Hypersensitivity Pneumonitis due to Metalworking Fluids: How to Find the Antigens

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Abstract

Most surveys of outbreaks of hypersensitivity pneumonitis (HP) in subjects with occupational exposure to water-based metalworking fluids (MWFs) were unable to detect a clear link between symptoms and the precise causative agents. We studied the case of a male 41-year-old industrial knife grinder with exposure to water-based MWFs since 12 years. The diagnosis of HP was made by typical work-related symptoms, the demonstration of high lymphocyte numbers in bronchoalveolar lavage and elevated IgG antibody concentrations to various molds in the patient's serum, and complete recovery after early exposure cessation. Whereas an environmental survey showed only low numbers of mold contamination in one sump sample, high antigenic activity was demonstrated in the same sample by antigen-specific IgG inhibition tests. We conclude that the detection of antigenic molds in water-based MWFs by culture methods may be limited. The link between occupational exposure to specific molds in MWFs and hypersensitivity pneumonitis can be established by the demonstration of antigenic activity by antigen-specific IgG inhibition tests.

Keywords

Actinomycetes • Bronchoalveolar lavage • Hypersensitivity pneumonitis • Metalworking fluids • Molds • Occupational exposure

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1 Introduction

Cases with hypersensitivity pneumonitis (HP) and occupational exposure to water-based metal-working fluids (MWFs) were reported repeatedly in literature (Robertson et al. 2007; Fishwick et al. 2005; Hodgson et al. 2001; Fox et al. 1999). However, environmental surveys were unable to detect a clear link between case definitions and qualitative exposure indices in most symptomatic workers. We report such a case by using a novel method in order to avoid pitfalls of hitherto existing diagnostic tools.

2 Case History

A 41-year-old industrial knife grinder with exposure to water-based MWFs since 12 years developed recurrent work-related 'flu-like' symptoms, fatigue, joint pain, and mild shortness of breath. A diagnosis of HP was made by his pneumologist about 2 months later. Whereas lung function and high resolution computed tomography were normal, bronchoalveolar lavage yielded 65 % lymphocytes, and elevated specific IgG antibody concentrations to various molds were found in the patient's serum. After immediate complete exposure cessation the patient was seen in IPA for a medical opinion shortly after the results of the environmental survey became available (see below). He had no symptoms and lung function was normal. The subject gave written informed consent to publish his case.

3 Methods

3.1 Environmental Survey – Sampling

A survey in the working environment was performed by the Statutory Accident Insurance of the Wood and Metal Industry a few weeks after the diagnosis. The patient had worked on grinding machines with separate MWFs sumps. Samples were taken from the sump of a machine without visible contamination (MWF1) and from the sump of a machine with visible snow flurry-like contamination (MWF2; two samples were taken from MWF2 with a 3 weeks interval). This machine was inoperative since about 2 months prior to the survey for technical reasons. Two different water-based MWFs, but no additional biocides were used in this plant.

Water-based MWFs samples were collected in sterile 500 mL bottles for the determination of colony counts and identification of bacteria and molds. MWF samples were transported to the laboratory within 24 h at temperatures of 2–8 °C. Microbiological analyses were carried out immediately after arrival of the samples.

3.2 Environmental Survey – Analyses of Bacteria

For the determination of total colony counts samples were serially diluted with 0.9 % NaCl and inoculated on tryptic-soy agar (TSA; Merck, Darmstadt, Germany) with 0.3 g/L cycloheximide (AppliChem, Darmstadt, Germany). Plates were incubated aerobically at 30 °C. Colonies were counted after 24 h up to 6 days. Predominant colonies on the total-count plates were picked and grown as a pure culture for further identification. Colony characteristics or morphological characteristics of spores were described as recommended by Bergey's Manual of Determinative Bacteriology (Holt 1994). Selected bacterial isolates were Gram-stained and further identified using the Crystal-System GP (Becton-Dickinson Diagnostics, Heidelberg, Germany) and the API-System 20NE (bioMérieux, Nuertingen, Germany). For total colony counts of thermophilic actinomycetes, samples were serially diluted with 0.9 % NaCl and 0.01 % Tween 80 and inoculated on glycerol-arginine agar (Danneberg and Driesel 1999). Plates were incubated at 36 °C and colony counting was performed up to 14 days. Mycobacteria were detected by microscopy and cultivation as described earlier (Medical Standards Committee 1996). Identification of certain species was carried out by polymerase-chain-reaction (PCR) and the detection of specific gene-sequences (Khan and Yadav 2004).

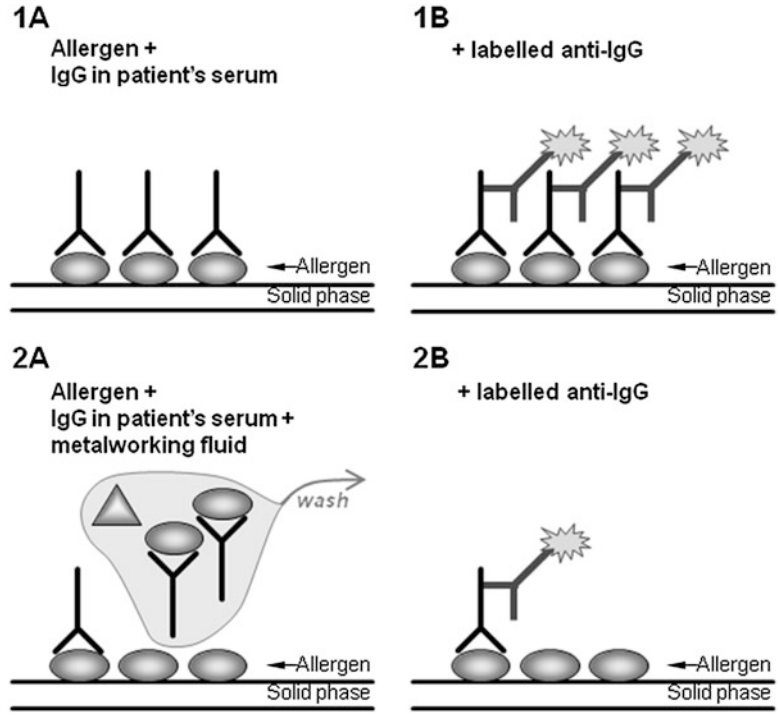

Fig. 45.1 Principle of IgG inhibition testing with metalworking fluid. Diagrams (**1A**) and (**1B**) show the direct binding of IgG antibodies in the patient's serum to the allergens bound to the solid phase. Preincubation with metalworking fluid in inhibition testing (**2A**) yields lower binding of IgG to the solid phase (**2B**). The binding differences (between **1B** and **2B**) are expressed as inhibition rates

3.3 Environmental Survey – Analyses of Molds

For the determination of total colony counts samples were serially diluted with 0.9 % NaCl and 0.01 % Tween 80 and inoculated on dichloranе-glycerol agar (DG 18, Oxoid, Wesel, Germany). Plates were incubated aerobically at 25 °C. Colonies were counted after 48 h, then every 24 h up to 7 days. Mold species were identified by microscopy and visual detection of conidia. For the detection of *Aspergillus fumigatus*, samples were inoculated on malt extract agar (Merck, Darmstadt, Germany) and incubated at 37 °C.

3.4 Immunological Testing

Specific IgG antibodies to various microorganisms were measured with IgG-ImmunoCAP (Phadia, Freiburg, Germany) in the patient's serum. Sump samples from MWF1 and MWF2 were used to inhibit binding of the patient's specific IgG antibodies to commercially available ImmunoCAPs. The method has been described in detail recently (Merget et al. 2009). Briefly, the sump samples were centrifuged at 2,400 × *g*, the pellets were homogenized by Precellys SK38 (Bertin, Montigny-le-Bretonneux, France) at 3 × 6,500 rpm, centrifuged at 15,800 × *g*, and the sterile filtrated supernatant was used for specific IgG inhibition (Fig. 45.1).

4 Results

4.1 Environmental Survey

The first MWF2 sample contained 6.3×10^2 colony forming units (CFU) of molds/mL (predominantly *Fusarium solani* and *Fusarium oxysporum*) and 1.2×10^6 CFU of bacteria/mL (predominantly *Micrococcus luteus* and *Mycobacteria* sp.), the second MWF2 sample showed similar contamination but was not cultivated. MWF1 did not show any growth of molds, but yielded 2.2×10^5 CFU of bacteria/mL (*Moraxella* sp. and *Mycobacteria* sp.). In none of the MWF samples thermophilic actinomycetes were detected. Air monitoring

Table 45.1 IgG antibody concentrations in the patient's serum and inhibition rates of IgG-binding to different microorganisms with sump samples of a grinding machine without (MWF1) and with (MWF2) visible snow flurry-like contaminations

Antigen	IgG-antibody concentration (mg/L)	Manufacturer's cut-off (mg/L)	Inhibition with MWF1 (%)	Inhibition with MWF2 (%)[a]
Alternaria alternata	70	12	0	63
Fusarium proliferatum	133	46	8	48
Aspergillus fumigatus	78	39	0	13
Aspergillus versicolor	38	100	ND	ND
Cladosporium herbarum	154	37	0	55
Penicillium sp.	85	50	ND	ND
Aureobasidium pullulans	123	22	8	78
Saccharopolyspora rectivirgula	53	10	5	27

ND not done, [a]MWF2 inhibition rates represent the arithmetic mean of two separate samples

did not show microbial contamination (data not shown). In summary, the environmental survey yielded very low mold contamination of MWF2.

4.2 Immunological Testing

The earlier findings of elevated specific IgG antibodies to molds in the patient's serum were reproduced. More than twofold higher specific IgG antibody concentrations than the manufacturer's cut-off values were detected with *Alternaria alternata* (Gm6), *Fusarium proliferatum* (m9), *Cladosporium herbarum* (Gm2), *Aureobasidium pullulans* (m12) and the actinomycete *Saccharopolyspora rectivirgula* (Gm22). Specific IgG inhibition experiments showed no inhibition with MWF1, but the preincubation of the patient's serum with MWF2 strongly inhibited the binding of the specific IgG antibodies to antigens of *Alternaria alternata*, *Fusarium proliferatum*, *Cladosporium herbarum* and *Aureobasidium pullulans* (Table 45.1).

5 Discussion

The diagnosis of occupational HP is often made after exposure cessation, because subjects experience work-related symptoms and realize that the work environment causes them. In view of the overall favorable prognosis of HP patients are referred for a definite diagnosis weeks or months after exposure cessation symptom-free, with normal chest x-ray or computed tomography and without lung function impairment. Also in the present case the diagnosis was based on typical symptoms, lymphocytosis of bronchoalveolar lavage, the demonstration of mold-specific IgG antibodies and the disappearance of symptoms after exposure cessation. Thus the diagnosis of HP can be made with reasonable certainty and challenge testing was not considered ethical.

Although the environmental survey showed only low numbers of molds and no actinomycetes in one sump probe and no relevant concentrations of microorganisms in air samples, the results of the inhibition tests demonstrate that antigenic activity was present in MWF2, but not in MWF1. This finding indicates that monitoring of MWFs by culturing of molds may be limited by producing false-negative results that may lead to diagnostic problems because cases with occupational HP often do not show the complete spectrum of the disease due to early exposure cessation. If the medical diagnosis is not certain and environmental surveys of the workplaces do not show relevant microbial contamination, the subjects may be not diagnosed correctly. This has also consequences for the prevention of further cases in the respective industrial setting.

The present results do not allow to define a single causative mold, but they demonstrate a clear link between disease and exposure. This is especially evident for molds which could be cultivated only in small numbers as *Fusarium* sp. in the contaminated MWF, but induced strong specific IgG responses and exhibited inhibitory capacity. As no immunologic tests with bacteria were performed (with the exception of *Saccharopolyspora rectivirgula*), we cannot exclude an additional role of these microorganisms for the development of the disease. However, in view of the identification of major well-known fungal antigens in the MWF such commercially unavailable tests were not considered.

Environmental surveys during outbreaks of HP showed no (Robertson et al. 2007) or low (Tillie-Leblond et al. 2011; Fishwick et al. 2005; Hodgson et al. 2001; Fox et al. 1999) fungal contamination of sump probes. Actinomycetes were not found in any of these studies. Consequentially none of the outbreak studies in the literature considered molds or actinomycetes as the primary antigens. Although the relevant antigens could not be clearly defined in these studies, the discussion focused on mycobacteria or various gram-negative bacteria (Tillie-Leblond et al. 2011; Fishwick et al. 2005). However, in the present study molds which are among the most frequent antigens for HP in various occupational settings (Kurup et al. 2006) were identified as antigenic sources in MWFs. Although we detected specific IgG antibodies to the actinomycete *Saccharopolyspora rectivirgula* in the patient's serum, inhibition rates with this bacterium as solid phase were low. Thus we cannot precisely estimate the causal role of actinomycetes in the present case. A recent study demonstrated antibodies in sera of affected workers with exposure to MWFs directed to various mold and actinomycete antigens (Fox et al. 1999). In view of the inability to culture these microorganisms in sump probes this finding was interpreted as generalized hyperimmune response. In the present study no generalized immune response can be assumed because both direct IgG antibody binding and inhibition tests with sump proteins varied considerably between single microorganisms.

Although we recognize that this is a case study and the results may not be generalized, we recommend to include immunologic testing to molds and possibly actinomycetes in the diagnostic work-up of workers with HP and exposure to MWFs. IgG inhibition tests may help to establish the link between exposure and disease for workers with suspected HP due to MWFs, irrespective of the results of culture methods.

Conflicts of Interest The authors declare that they have no conflict of interest.

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