

BDA20, a major bovine dander allergen characterized at the sequence level, is Bos d 2

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We have recently cloned and sequenced three bovine dander allergens. One of them, the predominant bovine dander allergen with a molecular weight of 20 kD (BDA20), was purified by affinity chromatography with monoclonal antibodies,¹ and the partial amino acid sequence of the natural protein was associated with a cloned complementary DNA sequence.² Although we had earlier observed that the molar amino acid composition of the natural BDA20 resembled that of Bos d 2, no definite link between the two proteins was previously shown. In this study, we show that the major bovine dander allergen BDA20 corresponds to the bovine allergen previously named Bos d 2.

METHODS

Antigen preparation

Natural (n) BDA20 and recombinant (r) BDA20 were prepared and purified as described previously.^{2,3}

Immunoblotting

Bovine dander extract, affinity-purified nBDA20, and rBDA20 were run in sodium dodecylsulfate-polyacrylamide gel electrophoresis, and the proteins were transferred to a Hybond-ECL nitrocellulose membrane (0.45 µm; Amersham International, Buckinghamshire, England) as described previously.³

After transfer, the standard proteins were stained with amido black (Merck, Darmstadt, Germany), and the nitrocellulose membrane was blocked in phosphate-buffered saline (PBS; pH 7.3) containing 1% gelatin and 0.1% Tween-20 (Fluka Chemie, Buchs, Switzerland) for 2 hours at 37°C. The membrane strips were incubated overnight at room temperature with serum from a patient with cow dander-induced asthma (diluted 1:20), with a monoclonal antibody (1:500 dilution of a hybridoma culture supernatant), or with a rabbit antiserum against Bos d 2 (Ag 3 of Prahl;⁴ serum received from Dr. H. Løwenstein, ALK A/S, Denmark; diluted 1:25,000). The strips were then washed in PBS-Tween-20 four times for 5 minutes and incubated for 1 hour with 1:1000 diluted peroxidase-labeled rabbit anti-human IgE (DAKO P295; DAKO A/S, Glostrup, Denmark), with

Abbreviations used

BDA20: Bovine dander allergen 20
PBS: Phosphate-buffered saline

1:2000 diluted rabbit anti-mouse Ig (DAKO P161), or with 1:30,000 diluted swine anti-rabbit Ig (DAKO P399). After washing, the reactive bands were visualized with ECL detection reagents (Amersham International).

Inhibition tests

Anti-Bos d 2 and the patient serum were pretreated with either nBDA20 or rBDA20 before use in the immunodetection as follows. Anti-Bos d 2 was diluted 1:5000 in PBS, and 100 µg of nBDA20 or 150 µg of rBDA20 was added to a microcentrifuge tube to make total volumes of 250 µl or 300 µl, respectively. Noninhibited anti-Bos d 2 and anti-Bos d 2 mixed with 100 µg of human serum albumin were used as controls. The tubes were gently shaken at room temperature for 6 hours. The mixtures were diluted 1:10 in PBS containing 1% gelatin and 0.1% Tween-20, and the nitrocellulose strips containing nBDA20 or rBDA20 were treated with the sera, followed by the ECL reagents as described above.

RESULTS

As shown in Fig. 1, serum IgE from a patient with cow dander-induced asthma, a mouse monoclonal antibody against nBDA20, and the rabbit anti-Bos d 2 antiserum all react strongly against a 20 kD protein in cow dander. Serum IgE binding shows that this protein is an allergen, and both anti-BDA20 and anti-Bos d 2 react with a protein of the same molecular weight. The result was confirmed by the reactions against affinity-purified nBDA20. Therefore the natural BDA20 shares epitopes with the Bos d 2 used by Prahl et al.⁴

Cloning of cDNA molecules encoding allergens has allowed us to produce recombinant allergens in *Escherichia coli*. Recombinant BDA20 was blotted and detected with anti-Bos d 2. The serum reacted strongly with rBDA20 (Fig. 1, lane 9). The weak bands below rBDA20 are most likely truncated forms or degradation products of rBDA20.

The specificity of the anti-Bos d 2 was verified by inhibition tests (Fig. 1, lanes 6 to 10). Natural BDA20 or rBDA20 was allowed to react with the anti-Bos d 2 before the sera were applied in Western blotting. The specific binding of the soluble antigens completely prevented the attachment of anti-Bos d 2 to the 46 kD fusion protein on the membrane. In addition, human serum

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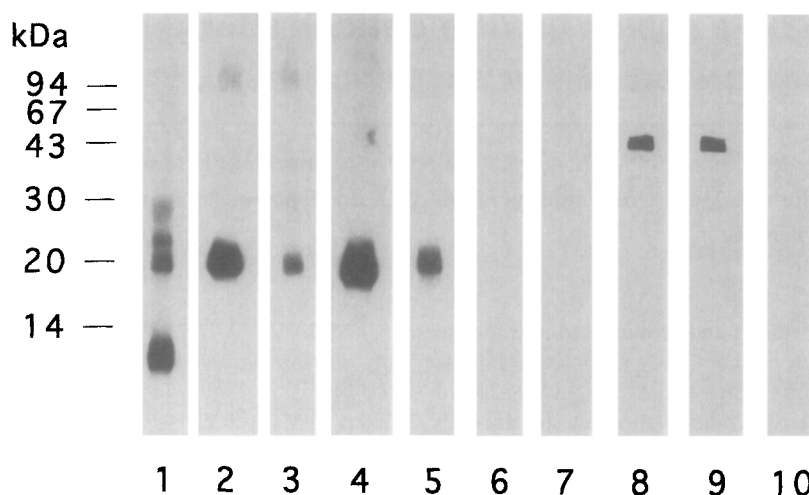


FIG. 1. Reactivity of antibodies in immunoblotting against bovine dander extract (lanes 1 to 3), nBDA20 purified from dander extract (lanes 4 and 5), and rBDA20 (lanes 6 to 10). Immunoblotting with serum IgE of patient with cow dander-induced asthma (diluted 1:20) (lane 1), with anti-BDA20 monoclonal antibody (culture supernatant dilution 1:500) (lanes 2 and 4), and with rabbit anti-Bos d 2 serum (diluted 1:25,000) (lanes 3 and 5). In the inhibition test, anti-Bos d 2 serum (final dilution, 1:50,000) was allowed to react with nBDA20 (concentration, 100 μ g/ml; lane 6), with rBDA20 (concentration, 150 μ g/ml; lane 7), and with human serum albumin (concentration, 100 μ g/ml; lane 8) before immunoblotting against rBDA20. Serum reactivity without inhibition (lane 9) and conjugate control (no primary antibody) (lane 10) are also shown.

albumin, which was used to control the nonspecific binding, did not affect the reactivity of the antibodies. The serum IgE from the patient with cow dander-induced asthma reacted identically in the inhibition test (data not shown). The inhibition test was also repeated with the blotted nBDA20. The result was similar to blotting against rBDA20 (data not shown).

DISCUSSION

Prahl et al.⁴ characterized bovine dander allergens by a combination of biochemical techniques. According to their findings, the most important cow-dander allergens were those with molecular weights of 24, 22, and 20 kd; and these allergens were named by the International Union of Immunological Societies Allergen Nomenclature Committee as Bos d 1, Bos d 3, and Bos d 2, respectively.⁵ According to our previous studies, the allergen we have named BDA20 is the predominant allergen in cow dander. We recently described its primary structure by using the sequences of cDNA clones and the partial amino acid sequence.² The homology comparisons revealed a relationship between BDA20 and lipocalins, carriers of small hydrophobic molecules.

We confirmed that BDA20 is the same protein as Bos d 2 in immunoblotting experiments. We had the opportunity to test the same rabbit anti-Bos d 2 polyclonal antibody that was used in experiments by Prahl et al.⁴ It reacted identically to the 20 kd protein in bovine dander extract immunoblotting with a monoclonal anti-BDA20. It also had a strong reactivity to nBDA20 and rBDA20 in

Western blot testing and to soluble nBDA20 and rBDA20 in an inhibition test.

In conclusion, our results demonstrated a close biochemical and immunologic relationship between Bos d 2 and BDA20, a major bovine dander allergen with a known sequence. Complying with the requirements of the International Union of Immunological Societies Allergen Nomenclature Committee, we have proposed that BDA20 be recognized as a completely sequenced allergen, Bos d 2.

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