A Case of Work-Related Donkey Milk Allergy

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Allergy to donkey milk (DM) is a rare clinical condition, not only because DM is not widely consumed, but also because of its hypoallergenic nature. In fact, the major cow milk (CM) allergens, κ-casein and αs1-casein, are very minor components of the casein contained in DM, which shows higher homology with the human counterpart than with the bovine one [1]. Because of its hypoallergenic properties, DM is considered a useful alternative for children affected by CM protein allergy [2] and by CM food protein–induced enterocolitis syndrome, as recently demonstrated in a pilot study [3].

We report the case of a 35-year-old woman with a history of cat dander allergy since childhood, seasonal allergic rhinitis, and asthma related to grass and olive pollen who was sensitized to house dust mites but had no history of food allergy. The patient developed respiratory allergy to DM characterized by rhinoconjunctivitis and asthma. The initial symptoms (rhinitis and mild wheeze) occurred 8-10 months after short but repetitive exposure to DM in the food analysis laboratory where she worked. Considering that she handled DM in both liquid form (raw and pasteurized) and powdered form (lyophilized DM), sensitization may have been percutaneous or by inhalation. A few months after the onset of respiratory symptoms, she experienced oral pruritus, cough, dyspnea, and wheezing immediately after tasting ultra-high-temperature (UHT) processed DM. Her symptoms resolved completely after 20 minutes with inhaled salbutamol (400 μg) and the oral antihistamine rupatadine (10 mg). From that episode on, she avoided all contact with DM but continued to tolerate CM and dairy products.

In order to characterize the patient's allergic reaction, we performed the following in vivo and in vitro tests: skin prick

tests (SPT) with whole CM, α-lactalbumin, β-lactoglobulin, and casein (Lofarma); prick-by-prick tests with CM (UHT) and DM (UHT); specific IgE for CM, sheep milk, and goat milk (ImmunoCap, Phadia AB; positive cut-off, 0.10 KU_A/L); basophil activation test (BAT) with CM extract (Lofarma) and raw DM. In addition, immunoblot analysis of raw DM and CM protein extract were performed to identify DM allergens and to verify possible cross-reactivity. DM and CM samples were separated under reducing conditions on 12% Nu-PAGE precast gel (1-dimensional electrophoresis [1DE]) (Invitrogen Life Technologies Ltd). DM 1DE gels were then electro-blotted and incubated with the patient's serum and with a negative control (serum of a person who was not allergic to either CM or DM), while the CM 1DE membranes were incubated with the same sera and with a pool of sera from children affected by CM allergy. To better separate DM reactive bands, we performed 2-dimensional electrophoresis (2DE) on DM (3-10 NL IPG strips [Bio-rad] and 12% Nu-PAGE precast gel [Invitrogen]). DM 2DE gel was electro-blotted and incubated with the patient's serum and with the negative control's serum. The reactive spots were excised, digested with trypsin, and identified using peptide mass fingerprinting in a Bruker Ultraflex II MALDI-TOF/TOF mass spectrometer (Bruker Daltonics).

The technique used to perform the BAT is described in Appendix 1, which is available in the Supplementary Material.

Prick-by-prick testing with DM yielded a positive reaction (wheal diameter, 7 mm; positive control wheal diameter, 4 mm), while the results of all the other cutaneous tests were negative. Specific IgE against cow, sheep, and goat milk was negative, as was recombinant IgE against α-lactalbumin, β-lactoglobulin, and caseins (CM). The BAT result was positive for raw DM (36.5% CD63-positive basophils) and negative for CM (8% CD63-positive basophils) (Figure E1 in Supplementary Material). The BAT result for the negative control was negative for both CM and DM.

Immunoblot analysis showed 6 bands recognized by the patient's serum on DM 1DE-I (Figure), while no band was recognized on CM 1DE-I (Figure E2 in Supplementary Material). The patient's serum recognized 10 reactive spots of DM proteins (Figure and Table E1 in Supplementary Material) separated by 2DE and electro-blotted (see above). These proteins were identified as whey proteins and casein fractions, namely, bovine serum albumin, lactotransferrin, β-lactoglobulin, lysozyme, β-casein, and α-S1-casein. Consistent with the patient's tolerance of CM, no cross-reactivity was detected by immunoblot analysis, probably owing to the low/moderate homology of these proteins with their CM counterparts [1].

Allergic manifestations to DM are rare, particularly in persons who tolerate CM. To our knowledge, this is the first case of occupational DM allergy. The only recently reported case of DM allergy involved a 25-year-old woman who developed anaphylaxis after consuming fresh DM. The patient did not report food allergy and tolerated CM, as in the present report. The authors hypothesized that the patient was sensitized percutaneously through repeated contact of her atopic skin with DM-based emollients [4]. The patient in the present report developed DM allergy following intermittent occupational contact and possibly inhalation of DM. Inhalation as a primary route of sensitization explains a substantial part of food allergies due to cross-reacting allergenic structures between inhalant and food allergens [5]. It was recently proposed that epicutaneous sensitization may also be the primary mechanism for the

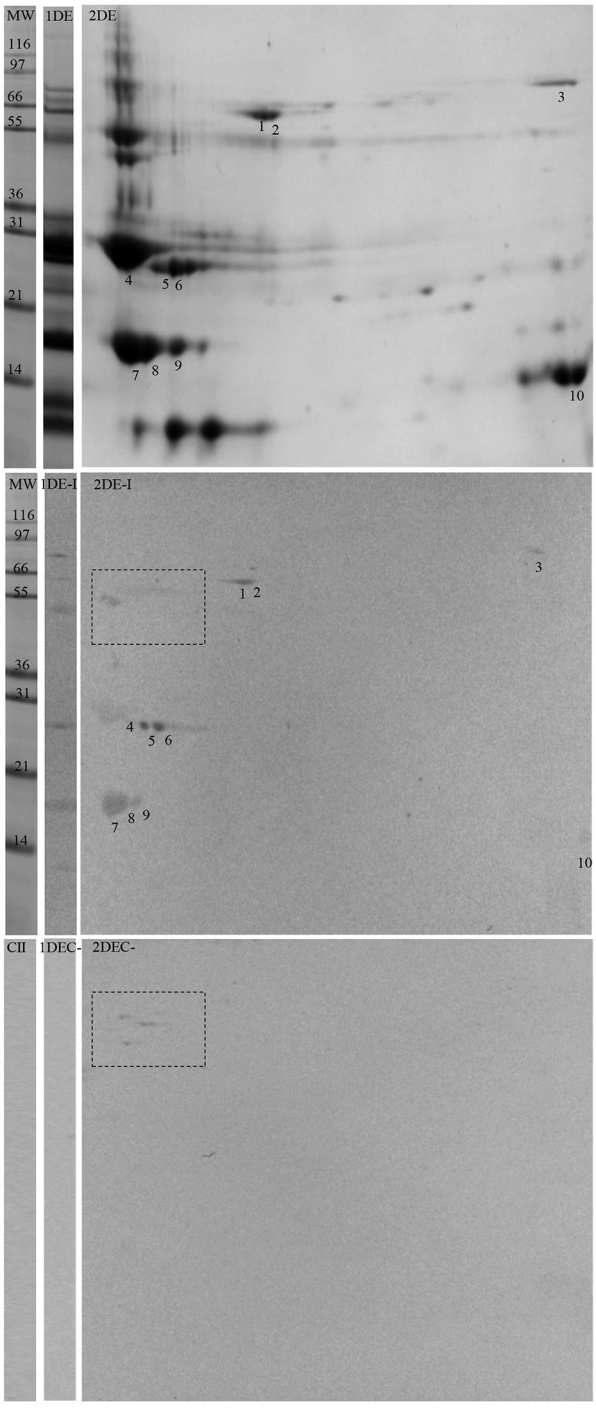

Figure. Immunoblotting of DM proteins incubated with patient serum. MW indicates molecular weight; 1DE, 1D-SDS PAGE; 2DE, 2-D SDS PAGE; 1DE-I, 1D-SDS PAGE immunoblotting; 2DE-I, 2-D SDS PAGE immunoblotting; CII, negative control without serum; 1DEC– and 2DEC–, negative control serum of a person who was not allergic to CM or DM. The spots in the dotted box were not considered because they also appeared in 2DEC–.

development of food allergy. Immediate reactions secondary to percutaneous sensitization have been reported with soy-based ingredients in cosmetic products, mare milk–based organic cosmetics [8,9], and oat proteins in topical products [10].

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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