also in the induction of allergic alveolitis. In two families both the husband and wife had allergic alveolitis following exposure to very moldy material. The wives became ill a few weeks after their husbands, following their performance of the work tasks previously done by their husbands. Thus it appears that a high single dose of inhaled microorganisms will cause an acute inflammatory reaction in any subject and that high exposures

which are repeated with sufficiently short intervals promote the development of allergic alveolitis. Evidence of transitional stages between the toxic syndrome and allergic alveolitis were found. Two farmers with the toxic syndrome who were otherwise not distinguishable from the other cases subsequently had several new exposures and febrile episodes following the original diagnosis, and they developed pulmonary chest radiographic changes compatible with allergic alveolitis.

These observations are compatible with current knowledge of pulmonary pathophysiology. Allergic alveolitis in farmers can be regarded as an example of an adaptive immune reaction to inhaled microorganisms. It involves the production of specific antibodies and antigen-specific T lymphocytes and the proliferation and activation of many other cells involved in the inflammatory response such as mast cells and fibroblasts. The pulmonary macrophages are highly activated. A large increase in mast cells has been observed in allergic alveolitis (6, 14), and the same phenomenon was also seen in seven farmers from the present study who underwent bronchoalveolar lavage (2). The exact mechanisms are not known. Complement activation may play an important role in initiating the reaction. Macrophages are activated by complement factor C5a and by antigen-coated microorganisms. Complement factor C5a and leukotriene B₄, elaborated by activated macrophages, may cause the influx and activation of granulocytes, which contribute to the early inflammatory reaction to mold inhalation. Thus, the fluid of bronchoalveolar lavage shows a transient granulocyte reaction following antigen challenge in cases of allergic alveolitis (5, 6). Macrophages also produce interleukin-1, which stimulates different lymphocyte populations, and interleukin-2, produced by activated lymphocytes, may further stimulate macrophages. Macrophages may play a key role in the initiating events, and it has been suggested that a defective function of macrophages may explain the lower incidence of allergic alveolitis in farmers who smoke tobacco. The chronic inflammatory state typical of allergic alveolitis may require the continued presence of antigen, which causes chronic stimulation of the lymphocyte and other inflammatory and repair cell systems.

Several aspects of the acute reaction of allergic alveolitis are similar to those of the toxic syndrome. In both conditions an acute inflammatory reaction with a decrease in gas transfer and a predominance of granulocytes is observed following exposure. In the toxic syndrome the events may be triggered by an activation of complement through the indirect pathway. Macrophage activation and granulocyte recruitment and activation may involve common pathways. Some of the subsequent events such as the release of fibronectin, interleukin-1, and other factors involved in the chronic inflammatory reaction may be similar in both events and provide a link between the two conditions. Specific antibodies are important in the defense against

inhaled particulate matter, and it is important to note that even apparently unexposed subjects (urban dwellers) have antibodies against most microbial antigens which are believed to cause farmer's lung (1). The reaction to inhaled microorganisms causing organic dust toxic syndrome in asymptomatic farmers may involve adaptive immune mechanisms. Findings of bronchoalveolar lavage in 10 of the reference farmers in the present study (7), as well as in other studies (8), indicate the presence of low-degree inflammatory changes in the lungs and the presence of an increased number of T cells. Endotoxin has been implied as a possible cause of the toxic syndrome. This component of the walls of gram negative bacteria is possibly more potent than other microorganisms in eliciting a febrile response following inhalation. The mechanisms proposed for the action of endotoxin are similar to those already described under natural immunity. The responsible agents for a type of reaction such as the organic dust toxic syndrome may therefore depend on the relative proportion and potency of different microorganisms in the inhaled dust. The abnormally high values found in the present study in environments not causing symptoms cause the concern that perhaps material other than endotoxin in the air samples may have influenced the measurements.

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