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Immunochemical cross-reactions between pentraxins of different species

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SUMMARY

Monospecific antisera were raised by immunization with pentraxins, C-reactive protein (CRP) or serum amyloid P component (SAP), that had been isolated from man and a number of different vertebrate species. These antisera were used to test sera or serous fluids from a range of other vertebrate species and also some invertebrates. Cross-reactions between species were comparatively rare but were seen among primates and among ruminants. There was also cross-reactivity between these two groups. Antisera to plaice pentraxins did not react with sera from higher vertebrates but did recognize antigens in sera from other flat fish. No cross-reactivity was observed between CRP and SAP either within or between species despite the known homologies of amino acid sequence and similarities of structure within the family. Several reactions occurred with sera in which either CRP or SAP had previously not been sought or in which only preliminary investigations had been performed. The proteins detected were: CRP in goat, cow, sheep, cat and lemon sole; SAP in monkey and dab. These findings significantly extend the range of species in which pentraxins are known to be present.

INTRODUCTION

C-reactive protein (CRP) and serum amyloid P component (SAP), members of the pentraxin family of plasma proteins (Osmand et al., 1977), are present in the sera of a wide range of vertebrate species (Pepys *et al.*, 1978; Baltz *et al.*, 1982; Robey, Tanaka & Liu, 1983). The evolutionary conservation of this family of proteins suggests that they have important biological functions, although these have not yet been precisely identified. However, no human individual has been reported with either partial or complete deficiency of either CRP or SAP.

CRP and SAP are probably the products of gene duplication, and in man both genes are located on the proximal long arm of chromosome 1 (Whitehead *et al.*, 1983; Floyd-Smith *et al.*, 1986). These human proteins share extensive homology of amino acid sequence, with 51% strict residue-for-residue identity rising to 66% when neutral substitutions are considered (Lei *et al.*, 1985; Mantzouranis *et al.*, 1985; Woo, Korenberg & Whitehead, 1985). Furthermore, in all vertebrate species from which they have been isolated, they have a similar electron microscopic appearance with subunits arranged in a cyclic pentameric disc (in some proteins with two such discs stacked face-to-face), hence the family name of pentraxins (Osmand *et al.*, 1977). In addition, all pentraxins studied so far have the capacity for calcium-dependent binding either to phosphoryl choline or to agarose (reviewed in Pepys & Baltz, 1983). A phosphoryl choline-binding protein has also been described in an invertebrate species, *Limulus polyphemus*, an arachnid known as the 'horseshoe crab'. This 'CRP' shares significant sequence homology with human CRP and other pentraxins (Nguyen *et al.*, 1986a, b), and has been reported to cross-react immunochemically with antiserum to rabbit CRP, although it is apparently hexameric (Fernandez-Moran, Marchalonis & Edelman, 1968) and has a more complex subunit structure than the known vertebrate pentraxins (Robey & Liu, 1981; Nguyen *et al.*, 1986b; Baltz *et al.*, 1982).

Earlier reports have identified immunochemical cross-reactivity between certain mammalian CRPs (Abernethy, 1937; Anderson & McCarty, 1951; Gotschlich & Stetson, 1960) and these reflect very close structural similarity in cases where the sequences are known, for example human and rabbit CRP (Wang *et al.*, 1982). However, despite the strong resemblances between CRP and SAP, no antigenic cross-reactivity between them has been described hitherto. We have attempted to reassess this situation and report here a systematic examination by immunodiffusion in gel of immunoprecipitation reactions between a number of different anti-pentraxin antisera and serum or serous fluid from a variety of vertebrate as well as some invertebrate species.

Abbreviations: CRP, C-reactive protein; SAP, serum amyloid P component.

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Table 1. Cross-reactions with antisera to CRP

Sera	Sheep anti-human	Rabbit anti-human	Sheep anti-rabbit	Rabbit anti-goat	Rabbit anti-bovine	Rabbit anti-dog	Rabbit anti-rat	Rabbit anti-plaice	Rabbit anti-lumpsucker
Sea urchin	–	–	–	–	–	–	–	–	–
Octopus	–	–	–	–	–	–	–	–	–
Dogfish	–	–	–	–	–	–	–	–	–
Skate	–	–	–	–	–	–	–	–	–
Brill	–	–	–	–	–	–	–	–	–
Lumpsucker	–	–	–	–	–	–	–	–	+
Turbot	–	–	–	–	–	–	–	–	–
Plaice	–	–	–	–	–	–	–	+	–
Flounder	–	–	–	–	–	–	–	–	–
Lemon sole	–	–	–	–	–	–	–	+	–
Dab	–	–	–	–	–	–	–	–	–
Cod	–	–	–	–	–	–	–	–	–
Rainbow trout	–	–	–	–	–	–	–	–	–
AP* mouse	–	–	–	–	–	–	–	–	–
AP rat	–	–	–	–	–	–	+	–	–
AP guinea-pig	–	–	–	–	–	–	–	–	–
Fruit bat	–	–	–	–	–	–	–	–	–
Fruit bat ascites	–	–	–	–	–	–	–	–	–
AP rabbit	+	–	+	–	–	–	–	–	–
Pigeon	–	–	–	–	–	–	–	–	–
Chicken	–	–	–	–	–	–	–	–	–
Cat	–	+	–	–	–	–	–	–	–
AP dog	–	–	–	–	–	+	–	–	–
Sheep	–	+	–	+	+	–	–	–	–
Pig	–	–	–	–	–	–	–	–	–
AP goat	–	+	–	+	–	–	–	–	–
AP cow	–	+	–	+	+	–	–	–	–
Horse	–	–	–	–	–	–	–	–	–
AP monkey	+	+	–	–	–	–	–	–	–
AP human	+	+	–	–	+	–	–	–	–

* AP, acute phase.

MATERIALS AND METHODS

Sera and serous fluids

The fish sera: dogfish (*Scyliorhinus caniculus* L.), skate (*Raja naevus*; Muller & Henle), brill (*Scophthalmus rhombus* L.), lumpsucker (*Cyclopterus lumpus* L.), turbot (*Scophthalmus maximus* L.), plaice (*Pleuronectes platessa* L.), flounder (*Platichthys flesus* L.), lemon sole (*Microstomus kitt* Walbaum), dab (*Limanda limanda* L.), cod (*Gadus morhua* L.), rainbow trout (*Salmo gardneri* Richardson), and sea urchin (*Echinus exculantus* or *Echinocardium cardatum*) coelomic fluid were provided by Dr T. C. Fletcher, NERC Institute of Marine Biochemistry, Aberdeen. Octopus (*Octopus vulgaris* Cuvier) haemolymph was supplied by Dr M. J. Wells, Department of Zoology, University of Cambridge. Acute-phase rat serum containing 800 μg CRP/ml was from a Sprague Dawley rat (Charles River U.K. Ltd, Margate, Kent), and the acute-phase mouse serum was a pool from several strains, containing approximately 100 μg SAP/ml. Fruit bat (*Eidolon helvum*) serum and ascites were a gift from Mr D. Simmonds, Pollards Wood Research Station, Institute of Cancer Research, Chalfont St Giles, Bucks, and the pigeon serum was donated by Dr J. Longbottom, Cardiothoracic Institute, Brompton Hospital, London. Acute-phase rabbit serum containing 108 μg CRP/ml was obtained from a lop-eared rabbit (Hylyne Rabbits Ltd, Northwich, Cheshire) that had received subcutaneous injections of casein solution. Normal chicken serum was purchased from Gibco Europe Ltd, Uxbridge, Middlesex. Acute-phase dog serum containing approximately 80 μg CRP/ml was provided by Dr R. Batt, Department of Veterinary Pathology, University of Liverpool, and normal cat serum was from the Royal Veterinary College, London. Normal pig and sheep serum, post-operative goat serum and bovine serum from a Jersey cow that had been injected with vitamin-free casein and lipopolysaccharide were provided by Dr A. Feinstein, AFRC Institute of Animal Physiology, Babraham, Cambridge. Horse serum was from Dr E. L. Gerring, Royal Veterinary College, London, and acute-phase monkey serum from animals that had undergone neurosurgical procedures was provided by Dr G. Ettlinger, Institute of Psychiatry, London. The acute-phase human serum was a clinical sample containing 108 μg CRP/ml. All sera and serous fluids were tested by immunodiffusion against a panel of antisera to CRP and SAP from different species.

Antisera

Antisera were raised by immunization of rabbits and sheep with the purified protein emulsified in Freund's complete adjuvant (Difco Laboratories, East Molesey, Surrey) followed by booster injections in incomplete Freund's adjuvant (Difco). Antiserum

Table 2. Cross-reactions with antisera to SAP

Sera	Sheep anti-human	Rabbit anti-human	Rabbit anti-guinea-pig	Rabbit anti-rat	Sheep anti-mouse	Rabbit anti-mouse	Rabbit anti-plaice
Sea urchin	–	–	–	–	–	–	–
Octopus	–	–	–	–	–	–	–
Dogfish	–	–	–	–	–	–	–
Skate	–	–	–	–	–	–	–
Brill	–	–	–	–	–	–	–
Lumpsucker	–	–	–	–	–	–	–
Turbot	–	–	–	–	–	–	–
Plaice	–	–	–	–	–	–	+
Flounder	–	–	–	–	–	–	+
Lemon sole	–	–	–	–	–	–	–
Dab	–	–	–	–	–	–	+
Cod	–	–	–	–	–	–	–
Rainbow trout	–	–	–	–	–	–	–
AP mouse	–	–	–	–	+	+	–
AP rat	–	–	–	+	–	–	–
AP guinea-pig	–	–	+	–	–	–	–
Fruit bat	–	–	–	–	–	–	–
Fruit bat ascites	–	–	–	–	–	–	–
AP rabbit	–	–	–	–	–	–	–
Pigeon	–	–	–	–	–	–	–
Chicken	–	–	–	–	–	–	–
Cat	–	–	–	–	–	–	–
AP dog	–	–	–	–	–	–	–
Sheep	–	–	–	–	–	–	–
Pig	+	–	–	–	–	–	–
AP goat	–	–	–	–	–	–	–
AP cow	+	–	–	–	–	–	–
Horse	–	–	–	–	–	–	–
AP monkey	+	+	–	–	–	–	–
AP human	+	+	–	–	–	+	–

* AP, acute phase.

Table 3. Cross-reactivity of antisera to CRP

Antiserum	Complete identity: Serum (1)	Complete identity: Serum (2)	Partial identity: Serum (1)	Partial identity: Serum (2)
Sheep anti-human CRP			Human	Monkey
			Human	Rabbit
			Monkey	Rabbit
Rabbit anti-human CRP	Sheep	Cow	Human	Monkey
	Sheep	Cat	Human	Sheep
	Sheep	Goat	Human	Goat
	Cow	Goat	Human	Cow
	Cat	Goat	Human	Cat
			Monkey	Sheep
			Monkey	Goat
			Monkey	Cow
			Monkey	Cat
			Cow	Cat
Rabbit anti-goat CRP	Goat	Sheep	Goat	Cow
			Sheep	Cow
Rabbit anti-bovine CRP	Human	Sheep	Cow	Human
			Cow	Sheep

specificity was tested by immunodiffusion in agarose. Sheep and rabbit antisera were raised against the following pentraxins, which had been isolated according to published methods: human CRP and SAP (de Beer & Pepys, 1982), rat CRP and SAP (de Beer *et al.*, 1982), plaice CRP and SAP (Pepys *et al.*, 1982), lumpsucker CRP (Fletcher, White & Baldo, 1977), mouse SAP (Pepys, 1979), guinea-pig SAP (Maudsley *et al.*, 1986), bovine and goat CRP (Maudsley *et al.*, 1987a, b), dog CRP (Caspi *et al.*, 1984), and rabbit CRP (Roe *et al.*, 1984).

Immunodiffusion

Double diffusion was conducted in 1% w/v Seakem ME agarose (FMC Corp., Miles Laboratories Ltd, Slough, Berks) in 0·07 M barbitone, 0·01 M EDTA, 0·1% w/v NaN_3, pH 8·6, and allowed to proceed for 24–48 hr at room temperature. The gels were then washed overnight at 37° in 5% w/v NaCl containing 0·1% w/v NaN_3, after which they were pressed, dried and stained with Coomassie blue.

RESULTS

Cross-reactions among species

The presence or absence of immunoprecipitation between the various reagents tested is summarized in Tables 1 and 2. Further

Table 4. Cross-reactivity of antisera to SAP

Antiserum	Complete identity		Partial identity	
	Serum (1)	Serum (2)	Serum (1)	Serum (2)
Sheep anti-human SAP	Cow	Pig	Human	Monkey
			Human	Pig
			Human	Cow
			Monkey	Pig
			Monkey	Cow
Rabbit anti-human SAP		Human	Monkey	
Rabbit anti-plaice SAP		Plaice	Flounder	
			Plaice	Dab
			Flounder	Dab

details of the type of reaction, whether of complete or of partial identity, are listed in Tables 3 and 4, the numbering of the serum samples used as sources of antigen being according to Fig. 1.

Rabbit anti-plaice CRP cross-reacted only with serum from the lemon sole (Fig. 2), however there was no fusion of the immunoprecipitation lines, only inhibition by the strong line formed with plaice CRP of the much weaker line with lemon sole serum.

The reactions of rabbit anti-human CRP with cat, goat, sheep and bovine sera were weak in contrast to those with monkey and human acute-phase sera. Rabbit anti-bovine CRP gave a line with normal sheep serum and against acute-phase but not normal human serum. These reactions were also weak. Rabbit anti-goat CRP gave quite strong reactions, bovine serum giving a slightly weaker line than sheep serum.

The reactions of pig and bovine serum with sheep anti-human SAP were quite definite, although monkey and human serum gave much stronger lines. There was no difference between normal and acute-phase monkey serum or between normal and acute-phase bovine serum in this respect. In the case of rabbit anti-plaice SAP, which gave strong lines against flounder and dab serum, the spur angle between the flounder and dab lines was usually slightly smaller than the angle between the plaice and the dab lines. This would suggest that dab SAP is more closely related to flounder than plaice SAP.

Absence of cross-reactivity between CRP and SAP

Human, monkey, bovine, rat and plaice serum are all known to contain readily detectable amounts of SAP and CRP, either in normal or during the acute-phase response. Acute-phase human and monkey sera gave reactions of non-identity when tested against anti-human CRP and anti-human SAP in adjacent wells. This was true for antisera raised in sheep or rabbits. The reaction of rabbit anti-human CRP and sheep anti-human SAP against bovine serum (normal or acute-phase) was also one of non-identity. However, interpretation of this was complicated by the fact that anti-CRP also reacted with sheep serum, therefore an extra line between the antiserum wells was observed which fused with the bovine CRP line (Fig. 3). Non-identity between plaice CRP and SAP and between rat CRP and SAP, using the same antisera as used in this study, has been reported previously by Pepys *et al.* (1982) and de Beer *et al.* (1982), respectively, and therefore was not repeated.

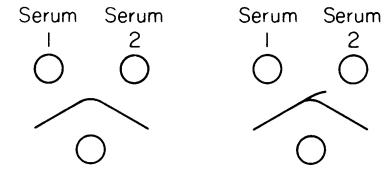

Figure 1. Types of cross-reactions listed in Tables 3 and 4. Left, complete identity; right, partial identity. In both cases antiserum is in the bottom well.

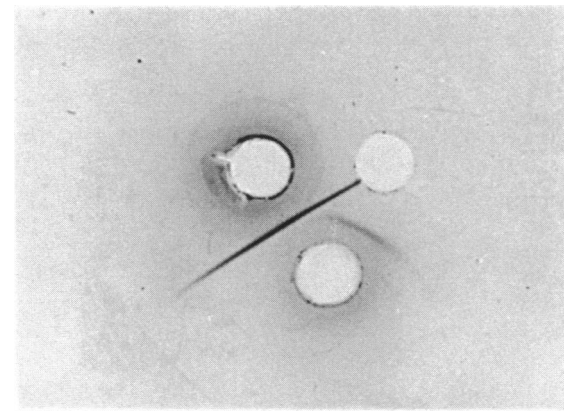

Figure 2. Cross-reaction of rabbit anti-plaice CRP with lemon sole serum. Above left, plaice serum; above right, lemon sole serum; bottom, rabbit anti-plaice CRP antiserum.

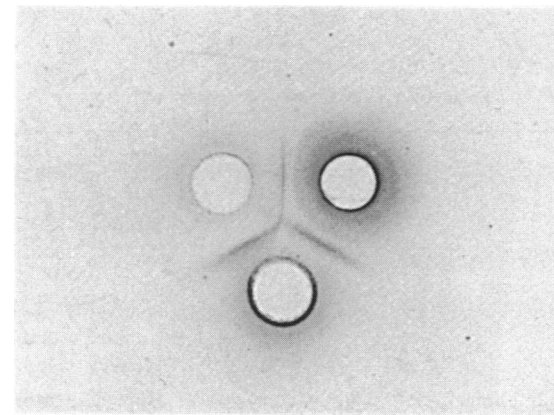

Figure 3. Immunological distinction between bovine CRP and bovine SAP, and cross-reaction between bovine CRP and sheep CRP. Above left, rabbit anti-human CRP antiserum; above right, sheep anti-human SAP antiserum; bottom, bovine serum.

DISCUSSION

The results of this study confirm the comparative rarity of cross-reactions between pentraxins of different species and the complete immunochemical distinction between CRP and SAP in the same species. Most of the sera tested were from placental mammals or teleost fish, the exceptions being two invertebrate

sera, two avian sera, and dogfish serum. Therefore it is not surprising that the majority of cross-reactions were amongst the mammalian sera, particularly primates and ruminants. The relationship between these two groups, however, was not expected, nor was that of cat CRP with both groups, considering the evolutionary distance between them. The reactions between fish pentraxins are also of interest because not only did antisera to plaice pentraxins fail to cross-react with sera from higher vertebrates, but they appeared to be selective for certain members of the flatfish group alone. This was in spite of the fact that a number of other teleost fish also possess pentraxins, e.g. turbot (Fletcher, White & Baldo, 1980), lumpsucker (Fletcher *et al.*, 1977) and rainbow trout (Winkelhake & Chang, 1982).

The host animal in which an antiserum is raised is an important factor with respect to its capacity to cross-react. This was evident in that rabbit but not sheep anti-human CRP cross-reacted with the ruminant sera and vice versa for anti-human SAP. Differences between individual animals of the same species in which the antiserum is raised can also determine whether it will cross-react or not. For example, of the four rabbit anti-human CRP sera tested, only three gave the cross-reactions described against ruminant and cat sera.

An analogous phenomenon may explain why Dillman & Coles (1966) were able to report cross-reactivity between rabbit anti-human CRP antisera and dog CRP, whereas we did not find any such cross-reactivity here. Alternatively, it may be that a reaction too weak to be seen by immunoprecipitation in gel was demonstrable by the more sensitive agglutination procedure. Another possibility is that latex tests are susceptible to false positive reactions. One or other of these mechanisms may explain why Usui (1964) and Sakamoto (1964) reported positive latex tests, using the same commercial kit as Dillman & Coles (1966), when they tested acute-phase guinea-pig serum and perilymph, whereas we have not been able to isolate any CRP in the guinea-pig (Maudsley *et al.*, 1986).

This type of study has unavoidable constraints, since positive precipitation reactions depend on the quality and titre of antibody response in individual animals, and negative results do not exclude the presence of the antigens in question. However, the present investigation has yielded valuable information. It facilitated the recognition or identification of pentraxins in seven species in which they had not previously been recognized: CRP in cat, goat, cow, sheep and lemon sole, and SAP in monkey and dab. The pentraxins of cow and goat have since been isolated and characterized in some detail (Maudsley *et al.*, 1987a, b), whilst the sheep pentraxin, which had been identified in a preliminary study as an SAP by virtue of its binding to agarose (Pepys *et al.*, 1978), was found to be more closely related immunochemically to human CRP.

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