

## **Baker's asthma**

### **Studies of the cross-antigenicity between different cereal grains**

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*(Received 25 October 1982; accepted for publication 19 February 1983)*

#### **Summary**

Specific IgE antibodies were demonstrated in the sera of six bakers with respiratory symptoms of asthma or bronchitis by the radioallergosorbent (RAST) assay. The specificity of the antibodies was found to be directed not only against the common flours (rye, wheat) used in the bakeries but also against triticale, barley, oat, corn and rice in some of the bakers. By using the RAST inhibition tests, cross-antigenicity was shown to exist between different cereal grains. The degree of cross-reactivity closely paralleled their taxonomic relationship and appeared to be in the following order of decreasing closeness: wheat, triticale, rye, barley, oat, rice and corn. The allergenic activity in the rye and wheat extracts was found to be distributed among various fractions of different molecular weights.

#### **Introduction**

In a previous article published in the *Journal*, we have presented evidence to indicate that baker's asthma is a form of allergic asthma mediated by IgE antibodies specific for cereal antigens (Block *et al.*, 1983). Although wheat and rye are the more common flours that they work with, bakers often have occupational exposure to flours from other cereal grains as well. It would be of clinical significance, therefore, to determine the extent of allergic sensitization of symptomatic bakers to different cereal flours and the degree of cross-reactivity between individual cereal grains.

#### **Materials and Methods**

##### *Sera*

As reported earlier, we have studied seven symptomatic bakers in detail and have established that there was a positive correlation between the level of serum IgE

\* Dr Block was the recipient of the British Columbia Christmas Seal Fellowship.

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antibodies specific for cereal antigens, the degree of non-specific bronchial reactivity and an individual baker's bronchial response to inhalation challenge with an extract of cereal flour (Block *et al.*, 1983). The sera from six of the seven symptomatic bakers were used in the present study; unfortunately, an insufficient quantity of serum was collected from one of the seven subjects. The clinical features of these bakers have been described in our previous article (Block *et al.*, 1983). Five had symptoms of asthma and one had bronchitis. Sera collected from another ten asthmatics who had no occupational exposure to flours were used as controls.

#### *Flour extract*

Rice flour and whole grains of rye, spring wheat, triticale, barley, oat and corn were bought from a natural food store. Durum wheat was kindly donated by Ogilvie Mills Ltd., Vancouver, B.C. The cereal grains were ground separately to give a fine powder. Individual flour extracts were prepared by stirring a 10% weight/volume (w/v) flour suspension in phosphate-buffered saline (PBS) at 4°C overnight. The solutions were then centrifuged and stored at -20°C.

#### *Fractionation of cereal extracts*

Eight mls each of a 10% w/v rye and spring wheat extracts were passed through a Sephadex G100 (Pharmacia Inc., Uppsala, Sweden) column, 2.5 × 90 cm in size. Aliquots of 2.5 ml were collected in each tube. The elution profiles were produced by plotting the absorbance at 280 nm against the eluted volume and are shown in Figs 1 and 2. The molecular weights of the protein peaks in the elution curves were determined by comparison with a calibration graph which was constructed by passing through the same column four standards of known molecular weights, namely, human

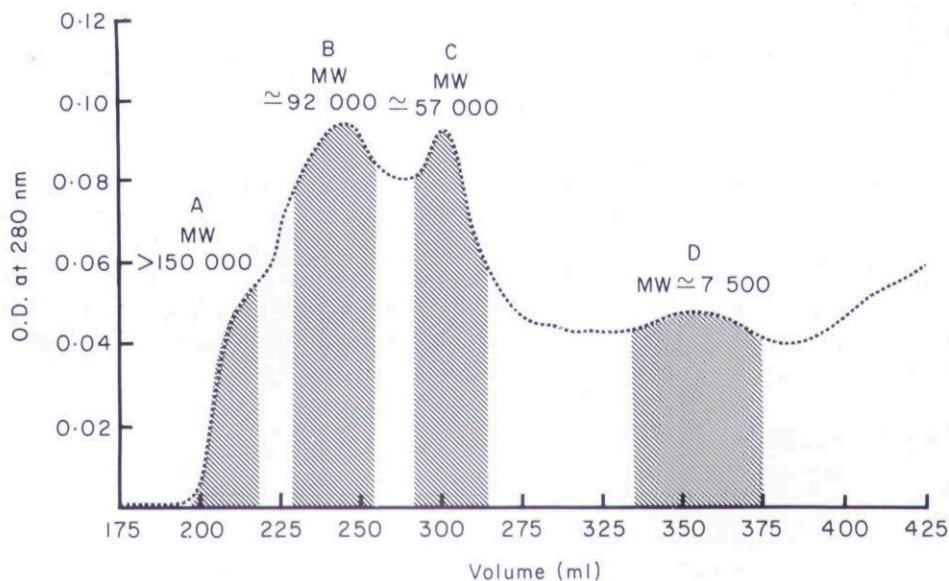


**Fig. 1.** The elution profile of a 10% w/v rye extract through a Sephadex G-100 column, 2.5 × 90 cm. The shaded areas indicate the volumes pooled for each fraction. The approx. mol. wt for fractions A, B, C, D are 150 000, 92 000, 57 000 and 17 500 daltons, respectively.

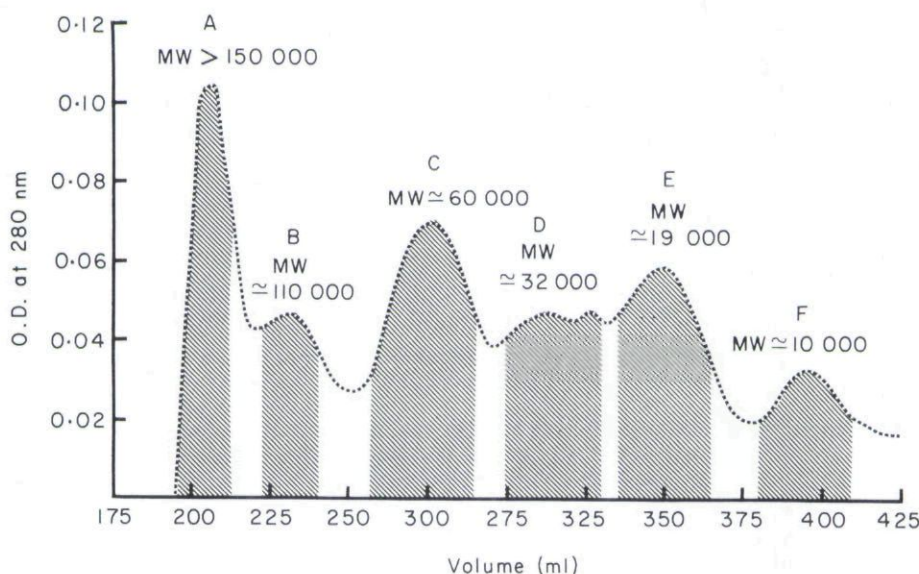


**Fig. 2.** The elution profile of a 10% w/v spring wheat extract through a Sephadex G-100 column, 2.5/90 cm. The shaded areas indicate the volumes pooled for each fraction. The approx. mol. wt for fractions A, B, C, D, E, F, are 150 000, 110 000, 60 000, 32 000, 19 000 and 10 000 daltons, respectively.

IgG (150 000 daltons), human serum albumin (67 000), chymotrypsinogen (25 000) and ribonuclease (12 500). The protein peaks were designated as A, B, C, D, etc., in decreasing molecular weights. The tubes corresponding to each protein peak were pooled and the eluates concentrated to 2.5 ml which was then kept frozen at  $-20^{\circ}\text{C}$  until used. By this method of fractionation, four fractions were obtained from the rye extract and six fractions obtained from the spring wheat extract.

#### *Preparation of allergen discs for RAST*

The method of coupling of flour extract to paper discs was modified from the method of Ceska, Ericksson & Varga (1972). Briefly, 6-mm-diameter discs were punched out from Whatman 541 filter papers. One hundred washed discs were put in a beaker containing 30 ml of distilled water. After 500 mg of cyanogen bromide was added to the solution, the pH was adjusted to 11.0 for 15 min with 1 N sodium hydroxide. The activated discs were washed in a Buchner funnel with cold distilled water, and then with cold 0.1 M bicarbonate buffer (pH 8.6).

Ten ml of the 10% w/v flour extract was added to the activated discs and mixed overnight at  $4^{\circ}\text{C}$ . The discs were washed five times with cold bicarbonate buffer; 20 ml of 0.5 M ethanolamine in the same buffer was then added to the discs and were mixed at room temperature for 2 hr. The discs were washed once with bicarbonate buffer, once with 0.1 M acetate buffer (pH 4.0); three times with PBS and once with RAST buffer (Pharmacia Inc.). The discs were stored in RAST buffer at  $4^{\circ}\text{C}$  until used.

#### *Radioallergosorbent (RAST) assay*

A 0.05 ml serum sample was pipetted in a tube containing an allergen disc. The tubes were shaken on a clinical rotator at room temperature for 3 hr and washed four times with RAST buffer. Then 0.05 ml of  $^{125}\text{I}$ -labelled rabbit anti-human IgE (Pharmacia

Inc.) was added to each tube and the tubes were again shaken on the rotator at room temperature overnight. The tubes were washed four times with RAST buffer and the radioactivity was measured by a gamma counter. The uptake of radioactivity of the allergen discs was expressed as the RAST value which was the ratio of the radioactivity of the baker's serum sample to that of normal control.

#### *Radioallergosorbent (RAST) inhibition tests*

The antigen specificity of the serum RAST activity was determined by the degree of inhibition of serum RAST values by prior absorption of the serum sample with a flour extract. Aliquots of a serum sample in 0.15 ml were mixed with different amounts of a 10% flour extract and left at room temperature for 3 hr. Then 0.05 ml of the sample was used for the RAST assay, using an allergen disc containing the same cereal antigen. The results were expressed as:

$$\% \text{ inhibition} = \frac{(B) - (A)}{(B)} \times 100\%$$

where (B) represents the serum RAST value before incubation with flour extracts and (A) represents the serum RAST value after incubation with flour extracts. Similarly, the antigenicity of the four fractions of the rye flour extract and the six fractions of the wheat flour extract obtained by fractionation through a Sephadex G-100 column was also measured by the ability of each fraction to inhibit the RAST value of a given serum sample.

The degree of cross-reactivity between differential cereal flours was also determined by the RAST inhibition test. In this case, 0.15 ml of the serum sample was pre-incubated with 0.05 ml of the cereal extract to be tested and then the RAST assay was carried out using rye or wheat allergen discs.

## Results

#### *Serum RAST values against different cereal flours*

These results are shown in Table 1. In comparison with the ten asthmatic controls who had no occupational exposure to flours, each of the six symptomatic bakers was found to have elevated serum RAST values for several of the cereal flours. As reported in our earlier article (Block *et al.*, 1983), four of six symptomatic bakers (bakers 1-4) had positive reactions while the remaining two had negative reactions to antigenic bronchoprovocation with rye or wheat flour extracts. The sera from bakers 1-4 were found to have considerably higher RAST values than the sera from bakers 5 and 6. These subjects not only had elevated serum RAST values for several of the taxonomically closely related cereals such as rye, wheat, durum wheat and triticale (a hybrid species between rye and wheat), but several of them also had very high values for barley (bakers 2, 3, 4), corn (bakers 3, 4), oat (baker 3) and rice (baker 4). The latter four cereal grains have a much more distant taxonomic relationship with rye and wheat. The taxonomic chart of different cereal grains has been published previously by Baldo, Krilis & Wrigley (1980).

#### *Antigenic specificity of the serum RAST activity*

The results of the RAST inhibition tests are shown in Fig. 3. Addition of increasing amounts of a particular flour extract to a serum sample from a baker prior to the RAST

Table 1. Serum RAST values against different cereal flours

| Baker                               | Years of flour exposure | Reaction to antigenic bronchoprovocation | Cereal antigens |              |             |            |             |             |             |           |
|-------------------------------------|-------------------------|------------------------------------------|-----------------|--------------|-------------|------------|-------------|-------------|-------------|-----------|
|                                     |                         |                                          | Rye             | Spring wheat | Durum wheat | Triticale  | Barley      | Oat         | Corn        | Rice      |
| 1                                   | 7                       | Positive                                 | 11.7            | 4.8          | 3.3         | 11.7       | 3.4         | 1.4         | 1.0         | 1.9       |
| 2                                   | 45                      | Positive                                 | 12.1            | 14.7         | 11.2        | 13.2       | 17.5        | 2.5         | 3.7         | 1.3       |
| 3                                   | 31                      | Positive                                 | 20.0            | 19.4         | 23.0        | 21.3       | 26.3        | 8.1         | 15.3        | 1.8       |
| 4                                   | 5                       | Positive                                 | 20.4            | 13.6         | 13.5        | 11.6       | 17.0        | 3.4         | 19.1        | 17.0      |
| 5                                   | 35                      | Negative                                 | 2.0             | 3.8          | 1.9         | 1.7        | 1.7         | 1.5         | 1.6         | 1.3       |
| 6                                   | 41                      | Negative                                 | 3.8             | 3.7          | 3.8         | 3.8        | 2.9         | 1.3         | 3.0         | 2.5       |
| Non-baker asthmatic controls (n=10) | 0                       | Negative*                                | 1.2 ± 0.4       | 1.34 ± 0.45  | 1.57 ± 0.91 | 1.53 ± 0.8 | 1.87 ± 1.37 | 1.24 ± 0.25 | 1.66 ± 1.04 | 1.3 ± 0.3 |

\* Antigenic bronchoprovocation with rye and wheat extracts was performed in four of the ten non-baker asthmatic controls and all had negative reactions.

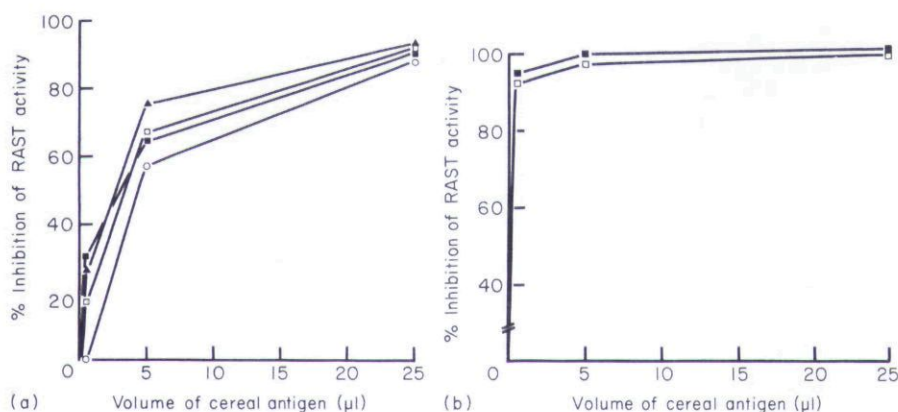


**Fig. 3.** (a) Antigenic inhibition of serum RAST activity against rye (▲), spring wheat (□), barley (■) and oat (○). Percent inhibition of anti-cereal RAST activity was plotted against the volume of corresponding flour extract pre-incubated with the serum of baker 3 before the RAST assay. (b) Antigenic inhibition of serum RAST activity against corn (■) and rice (□). Percent inhibition of anti-cereal RAST activity was plotted against the volume of the corresponding flour extract pre-incubated with the serum of baker 4 before the RAST assay.

procedure resulted in dose-dependent inhibition of the serum RAST value for the corresponding cereal antigen. This would indicate that the serum RAST activity was indeed cereal antigen-specific because the binding of specific IgE antibodies by the addition of flour antigens to serum lead to a suppression of the specific antibody activity as measured by the RAST.

#### *Cross-reactivity between different cereal antigens*

The extent that different flour extracts are able to inhibit the serum RAST activity against rye or wheat could be used as an index of the degree of cross-antigenicity between cereal antigens. The results of the RAST inhibition tests are shown in Table 2, using the sera from the four bakers with particularly high RAST activities for the experiments. The degree of cross-reactivity between the various cereal grains seemed to have a direct correlation with the closeness of their taxonomic relationship. Thus, triticale appeared to cross-react completely with rye in the RAST inhibition tests. This was not unexpected because triticale is a hybrid species between rye and wheat. Wheat

**Table 2.** Percent inhibition of serum RAST values for rye by absorption with different cereal antigens\*

| Baker    | Rye  | Spring wheat | Durum wheat | Triticale | Barley | Oat  | Corn | Rice |
|----------|------|--------------|-------------|-----------|--------|------|------|------|
| Rye disc |      |              |             |           |        |      |      |      |
| 1        | 100  | 32.3         | 58.3        | 100       | 32.2   | 54.8 | 0    | 0    |
| 2        | 100  | 100          | 100         | 100       | 81.3   | 42.3 | 0.3  | 0    |
| 3        | 97.0 | 88.9         | 92.8        | 92.2      | 56.4   | 30.2 | 16.1 | 0    |
| 4        | 100  | 100          | 100         | 100       | 75.3   | 45.5 | 47.9 | 34.1 |

\* Rye allergen discs were used for the RAST assay.

and barley were also found to have a high degree of cross-reactivity with rye as compared with oat, corn and rice in the RAST inhibition tests. Again, this finding fits well with the fact that wheat and barley are more close to rye taxonomically than oat, corn and rice.

*The antigenicity of various fractions of rye and wheat extracts*

Results of the RAST inhibition tests using the various fractions of rye and wheat extracts are shown in Tables 3 and 4 and indicate that the lower molecular-weight fractions are relatively more antigenic than the higher molecular weight fractions. Fractions C and D of the rye extract (approx. mol. wt 57 000 and 17 500 daltons,

**Table 3.** Percent inhibition of serum anti-rye RAST values by prior absorption with different fractions of a rye extract\*

| Baker | Fractions |      |      |      |
|-------|-----------|------|------|------|
|       | A         | B    | C    | D    |
| 1     | 28.4      | 62.5 | 72.9 | 88.5 |
| 2     | 6.3       | 15.0 | 15.7 | 80.3 |
| 3     | 16.2      | 21.4 | 75.1 | 19.7 |
| 4     | 4.1       | 0    | 15.4 | 79.3 |
| 5     | 12.7      | 28.2 | 73.3 | 82.4 |
| 6     | 23.6      | 48.7 | 68.6 | 86.7 |
| Mean  | 15.2      | 29.3 | 53.5 | 72.8 |

\* Rye allergen discs were used for the RAST assay.

**Table 4.** Percent inhibition of serum anti-wheat RAST values by prior absorption with different fractions of a wheat extract\*

| Baker | Fractions |      |      |      |      |      |
|-------|-----------|------|------|------|------|------|
|       | A         | B    | C    | D    | E    | F    |
| 1     | 18.8      | 23.4 | 53.5 | 58.1 | 48.4 | 25.0 |
| 2     | 0         | 0    | 21.2 | 32.9 | 48.4 | 32.4 |
| 3     | 25.1      | 40.6 | 40.1 | 27.1 | 20.1 | 7.1  |
| 4     | 3.8       | 7.9  | 16.5 | 33.3 | 54.3 | 63.6 |
| 5     | 37.6      | 37.4 | 50.1 | 42.8 | 84.0 | 53.7 |
| 6     | 52.6      | 38.9 | 78.6 | 82.8 | 70.8 | 51.5 |
| Mean  | 23.0      | 24.7 | 43.3 | 46.2 | 54.3 | 38.9 |

\* Spring wheat allergen discs were used for the RAST assays.

respectively) and fractions D and E of the wheat extract (approx. mol. wt 32 000 and 19 000 daltons, respectively) induced a higher degree of RAST inhibition than other fractions. It was evident, however, that the major antigenic component of the rye and wheat extracts could not be isolated by fractionation through molecular sieving using a Sephadex G-100 column because the results of the RAST inhibition tests clearly showed that the antigenic activity was identifiable throughout the various fractions of the flour extracts.

## Discussion

The results of this study have clearly demonstrated the presence of cross-reactivity between different cereal flours. The degree of cross-antigenicity between different cereal grains as determined by the RAST inhibition assay closely parallels their taxonomic relationship. The order of taxonomic relationship in decreasing closeness is as follows: wheat, triticale, rye, barley, oat, rice, corn (Baldo, Krilis & Wrigley, 1980). Closely related species such as wheat, rye and triticale were found to have a very high degree of cross-antigenicity. Even comparing more distantly related species such as rye, barley and oat, the degree of cross-reactivity between them was still remarkable. Our results are in agreement with those of other investigators who also reported the presence of cross-reactivity between rye, wheat, barley and oat (Baldo, Krilis & Wrigley, 1980) as well as between corn and rice (Hoffman, 1975). By employing the method of two-dimensional cross-immunoelectrophoresis between an extract of wheat or rye flour and the corresponding anti-serum raised in rabbits, Blands *et al.* (1976) were able to identify about forty antigenic components (as distinguished by different bands of immunoprecipitates) in the wheat extract and some of the components were partially identical to that in the rye extract. The existence of cross-reactivity between different cereal flours has important clinical implications. It would mean that bakers, once they are sensitized from occupational exposures, are likely to develop allergies to multiple cereal grains. The present study has indeed confirmed this point. We found that symptomatic bakers commonly developed specific IgE antibodies to a wide range of cereal grains (see Table 1). For sensitized bakers to avoid further occupationally induced respiratory symptoms, they would have to quit working in the bakery altogether. Simply changing their work exposure from one type of flour to another would be an ineffective way to manage the problem.

The allergenic activity of rye and wheat does not appear to be confined to a single component but rather it is distributed among various fractions of different molecular weights of the rye and wheat flour extracts (see Tables 3 and 4). In general, the low molecular-weight fractions seem to be more allergenic, with respect to their reactivity with the specific IgE antibodies of allergic bakers. Other investigators have also reported similar results. When the water-soluble proteins of wheat were fractionated into albumin and globulin fractions by salt precipitation, both fractions were reactive with the specific IgE antibodies from subjects with baker's asthma although the albumin fraction was found to have a higher degree of reactivity (Baldo & Wrigley, 1978). Similarly, the allergenicity of rice was also found to be distributed among various fractions of rice proteins (Shibasaki *et al.*, 1979).

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