Production of Monoclonal Antibodies against Wheat Flour Components

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It has been known for more than 50 years that immediate-type allergic reactions may occur after inhalation of wheat flour [6, 11, 13]. This disease, known as baker's asthma, has been paid considerable attention by various investigators [3, 4, 8, 12]. However, only little progress has been made in the isolation and characterization of specific allergenic wheat flour proteins. It has been shown that crude flour extract contains at least 40 different antigens. Screening the sera of 13 allergic bakers, Blands et al. [4] demonstrated 18 different allergens by means of crossed radioimmuno-electrophoresis. Among these, 3 have been classified as 'major allergens'. Sutton et al. [14] presented evidence for the importance of the water-soluble cereal proteins as allergens. Especially the 'albumin fraction' seems to play an outstanding role in the induction of IgE antibodies. The above-mentioned data, however, cannot be compared or related to each other since a standardization of the allergen preparations has not yet been achieved. In order to overcome this problem, several methods have been proposed [1-3]. Basically, all promising suggestions involve affinity chromatography, using various ligands. Introducing a monoclonal antibody as a ligand offers the advantage of high specificity without the necessity of purifying the allergen first. Furthermore, such a monoclonal antibody would provide an excellent tool for the standardization of allergen extracts.

SDS gel electrophoresis was performed as described by König and Henn [10]. Protein coupling to

1 This paper is dedicated to Prof. Paul Kallós on the occasion of his 80th birthday.

paper discs was carried out according to Cuatrecasas [7]. Histamine release in vitro from human basophilic leukocytes (LMBs) was measured as described by König and Henn[10].

The plasmocytoma cell line, culture conditions and cell fusion have been described in detail by Bohn and König [5].

Wheat flour, type 405, was extracted with (4 g/ml) 10% (w/v) sodium chloride solution. After an incubation period of 1 h at room temperature, the suspension was centrifuged at 3,000 g for 20 min. The supernatant was decanted and stored in aliquots at -20°C without dialysis. The protein

content was 2.75 mg/ml as estimated by the method of Lowry et al. [15]. A qualitative analysis was carried out by SDS gel electrophoresis. 34.5 µg protein per gel was applied; the gel consisted of 15% acrylamide. After staining, multiple protein bands were detected, as is shown in figure 1. The molecular weight of these proteins ranges between 5,000 and 100,000 daltons. The crude flour extract (11 mg) was incubated with about 500 CNBr-activated paper discs at 4°C for 16 h. About 60% of the protein was coupled, which yields 13 µg of bound protein per disc. These 'allergen discs' were used as solid-phase reactants in the RAST. As can be seen in figure 2, a specific uptake of radiolabeled anti human IgE took place with the allergen discs, previously incubated with the serum of a wheat-allergic baker.

In a first attempt to functionally purify the specific allergen, the crude flour extract was precipitated with ammonium sulfate. Precipitation was carried out at

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20, 40, 60 and 80% saturation. The resulting fractions were coupled to paper discs as described above and used in the RAST. Our data (not shown) demonstrated the highest uptake of iodinated anti human IgE with the 20% precipitate. Since the existence of antigen-specific circulating IgE does not necessarily prove its biological relevance, we analyzed the wheat flour fractions for histamine release from human LMBs in vitro. Basophils from an allergic baker were taken and purified as described by König and Henn [10]. Whole flour extract and the flour precipitates were analyzed in dilutions of 1:5, 1:50, 1:500 and 1:5,000. The protein concentrations of the undiluted fractions were 12–20 mg/ml. The results are shown in figure 3. As is apparent, optimal histamine release was achieved with the 80% precipitate. This finding contrasts with the results obtained by the RAST and might be due to the fact that serum and cells were taken from different individuals. A qualitative analysis by SDS gel electrophoresis showed that all precipitates were still quite heterogeneous protein mixtures (data not shown). Therefore, we attempted to produce hybridomas against flour crude extract. BALB/c donor mice were immunized subcutaneously or intraperitoneally with

Top

Fig. 1. Gel pattern of crude wheat flour extract on SDS-Page (15%acrylamide); cathode to the left, anode to the right.

2 -

◆999999999◆999999999◆

Con. 5

Serum dilutions

Fig. 2. Binding of radiolabeled antihuman IgE to protein-coupled paper discs after incubation with various dilutions of serum from an allergic baker. NSB = Non specific binding, Con = concentrated.

000

Fig. 3. Histamine release from human LMBs equals 100%. The protein fractions, which indicated.

%

400 -

300

200

Extract

20%

000

500

40%

5,000

60%

500

Preα₁†ale

a

Top

Bottom

in vitro; spontaneous histamine release were used as stimuli, were diluted as

Fig. 4. SDS gel electrophoresis of two isolated wheat flour antigens. The Rf value of the larger protein (a) is calculated to be 0.109, which corresponds to a molecular weight of approximately 100,000 daltons (J). The second gel shows two bands with Rf values of 0.725 and 0.750 (5,000–

10,000 daltons) (b). The presence of two protein bands on the gel might be due to the separation of noncovalently bound polypeptide chains by SDS. (↓) The molecular weight (daltons) was calculated from a reference gel containing the proteins aprotinin (6,500), cytochrome (12,500), α -chymotrypsinogen (21,500), ovalbumin (48,000), bovine serum albumin (68,000).

b

Top

I

Bottom

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0.2 mg of crude wheat flour extract emulsified in complete Freund's adjuvant. The animals were then treated with the same amount of protein at days 15, 22 and 31. At day 45, the mice received 1.4 mg extract i.v. Their spleens were taken 4 days later. The plasmacytoma cell line used, culture conditions and cell fusion protocol have been described in detail elsewhere [5].

Culture supernatants of the hybridomas were assayed for specific immunoglobulin by means of an enzyme-linked immunosorbent assay, ELISA, which revealed 16 different antibody-secreting hybridomas. Most of these were secreting IgM antibodies. The cell lines were expanded and cloned. In preliminary experiments we managed to isolate two proteins with molecular weights of 5,000–10,000 daltons and approximately 100,000 daltons, respectively (fig. 4). The purification of the proteins was performed by affinity chromatography. A qualitative analysis was carried out by SDS gel electrophoresis; no impurities were detectable.

The biological relevance of the components obtained by hybridoma technology for the allergic disease process is currently under study.

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