

COMMON ANTIGENIC AND ALLERGENIC DETERMINANTS ON CODFISH PROTEINS DETECTED WITH MOUSE MONOCLONAL IgG AND HUMAN IgE ANTIBODIES

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Abstract—The antigenic and allergenic profiles of codfish extract have been examined and a comparison made of the specificities of the determinants defined by mouse monoclonal antibodies and human IgE antibodies. By gel electrophoresis, codfish extract was found to comprise a heterogeneous mixture of proteins, in which the principal component and allergen was Gad cI (allergen M). Using monoclonal antibodies and sera from human cod-allergic subjects as immunological probes, common antigenic and allergenic determinants were demonstrated on some codfish proteins. It was also established that, although two monoclonal antibodies recognized the same determinant on Gad cI, there was no cross-reactivity between this determinant and those specified by IgE antibodies in the sera of cod-allergic patients. The specificity of IgE populations directed against Gad cI was found to vary from patient to patient, and was indicative of the existence of two types of allergenic determinants: those unique to a particular allergen and those shared by other proteins in the extract. These studies promote speculation regarding the relative immunogenicity of antigenic and allergenic determinants and the size and diversity of the IgE repertoire, given the potential immunogenicity of the entire protein surface.

INTRODUCTION

Many studies in immunology have focused on the analysis of antigenic determinants in proteins. However, the characterization of the allergenic, or IgE-binding, determinants has proceeded more slowly and few comparisons of allergenic and antigenic determinants have been made. Although allergenic sequences have been identified in codfish allergen Gad cI (allergen M) (Elsayed and Apold, 1983), ragweed allergen Ra3 (Atassi and Atassi, 1985, 1986) and the allergenic chironomid haemoglobins (Baur *et al.*, 1986), little is known about the relationship between allergenic and antigenic determinants on different proteins. In particular, it is unclear whether the diversity of IgE-binding proteins from a single allergen source, such as one species of pollen, mite, fungi, etc., reflects a large number of structurally distinct determinants or a smaller number of similar, or identical, determinants on otherwise different proteins.

Studies based on the use of monoclonal antibody (Mab) probes indicate that common cross-reacting determinants are present on the allergens Df29 and Dp28 from house dust mite species, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, respectively (Ley *et al.*, 1986). Cross-reactivity between the house dust mite allergens, Df29 from

D. farinae and Dm6 from *D. microceras*, has been proposed on the basis of crossed line immunoelectrophoresis (Lind, 1986). It has also been established that at least two antigenic determinants of the allergen Df29 are coincident with, or close to, the IgE-binding determinants identified using allergic sera (Ley *et al.*, 1986).

In Gad cI, the major allergenic component of codfish, at least five immunologically reactive sequences, associated with IgE-binding activity, have been described (Elsayed and Apold, 1983). We have investigated the allergenicity of the other components of codfish, using sera from subjects allergic to codfish, and two Mabs directed against Gad cI. In addition to characterizing the antigenic and allergenic profiles of cod and reactivity of the two anti-allergen Gad cI Mabs, this paper presents a methodological approach to ascertaining whether the determinants recognized by Mabs are related to the allergenic determinants specified by IgE present in allergic sera.

MATERIALS AND METHODS

Preparation of antigens and immunogens

Cod DIII was prepared from crude cod myogen tissue by a combination of DEAE-cellulose fractionations and repeated gel filtrations. Gad cI (Marsh *et al.*, 1988) was subsequently purified from cod DIII fraction by repeated gel filtrations, followed by

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preparative IEF (Aas and Jebsen, 1967; Elsayed and Aas, 1971).

For immunization purposes, Gad cI (1 mg/ml), dissolved in 0.5 ml saline, was emulsified with an equal volume of Freund's complete adjuvant (Difco, Detroit, U.S.A.) and 200 μ l was injected i.p. into female BALB/c mice. Boosting was carried out twice at 6 weeks and then at 16 weeks with 200 μ l of the protein in saline. Animals were used for the preparation of hybridomas 3 days after the final boost.

Preparation of hybridomas

This was carried out as previously described (Underwood, 1982; Leitzke and Unsicker, 1985).

Radioimmunoassays

Antibodies in sera or culture supernatants binding to cod proteins were detected in a solid-phase radioimmunoassay (Wide *et al.*, 1967), using either commercial cod allergen discs (Pharmacia, Uppsala, Sweden), prepared by coupling cod Gad cI to CNBr activated paper discs, or discs (6 mm) prepared by adsorbing 10 μ g of cod DIII onto 0.45 μ m nitrocellulose (NC).

In experiments designed to compare the binding specificity of the Mabs, the binding curve for each antibody was calculated over the concn range 0.6–10 μ g/ml. The additivity index (*AI*) was calculated according to the formula given by Friquet *et al.* (1983) and Steele *et al.* (1985), at a point where both antibodies had reached maximal binding:

$$\% AI = 100 \left(\frac{2A_{1+2}}{A_1 + A_2} - 1 \right).$$

A_1 and A_2 are the amounts of each antibody bound independently and A_{1+2} is the total amount bound when both antibodies are presented. The result was compared to a baseline % *AI*, in which A_{1+2} in the above formula was replaced by A_1 or A_2 , according to which antibody displayed the higher affinity binding.

Sera

Sera from patients allergic to cod and birch pollen were collected at the University Hospital of Bergen, Norway. Sera from patients displaying clinical allergy to fish were collected at the Allergy Clinic, Royal North Shore Hospital, Australia.

Gel electrophoresis, transfer to NC and probing of membrane strips

The procedures already described were followed (Towbin *et al.*, 1979; Tovey and Baldo, 1984, 1987; Tovey *et al.*, 1987).

Inhibition assays

Inhibition of the binding of Mab 17/20 to cod DIII proteins by anti-cod IgE antibodies in sera, Marr and At(47), was investigated using immunoassay procedures or western blotting. In both systems, the disc or strip from the transfer of cod DIII, separated by SDS-PAGE, was incubated overnight with a solution of the Mab (10 μ g/ml) or atopic serum (50% in PBS), or both. The extent of binding and its inhibition was determined using labelled second antibodies (either anti-mouse or anti-human). Controls accounting for non-specific binding were included in the assays.

Elution of IgE bound to cod proteins transferred to NC

IgE was eluted from cod antigens by shaking the appropriate band on NC with glycine-HCl, pH 2.5, in 1% BSA for 8 min. Immediately following elution, the solution was neutralized with a known amount of tris buffer and the activity of the eluted antibodies was ascertained by their rebinding to cod proteins, separated by SDS-PAGE and transferred to NC.

RESULTS

Hybridoma characteristics

From 580 wells growth tested for antibody production, 104 produced antibody reactive with Gad cI. Seven of these showed high titres and were cloned and grown as ascites. Hybridomas 17/20 and 21/4 were selected for further study on the basis of their performance in RAST immunoassays and their affinity for cod DIII extract in dot-binding studies. The characteristics of these Mabs are summarized in Table 1. Mab 17/20 is an IgG_{2b} antibody and Mab 21/4 is an IgG_{2a} antibody. In dot-binding studies, using ascitic fluid from mice immunized with the hybridomas, the limit of detection of Gad cI was 32 ng using a 1:10,000 dilution of Mab 17/20, and 250 ng using a 1:5000 dilution of Mab 21/4. No reactivity could be detected using a non-cod IgG Mab as a control.

Table 1. Characteristics of murine anti-Gad cI monoclonal antibodies

Antibody ^a	Subclass	pI	Percentage uptake of ¹²⁵ I-anti-mouse ^b		
			a ^c	b ^c	c ^c
17/20	G _{2b}	6.6	11	5.2	6.3
21/4	G _{2a}	6.9	12	5.6	5.6
Control	G _{2a}	—	3	0.5	2.1

^aAntibodies were used at a concn of 10 μ g/ml.

^bIn the presence of the antibody and using Gad cI as the antigen.

^ca, b, c: goat anti-mouse whole Ig; sheep anti-mouse whole Ig; and sheep anti-mouse F(ab')₂, respectively.

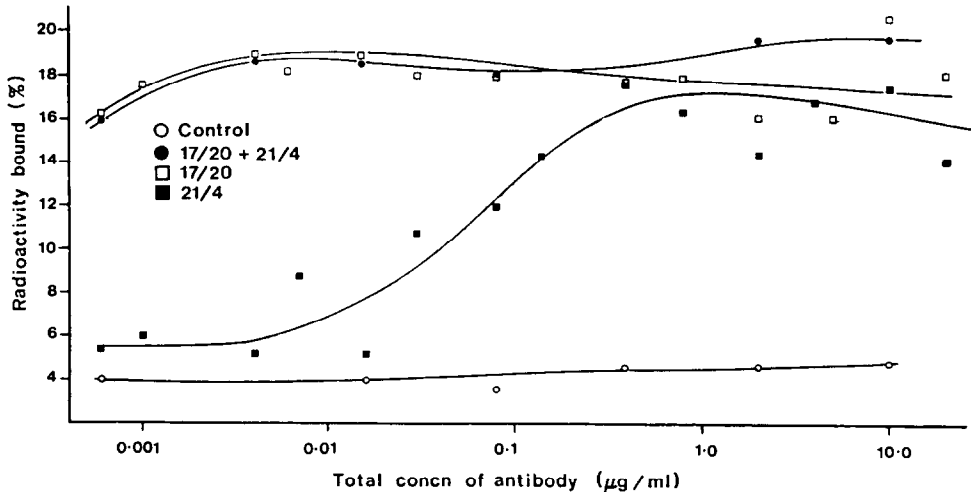


Fig. 1. Concn dependence of the binding of Mabs 17/20 and 21/4 to Gad cI, coupled to CNBr activated paper discs. Binding, which was detected with ^{125}I -goat anti-mouse Ig, is expressed on the vertical axis as the % radioactivity bound.

The concn dependence of the binding of Mabs 17/20 and 21/4 to cod proteins was investigated by radioimmunoassay and the results are presented in Fig. 1. Both clones produced uptakes which were well in excess of those observed with two non-cod Mabs used as controls. Mab 17/20 had almost constant activity over the concn range 0.6–10 $\mu\text{g/ml}$, whilst that of Mab 21/4 increased gradually with concn, reaching a plateau at approximately 0.4 $\mu\text{g/ml}$. When the two Mabs were incubated together with the cod discs, the binding curve asymptoted towards that for 17/20 alone. The additivity index for the two Mabs at 80 ng/ml was 20%. The corresponding baseline additivity was 19%. This indicates a lack of significant additivity and suggests that the determinants recognized by the two antibodies are either identical or located very close to each other.

Electrophoresis of cod DIII extract

The separation of the components present in the cod DIII extract by SDS-PAGE is shown in Fig. 2 and the results are summarized in Table 2. Under reducing conditions, cod DIII extract separated into approximately 15 readily identifiable components (Fig. 3a), all of which transferred to NC (Fig. 3b). The major component, Gad cI, was identified from its location near mol. wt 12,000, which was consistent with the mol. wt of 12,328 reported in the literature (Elsayed and Bennich, 1975). In addition to Gad cI, the most strongly staining components in the protein stain occurred at mol. wts of 17,000 and 47,000. Differences between the protein stains of the gel and NC transfer, for example for the two components at mol. wts of 21,000 and 24,000, probably reflect different affinities of the components for the Coomassie and amido black stains.

Probing of transferred proteins from cod DIII extract

Proteins transferred to NC, following SDS-PAGE of cod DIII extract, were probed with Mabs directed against unknown determinants on Gad cI and with sera from patients allergic to codfish or seafood. These results are presented in Fig. 3 and Table 2.

Two Mabs, 17/20 and 21/4, presented very similar recognition profiles, which included most of the 15 components detected in the protein stain (Fig. 3a). Mab 17/20 (Fig. 3b) differed from Mab 21/4 (Fig. 3c) mainly in its intensity of recognition, in the recognition of two minor bands near mol. wts of 52,000 and 57,000 and in the recognition of two bands not present in the protein stain between mol. wts of 24,000 and 33,000. Non-specific interactions occurring at mol. wts of 71,000, 47,000 and 17,000 were detected by probing without antibody (Fig. 3d) and with non-cod Mabs (Fig. 3e and f).

Sera from two cod- and one fish-allergic patients were used to probe for IgE-binding components. All three allergic sera presented the same recognition profile, consisting of six bands at mol. wts of 61,000, 47,000, 33,000, 24,000, 21,000 and 12,000 (Gad cI) (Fig. 3g, h and i). The most intense bands occurred with Gad cI and the 24,000 mol. wt component. A fourth patient, So(46), allergic to birch pollen but not to cod, presented with very faint recognition of the 47,000 mol. wt component and Gad cI (Fig. 3j). No non-specific interactions could be detected in the absence of antibody, or when a non-allergic human serum was used as a control (Fig. 3k and l). Recognition of Gad cI by either cod-allergic sera or cod-specific Mabs occurred as a broad diffuse band, centred on a region of high intensity at mol. wt 12,000.

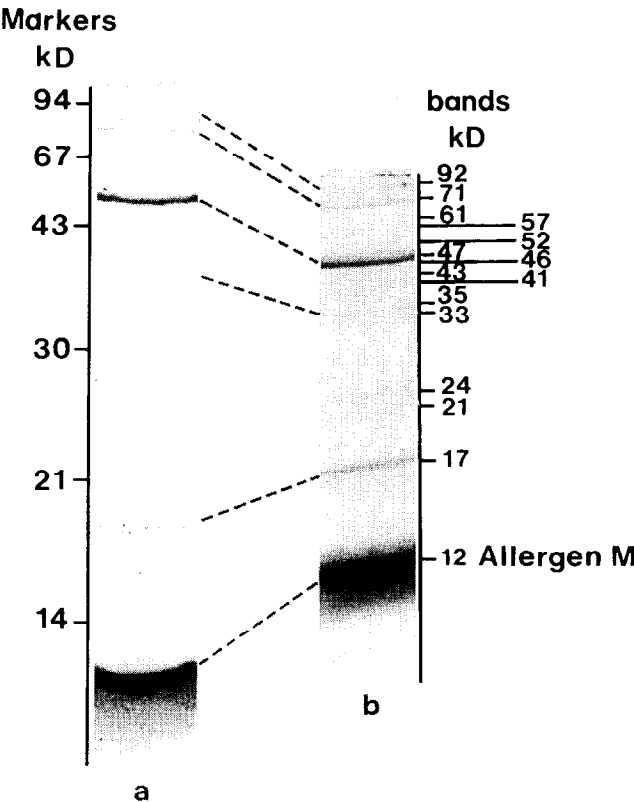


Fig. 2. Separation of proteins present in cod DIII extract by SDS-PAGE on small (8 × 6 cm) 18% homogeneous gels. (a) Protein stain of untransferred proteins; (b) protein stain of transferred proteins. Low mol. wt markers (Bio-Rad) are shown on the left-hand side. Corresponding protein bands on gel and NC transfer are indicated by dotted lines.

Inhibition studies

An attempt was made to assess the extent of cross-reactivity between the Mab 17/20 and the anti-cod IgE repertoire, represented by the allergic patients Marr and At(47). The results are summarized in Table 3. Neither allergic serum significantly inhibited binding of Mab 17/20 to cod discs; nor did Mab 17/20 appreciably reduce binding of IgE to cod discs. Controls suggest that some inhibition can be

achieved through non-specific interactions arising from components in normal human serum.

These results were supported by a western blotting study (data not shown), in which binding to specific bands by either Mab 17/20 or cod-allergic serum Marr, singly or in the presence of each other, was compared. No change could be detected relative to the controls, in which only one of the probes was present.

Table 2. Antigenic and allergenic components of cod DIII extract separated by SDS-PAGE

Antibody or antiserum used to determine binding ^a											
Band	mol. wt	17/20	21/4	Anti-mouse system			Anti-IgE system				Normal
				none	anti-heparin	anti-insulin	Marr (6%) ^b	At(47) (20%) ^b	Mo(40) (20%) ^b	So(46) (2%) ^b	
1	92,000	+	+								
2	71,000	+	+	+	+	+					
3	61,000	++	++				+	+	+		
4	57,000	+									
5	52,000	+									
6	47,000	+	+	+	+	+	+	+	+	+	
7	46,000										
8	43,000	+	+								
9	41,000	+	+								
10	35,000	+	+								
11	33,000	++ ^c	++				+	+	+		
12	24,000	++	++				++	++	++		
13	21,000	++	++				++	++	++		
14	17,000	+	+	+	+	+					
15	12,000	++++	++++				++++	++++	++++	+	

^aReactivity in the table is indicated by the number of “+” s.

^bPercentages refer to RAST scores of the sera with cod allergen discs and ¹²⁵I-anti-IgE.

^cTwo extra bands between mol. wts of 33,000 and 24,000 were present when 17/20 was used as the probe.

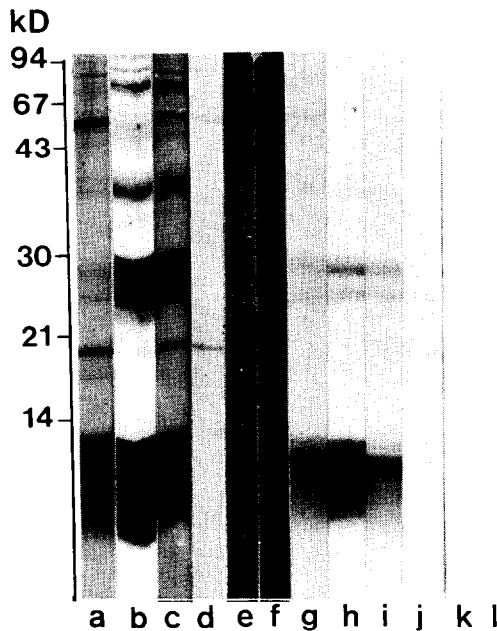


Fig. 3. Recognition profiles of murine Mabs and cod-allergic sera used to probe protein blots of cod extract separated by SDS-PAGE. (a) Protein stain; (b) Mab 17/20; (c) Mab 21/4; (d) no antibody; (e and f) murine non-cod Mabs; (g) serum Marr; (h) serum At(47); (i) serum Mo(40); (j) serum So(46); (k) no antibody; (l) normal human serum.

Rebinding of eluted IgE antibodies

IgE antibodies, which were present in the sera of At(47) and Marr and had bound to the Gad cI band in cod blots, were eluted from that band with low pH buffer. Upon neutralization and reincubation of the eluted IgE with protein blots of cod, it was observed that IgE eluted from the blot probed with At(47) (Fig. 4Aa) rebound only to the Gad cI band (Fig. 4Ab), whilst that eluted from the blot probed were Marr (Fig. 4Bb) rebound to Gad cI and the bands at mol. wts of 17,000, 21,000, 24,000 and 47,000 (Fig. 4Bc, d and f). A comparison with the original protein stain is provided in Fig. 4Ba. Material eluted from the regions of the transfer, in which no IgE-binding could be detected, did not produce bands on rebinding (Fig. 4Ac and 4Be).

Elution of IgE from the Gad cI band was complete in the case at At(47), but some residual IgE-binding, following IgE elution, could be detected in the case of Marr (Fig. 4Bg).

DISCUSSION

The similarity in the recognition profiles of Mabs 17/20 and 21/4, when used as probes of cod extract separated by SDS-PAGE (Fig. 3b and c), suggested that both antibodies recognized the same antigenic determinant on Gad cI. This was supported by the fact that, in inhibition studies, the binding curve for Mabs 17/20 and 21/4, together, converged on that for 17/20 alone (Fig. 1). The additivity index also indicated that both antibodies bound to the same

Table 3. Competition between binding of Mab 17/20 and human cod-allergic serum (Marr) to cod allergen discs

Antisera	Percentage uptake radioactivity with		Remarks
	¹²⁵ I-anti-mouse	¹²⁵ I-anti-IgE	
(a) 17/20 ^a	25	—	Normal level of binding of Mab 17/20
(b) At(47) ^b	—	12	Normal levels of anti-cod IgE activity with two different cod-allergic sera
(c) Marr ^b	—	7	Level of non-specific binding by radio-labels to components in serum
(d) Normal human serum	7	1	Non-specific displacement of 17/20 by normal serum components
(e) 17/20 + normal human serum	16	—	Non-specific displacement of anti-cod IgE by control Mab
(f) At(47) + control Mab	—	8	No difference relative to (a) or (b): implies 17/20 and anti-cod IgE in At(47) specify different determinants
(g) 17/20 + At(47)	22	12	No difference relative to (c) and (e): implies non-specific displacement of 17/20 by normal serum components; IgE and 17/20 determinants are different
(h) 17/20 + Marr	16	7	

^aMab 17/20 was used at a concn of 10 µg/ml.

^bAllergic sera were used at concn of 1 in 2.

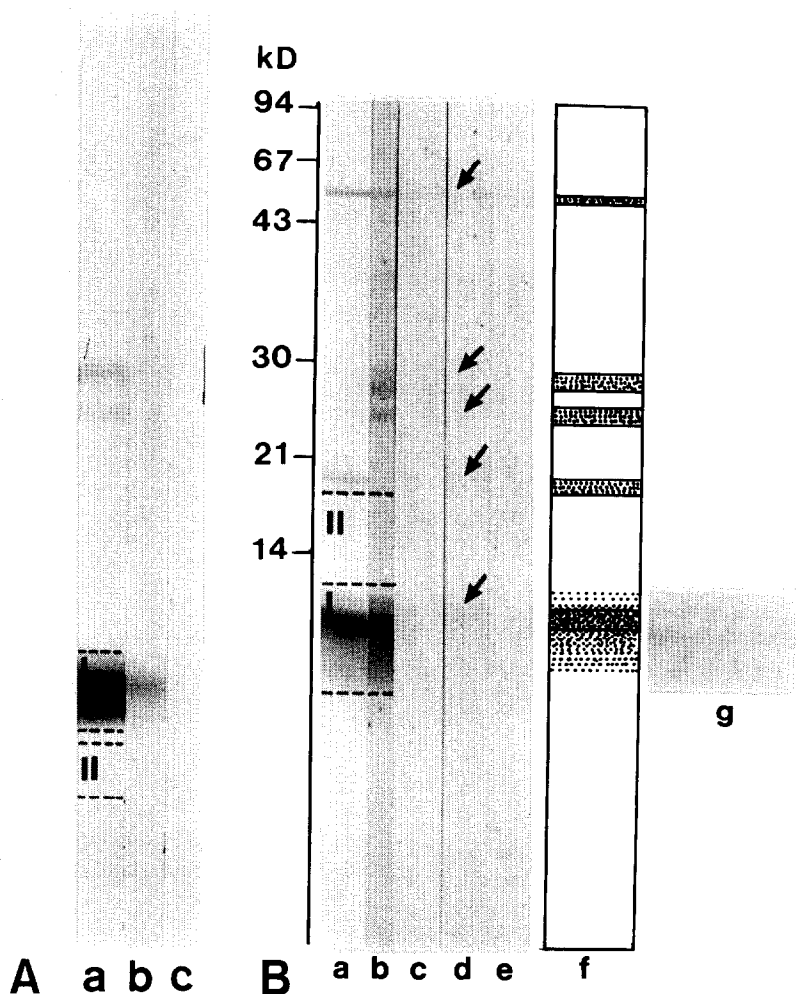


Fig. 4. (A) Rebinding of IgE eluted from Gad cI band to protein blots of cod extract separated by SDS-PAGE. Initial IgE-binding was performed with serum from At(47). (a) Allergic profile for At(47) prior to elution; (b) rebinding of IgE eluted from region I to protein blots of cod; (c) control strip: rebinding of material eluted from region II to protein blot of cod. (B) Rebinding of IgE eluted from Gad cI band to protein blots of cod extract separated by SDS-PAGE. Initial IgE binding was performed with cod-allergic serum, Marr. (a) Protein stain; (b) allergic profile for Marr serum prior to elution; (c and d) rebinding of IgE eluted from region I to protein blots of cod. Arrows indicate where rebinding occurred; (e) control strip, rebinding of material eluted from region II to cod protein blots; (f) pictorial representation of (c) and (d) illustrating bands produced on rebinding; (g) excised region I stained for residual IgE-binding, following elution of IgE from it.

site, although Mab 17/20 was of higher affinity. Recognition of both the denatured and native cod proteins by Mab 17/20 suggests that the determinant is resistant to denaturation.

Although the monoclonality of Mabs 17/20 and 21/4 was established in IEF, western blots of cod proteins separated by SDS-PAGE showed that both Mabs recognized other proteins in addition to Gad cI. Similar observations with Mabs in other systems have been reported frequently in the literature (Turner, 1983; Ogata *et al.*, 1983; Ekramoddoullah *et al.*, 1986; Mourad *et al.*, 1986; Luca *et al.*, 1986) and have been interpreted as being either non-specific interactions, or being due to the presence of identical, or closely related, determinants on different proteins (Ghosh and Campbell, 1986). In the present study,

although some of the cross-reactivity can be accounted for by non-specific interactions with the components at mol. wts of 71,000, 47,000 and 17,000, the high intensity of binding between the two Mabs and the components at mol. wts of 61,000, 24,000 and 21,000 suggests that these proteins share at least one antigenic determinant with Gad cI.

Gad cI is the major protein in the DIII fraction of cod and has been described as the sole allergenic component. The application of PAGE and immunoblotting to the analysis of the DIII fraction has permitted a reassessment of these data. By SDS-PAGE, cod DIII extract was shown to be a heterogeneous mixture of proteins, the principal component of which was Gad cI (Fig. 2). Although Gad cI was also shown to be responsible for most of the

allergenicity of the extract, five other IgE-binding bands were identified in immunoblots of DIII separated by SDS-PAGE, using sera from three different cod-allergic patients.

It is of particular interest that identical allergenic profiles were obtained for the two cod-allergic patients, At(47) and Mo(40) from Norway, and the one fish-allergic patient, Marr, from Australia. This observation suggests that common determinants exist between Gad cI and proteins from other fish, since patient Marr had no previous history of exposure to cod. The fact that three different allergic sera recognized the same bands suggests that the number of allergenic sites on the cod proteins and the specificity of the IgE response to them is more restricted than it appears from the immunoblots. A similar observation was made in the case of the Norwegian response to birch pollen, which was essentially limited to a few components, except when the initial or primary response was complicated by exposure to multiple allergens (Hemmens *et al.*, 1989).

Protein blotting studies, which use sera from allergic patients to probe transfers of electrophoretically separated proteins, have demonstrated that extracts from a number of different allergen sources (including pollens, mites, fungi and venoms; Tovey and Baldo, 1987) generally contain several allergenic proteins distributed over a wide range of mol. wts. Although the allergenicity of four antigenic determinants in ragweed allergen Ra3 (Atassi and Atassi, 1985; 1986), and of two antigenic determinants in allergen Df29 from *D. farinae* (Ley *et al.*, 1986), have been established, it remains unclear whether the many different IgE-reactive bands detected on blots in this study represent distinct allergens, each with different allergenic determinants, or proteins of different molecular size, but with the same (or a restricted number of) allergenic determinants. Demonstration of a common complement of six allergenic codfish components recognized by the IgE antibodies in the sera from two cod-allergic and one fish-allergic subjects suggests that, for codfish proteins, some common allergenic determinants are involved in the reactions with IgE antibodies.

However, the results of our IgE elution experiments indicate that the specificity of IgE populations directed against a single allergenic band in an immunoblot varies from person to person. In the case of the allergic patient At(47), it was shown that the IgE antibodies which bound to Gad cI were specific for determinants which were unique to this particular component of cod. However, in the case of the allergic patient Marr, who presented with the same IgE-binding profile in cod immunoblots as At(47), at least some of the IgE populations directed against Gad cI were shown to be multispecific and recognized determinants common both to Gad cI and other components of cod. Although it was not possible in either case to predict the number of different IgE populations which were eluted, the results with

At(47) show that unique allergenic determinants do exist, whilst the results with Marr affirm the presence of common allergenic determinants on different allergenic components of cod. This, in turn, suggests that in some examples the IgE antibody repertoire for a particular allergen may be less diverse than predicted from the current concept of protein immunogenicity, in which the entire surface of a protein is considered to be immunogenic (Benjamin *et al.*, 1984).

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REFERENCES

- Aas K. and Jebsen J. W. (1967) Studies of hypersensitivity to fish. Partial purification and crystallization of a major allergenic component of cod. *Int. Archs Allergy* **32**, 1–20.
- Atassi H. and Atassi M. Z. (1985) Localization of the continuous allergenic sites of ragweed allergen Ra3 by a comprehensive synthetic strategy. *FEBS Letts* **188**, 96–100.
- Atassi H. and Atassi M. Z. (1986) Antibody recognition of ragweed allergen Ra3: localization of the full profile of the continuous antigenic sites by synthetic overlapping peptides representing the entire protein chain. *Eur. J. Immun.* **16**, 229–235.
- Baur X., Aschauer H., Mazur G., Dewair M., Prelicz H. and Steigemann W. (1986) Structure, antigenic determinants of some clinically important insect allergens: chironomid hemoglobins. *Science* **233**, 351–354.
- Benjamin D. C., Berzofsky J. A., East I. J., Gurd F. R. N., Hannum C., Leach S. J., Margoliash E., Michael J. G., Miller A., Prager E. M., Reichlin M., Sercarz E. E., Smith-Gill S. J., Todd P. E. and Wilson A. C. (1984) The antigenic structure of proteins: a reappraisal. *A. Rev. Immun.* **2**, 67–101.
- Ekrasmoddullah A. K. M., Kisil F. T. and Sehon A. H. (1986) Partial characterization of an antigenic site of high molecular weight basic antigen, a ryegrass pollen allergen, using a monoclonal antibody. *Molec. Immun.* **23**, 111–117.
- Elsayed S. and Aas K. (1971) Isolation of purified allergens (cod) by isoelectric focusing. *Int. Archs Allergy appl. Immun.* **40**, 428–438.
- Elsayed S. and Apold J. (1983) Immunochemical analysis of cod fish allergen M: locations of the immunoglobulin binding sites as demonstrated by the native and synthetic peptides. *Allergy* **38**, 449–459.
- Elsayed S. and Bennich H. (1975) The primary structure of allergen M from cod. *Scand. J. Immun.* **4**, 203–208.
- Friquet G., Djavadi-Ohanian L., Pages J., Bussard A. and Goldberg M. (1983) A convenient enzyme-linked immunosorbent assay for testing whether monoclonal antibodies recognise the same antigenic site. Application to hybridomas specific for the B₂-subunit of *Escherichia coli* tryptophan synthase. *J. Immun. Meth.* **60**, 351–358.
- Ghosh S. and Campbell A. M. (1986) Multispecific monoclonal antibodies. *Immun. Today* **7**, 217–222.
- Hemmens V. J., Baldo B. A., Elsayed S. and Bass D. (1989) Allergic response to birch and alder pollen allergens influenced by geographical location of allergic subjects. *Int. Archs Allergy appl. Immun.* **87**, 321–328.
- Leitzke R. and Unsicker K. (1985) A statistical approach to determine monoclonality after limiting cell plating of a hybridoma clone. *J. Immun. Meth.* **76**, 223–228.
- Ley V., Saiz J. C. and Carreira J. (1986) Isolation and characterization of the main allergen of *Dermatophaga*

- goides farinae* by monoclonal antibodies that recognize IgE related epitopes. *Molec. Immun.* **23**, 1311–1318.
- Lind P. (1986) Demonstration of close physicochemical similarity and partial immunochemical identity between the major allergen, Dp42, of the house dust mite, *D. pteronyssinus*, and corresponding antigens of *D. farinae* (Df6) and *D. microceras* (Dm6). *Int. Archs Allergy appl. Immun.* **79**, 60–65.
- Luca F. C., Bloom G. S. and Vallee R. B. (1986) A monoclonal antibody that cross-reacts with phosphorylated epitopes on two microtubule-associated proteins and two neurofilament polypeptides. *Proc. natn. Acad. Sci. U.S.A.* **83**, 1006–1010.
- Marsh D. G., Goodfriend L., King T. P., Lowenstein H. and Platts-Mills T. A. E. (1988) Allergen nomenclature. *Int. Archs Allergy appl. Immun.* **85**, 194–200.
- Mourad W., Pelletier G., Boulet A., Islam N., Valet J.-P. and Hebert J. (1986) Allergenicity and cross-reactivity of rye grass pollen extracts revealed by monoclonal antibodies. *J. Immun. Meth.* **89**, 53–59.
- Ogata K., Arakawa M., Kasahara T., Shioiri-Nakano K. and Hiraoka K. (1983) Detection of toxoplasma membrane antigens transferred from SDS-polyacrylamide gel to nitrocellulose with monoclonal antibody and avidin-biotin, peroxidase, anti-peroxidase and immunoperoxidase methods. *J. Immun. Meth.* **65**, 75–82.
- Steele J. G., Dalton B. A., Hoffman H., Underwood P. A. and Rathjen D. (1985) Monoclonal antibodies to nerve growth factor from bovine seminal plasma. *Molec. Immun.* **22**, 1061–1072.
- Tovey E. R. and Baldo B. A. (1984) Standardization of allergens. Qualitative definition of house dust mite extracts following electroblotting and detection of components with antibody and lectin probes. *Int. Archs Allergy appl. Immun.* **75**, 322–329.
- Tovey E. R. and Baldo B. A. (1987) Characterization of allergens by protein blotting. *Electrophoresis* **8**, 452–463.
- Tovey E. R., Ford S. A. and Baldo B. A. (1987) Protein blotting on nitrocellulose: some important aspects of the resolution and detection of antigens in complex extracts. *J. Biochem. Biophys. Meth.* **14**, 1–17.
- Towbin H., Staehelin T. and Gordon J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350–4354.
- Turner B. M. (1983) The use of alkaline-phosphatase-conjugated second antibody for the visualization of electrophoretically separated proteins recognized by monoclonal antibodies. *J. Immun. Meth.* **63**, 1–6.
- Underwood P. A. (1982) Mapping of antigenic changes in the haemagglutinin of Hong Kong influenza (H3N2) strains using a large panel of monoclonal antibodies. *J. gen. Virol.* **62**, 153–169.
- Wide L., Bennich H. and Johansson S. G. O. (1967) Diagnosis of allergy by an *in vitro* test for allergen antibodies. *Lancet* **ii**, 1105–1107.