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Pulmonary illness as a consequence of occupational exposure to shrimp shell powder

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A B S T R A C T

Objectives: An employee with no prior history of allergy or asthma, experienced respiratory and flu-like symptoms during production of shrimp shell powder in a seafood savory factory in Norway. We aimed to clarify the diagnosis and to identify the cause of the symptoms by specific inhalation challenge (SIC) and by characterizing the powder's biocontaminants, particle size fractions and inflammatory potential.
Methods: Respiratory and immunological responses were measured the day before and after each of four challenges with 20–150 g shrimp shell powder during three consecutive days. The powder was analyzed for endotoxin, microorganisms and particle size fractions by standardized laboratory methods. Total inflammatory potential was quantified by reactive oxygen species (ROS) production in a granulocyte assay.
Results: The patient had elevated IgG, but not IgE, towards shrimp shell powder. 20 min challenge with 150 g shrimp shell powder induced 15% decrease in FVC, 23% decrease in FEV_1 and increased unspecific bronchial reactivity by methacholine. Neutrophils and monocytes increased 84% and 59%, respectively, and the patient experienced temperature increase and flu-like symptoms. The shrimp shell powder contained 1118 endotoxin units/g and bacteria including *Bacillus cereus*, and 57% respirable size fraction when aerosolized. The ROS production was higher for shrimp shell powder than for endotoxin alone.
Conclusions: Endotoxin and other bacterial components combined with a high fraction of respirable dust might be the cause of the symptoms. The patient's characteristics and response to SIC were best compatible with occupational asthma and organic dust toxic syndrome, while hypersensitivity pneumonitis could not be excluded.

1. Introduction

Occupational exposure to bioaerosols may be associated with increased inflammation of the airways. Occupational exposures are often characterized with peak or continuous exposure to much higher levels of contaminants and dust than usually encountered outside the working environment. Thus, occupational bioaerosol exposure may cause severe health effects that may be either temporary or permanent, depending on host factors and the type and duration of exposure. In addition to nonspecific symptoms of mucous membrane irritation, workers may be at risk of pulmonary illnesses such as occupational asthma and bronchitis, organic dust toxic syndrome (ODTS), and hypersensitivity pneumonitis (HP) (Granslo et al., 2009; Madsen et al., 2012; Morell et al., 2011; Raulf et al., 2014; Sigsgaard and Schlunssen, 2004; Smit et al., 2006; Spaan et al., 2006; Villar et al., 2014). The different disease presentations may depend on the site of the dusts' deposition in the respiratory system; bronchitis and asthma are associated with particle deposition in the bronchi and upper airways, while ODTS and HP are typically associated with particles depositing in the alveoli (Raulf et al., 2014).

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Workers in food production, food processing and other industries involving biological agents are typically exposed to bioaerosols with proteins (allergens) from various sources, as well as a range of contaminants including endotoxins, insect fragments, and microorganisms (Abdel Rahman et al., 2012; Basinas et al., 2012; Baur and Bakehe, 2014; Gautrin et al., 2010; Helaskoski et al., 2014; Kogevinas et al., 2007; Malo et al., 1997; Mapp et al., 2005; Thorne et al., 2004). The bioaerosols may lead to diseases such as asthma (e.g. baker's asthma) and HP (e.g. farmer's lung) (Basinas et al., 2012; Baur and Bakehe, 2014; Gautrin et al., 2010; Madsen et al., 2012; Malmberg et al., 1988; Morell et al., 2011; Rylander et al., 1989; Seifert et al., 2003; Thorne et al., 2005; Villar et al., 2014). Chitin is a major component of crustacean shell. Since lack of the chitin degrading enzyme chitinase is associated with an increased risk for asthma (Brinchmann et al., 2011), chitin has been discussed as a causative agent for asthma specifically for workers involved in crustacean processing. Human experiments indicate that chitin might have an immunomodulatory effect, driving the immune system away from a type 2 response, hence, diminishing the tendency to sensitization in the workforce (Sigsgaard et al., 2015). However, with regard to respiratory illness in the working environment, tropomyosin and not chitin appear to be the most likely offending agent (Bonlokke et al., 2012; Cartier et al., 2004; Malo et al., 1997). High levels of aerosolized allergens such as tropomyosin have been described as important risk factor for the development of occupational asthma, as reported from snow crab processing plants (Abdel Rahman et al., 2012; Bonlokke et al., 2012; Cartier et al., 2004; Gautrin et al., 2010). Endotoxin is a constituent of the cell wall of Gram-negative bacteria and are ubiquitous in the environment, but very high exposure (e.g. $>10{,}000$ Endotoxin units (EU)/m^3) has mainly been reported for specific occupational groups, in particular within agriculture and life-stock farming (Basinas et al., 2012, 2013, 2015; Smit et al., 2006; Spaan et al., 2006), and for very specific production works, such as grass seed and bacterial proteins for animal and fish feed (Madsen et al., 2012; Sikkeland et al., 2008) and in cotton and other textile plant manufacturing (Er et al., 2016; Hinson et al., 2014). Exposure to endotoxin is associated with airway symptoms including nasal symptoms, bronchoconstriction and lung function decline, and high exposure levels may cause HP and ODTS (Madsen et al., 2012; Michel, 2000, 1997; Rylander et al., 1989; Sigsgaard et al., 2005; Thorne, 2000; Timm et al., 2009). Long term occupational exposure to high levels of endotoxin may cause permanent lung damage (Skogstad et al., 2012) and specific illnesses such as byssinosis (Er et al., 2016).

In the present study, we investigated one patient referred to our clinic experiencing fever, chills, and respiratory symptoms while working with shrimp shell powder production in a seafood savory factory. The aim of the study was to clarify the diagnosis and to identify the cause of the symptoms by specific inhalation challenge (SIC) and by characterizing the powder's biocontaminants, particle size fractions and inflammatory potential.

2. Material and methods

2.1. Production facility

The production plant is part of a special marine ingredients company producing 100% natural ingredients to the savory and nutritional markets. The plant produces different marine savory seafood ingredients, like seafood and shellfish powders and granules from codfish, arctic fish, shrimp, and shrimp shell. Shrimp powder is produced by cooking, drying and grinding whole shrimps in the production facility in Norway, whereas shrimp shell powder is produced from pre-made granules (coarse powder). The granules originate from dried and grinded shell removed from cooked shrimps. The production plant receives shrimp shell granules from two Canadian and one Norwegian company, which all use the same shrimp species, *Pandalus borealis*. The shrimp shell granules are shipped in “big-bags” to the production facility where they are grinded into powder and packed in smaller bags. The product specification for seafood savory powder in general states that 95% of the product should be below 200 μm, whereas for the shrimp shell powder 85% is below 100 μm, the latter being mainly inhalable. The raw material and end product is tested for microorganisms (total bacteria count, Enterobacteriaceae, *E. coli*, *Salmonella*, mould/yeast) and approved for distribution only if the concentrations are below pre-defined limits set to avoid food-related health hazards. The quality control does not include tests for microbial byproducts, such as endotoxin.

3. Specific inhalation challenge

3.1. Subject

The patient, a 48 year old never-smoking woman, with no prior medical history of allergy or asthma, was referred to Department of Occupational Medicine at Haukeland University Hospital in Bergen, Norway, due to respiratory (dyspnea, cough with phlegm, occasional wheezing) and flu-like symptoms (fever, joint aches, chills, fatigue) during the workdays and in the evenings and nights following work with shrimp shell powder. The patient did not experience symptoms when involved in the production of other marine savory seafood ingredients including cod powder and powder from whole shrimp. The patient reported to have had respiratory and flu-like symptoms from the very first week the company started producing shrimp shell powder (approximately one year prior to referral). All the symptoms got progressively worse during a work week, with some lessening during weekends, but could last as long as three weeks after exposure. Examination prior to SIC, at which time the patient had been out of exposure for several weeks, showed a healthy individual with no observed or reported respiratory symptoms. Forced Volume Capacity (FVC), Forced Volume in 1 second (FEV_1), carbon monoxide diffusing capacity of the lung (DLCO) and fraction of exhaled NO (FeNO) were within reference values and there was no sign of bronchial hyperresponsiveness with a methacholine provocation test ($PC_{20} > 16$). The patient had a total IgE level of 17 kU/L, negative Phadiatop® and food allergy panel, and no elevated serum IgE towards shrimp. She could consume shrimps without having any symptoms.

3.2. Specific inhalation challenge

Specific inhalation challenge was performed in an inhalation chamber (12.8 m^3). On the first day with challenge, the patient was exposed to lactose powder (Lactose monohydrat GPR RECTAPUR (product number: 24945.360), VWR Chemicals, Leuven, Belgium) by dust tipping from one tray to another 30 cm distance from the face for 10 min as a control (placebo) (E_0). In the absence of any significant change in FEV_1 within the next hour, the patient was exposed to 20 g shrimp shell powder granules mixed with 150 g lactose powder for 10 min (E_1). The challenges continued with increasing exposure (increased concentration of shrimp shell powder and increased duration of challenge) over the next two days (Appendix Table A.1).

Shrimp shell powder produced in the factory was used during SIC. For dosage we took into account the endotoxin concentration in the shrimp shell powder and assumed a similar aerosol concentration in the air as previously measured by a direct reading