explanation for our findings in the study. In conclusion, we extended here our original findings of the effect of the probiotic strain in reducing risk of eczema through early childhood. Future studies are required to establish mechanisms of probiotic actions, also in the light of the novel barrier dysfunction theory, and their applicability in prevention of atopic disease.

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Characterization of allergens from the fish bait *Galleria mellonella*

To the Editor:

Larvae of insects and worms used as live fish bait can be a cause of allergy in amateur fishers and occupationally exposed workers. Asthma, rhinitis, and contact urticaria have been associated with exposure and sensitization to *Galleria mellonella* larvae (bee moth) in anglers and breeders.[1-4] Villalta et al[4] described that bee moth's allergenic proteins are thermolabile, and their presence depends on the stage of the larval development, the bee moth's hemolymph being the most likely source.

We report on a 49-year-old amateur fisher with a history of smoking and a fixed-drug eruption to tetracycline. In the previous 4 years, he had experienced several acute episodes of sneezing, nasal itching, watery nose, and tearing. During the previous year, he had also developed exercise-induced asthma. He employed only bee moth as bait. His symptoms were so bothersome that he had to stop fishing on some occasions, but he took no medications.

Physical examination and blood tests results were normal. Total serum IgE concentration was 152 IU/mL. Skin prick tests with common aeroallergens were positive to grass pollen. A skin prick test was performed by puncturing the live wax moth larvae and then pricking the subject's skin with the same lancet (''prick-by-prick'' technique). A positive skin response (15-mm diameter wheal) was obtained in the patient, whereas it was negative in 10 healthy control subjects not exposed to the bee moth.

Spirometry was normal. Because of the possible exercise-induced asthma, a methacholine inhalation test was performed, which showed no airway hyperresponsiveness (PC_{20} > 16 mg/mL). Induced sputum was obtained and analyzed by flow cytometry as previously described.[5] At baseline, induced sputum showed 1.14% eosinophils, 53.35% neutrophils, 1.13% lymphocytes, 42.62% macrophages, 0.02% eosinophilic precursors, and 0.70% activated basophils. Real-time polymerase chain reaction in induced sputum cells (Applied Biosystems, Foster City, Calif) revealed relative mRNA expression of IL-5 (0.47), IL-10 (1.12), IL-13 (0.36), and vascular endothelial growth factor (VEGF) (1.13). mRNA values were normalized with rRNA gene used as endogen.

Specific inhalation challenge (SIC) with wax moth extract was performed as previously described[6] in the patient and in 1 control nonatopic subject after obtaining written informed consent. The starting concentration for SIC was that eliciting a 3-mm wheal by end-point skin titration (0.01% weight/volume [wt/vol]). FEV_1 was then measured at 30 seconds, 5 minutes and 10 minutes after inhalation of the extract. Then, the extract concentration was duplicated until an FEV_1 fall of 20% or greater in the first 60 minutes was obtained. Rhinorrea, tearing, and sneezing together with a 20% fall in FEV_1 was observed 30 minutes after the challenge (2.5% wt/vol). Subsequently, FEV_1 was monitored with a computerized asthma monitor (AM1 Jaeger, Hoechberg, Germany) every hour for the following 24 hours, and no late asthmatic response was obtained. SIC was negative in the control subject.

Twenty-four hours after SIC, bronchial hyperresponsiveness to metacholine increased (PC_{20}, 3.5 mg/mL) only in the patient. The development of bronchial hyperresponsiveness to methacholine after SIC may explain why the patient experienced exercise-induced asthma, which may be related to recurrent exposures to bee moth when fishing.

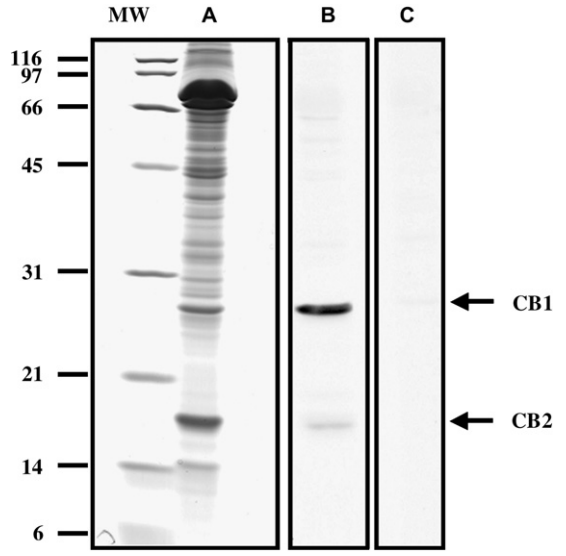

FIG 1. *Galleria mellonella* protein identification. **A,** SDS-PAGE with *G mellonella* extract. **B,** Immunoblot with patient's serum. **C,** Immunoblot with control's serum. *CB1,* Apolipophorin III (24 kd); *CB2,* 27k hemolymph protein (18 kd).

Induced sputum showed 5.6% eosinophils, 41.3% neutrophils, 3.5% lymphocytes, 49.36% macrophages, and 0.06% activated basophils. Real-time quantitative polymerase chain reaction in sputum cells showed an augmented mRNA expression of IL-5 (0.72) and IL-13 (0.97), whereas IL-10 (0.52) and VEGF (0.95) expression did not change significantly.

Sodium duodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) revealed several protein bands ranging from 6 kd to more than 116 kd. Immunonoblotting was performed using the patient's serum diluted at 1/10, incubated overnight, and revealed with a second antibody (antihuman IgE; horseradish peroxidase [HRP], Serotec, Oxford, UK) diluted at 1/5000. Two IgE-binding bands were identified in the *G mellonella* extract of about 24 kd and 18 kd. These bands were extracted from the gel, digested with trypsin, and the proteins were identified by mass spectrometry (MS) using matrix-assisted laser desorption ionization-time of flight and/or liquid chromatography-electrospray tonic tramp (liquid chromatography-mass spectrometry/mass spectrometry).[7] We used the National Center for Biotechnology Information database to compare the proteic sequences. These proteins corresponded to 27 kd hemolymph protein and apolipophorin III, respectively (Fig 1).

IgE-mediated allergy to *G mellonella*, a fishing bait widely used in Europe, has been previously reported.[1-4] Despite the fact that this arthropod is used in immunobiology to evaluate microbial pathogenicity, only a few of their proteins have been detected, purified, and characterized.[8]

We carried out the present study with the larval stage that the patient manipulated. When challenged, the patient developed a positive rhinoconjunctival and asthmatic reaction. Proteins recognized as allergens by this patient corresponded to a 27k hemolymph protein, a glycoprotein precursor with an unknown function, and apolipophorin III, a protein that plays an important role in lipid transport and lipoprotein metabolism in insects.

We report 2 new allergens, 27k hemolymph protein and apolipophorin III, from *G mellonella* used as fish bait in a patient with rhinoconjunctivitis due to manipulation of this wax moth. The clinical and immunological studies confirmed the involvement of these allergens in the patient's respiratory symptoms.

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Antiviral activity of human β-defensin 3 against vaccinia virus

To the Editor:

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that is associated with recurrent bacterial and viral skin infections.[1] This has prompted the Centers for Disease Control and Prevention to exclude individuals with AD from smallpox vaccination with vaccinia virus (VV)