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Opsonization of yeast by human serum IgA anti-mannan antibodies and phagocytosis by human polymorphonuclear leucocytes

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SUMMARY

The ability of sera from 72 patients with liver disease to opsonize yeast for phagocytosis by normal polymorphonuclear leucocytes has been studied. Seven showed defective opsonization. The opsonic activity of all but two sera was decreased markedly by heating at 56°C for 1 h. When the two sera with heat stable opsonic activity were fractionated by gel filtration and by ion exchange chromatography, the activity copurified with IgA, not with IgG. The purified IgA, radiolabelled with ^{125}I was shown to bind in a saturable manner to the yeast. Both sera had high levels of anti-yeast mannan IgA detected by an ELISA. In one case most of the anti-mannan activity was due to monomeric IgA, in the other it was dimeric. This was consistent with the observation of an apparent molecular weight of the opsonin of approximately 180 kD in one serum and 300–400 kD in the other.

Keywords IgA neutrohil opsonin phagocytosis liver disease

INTRODUCTION

The phagocytosis of yeasts and bacteria by polymorphonuclear leucocytes (PMN) is one of the first lines of defence against infection. For effective phagocytosis the micro-organisms must first be opsonized by interaction with serum proteins. The major opsonins are certain subclasses of IgG and fragments of the complement protein C3, for which there are receptors on the PMN surface. There is considerable evidence that efficient phagocytosis only occurs when the micro-organisms are opsonized with both IgG and C3 (Scribner & Fahrney, 1976; Newman & Johnston, 1979; Roos *et al.*, 1981).

Roos *et al.* (1981) showed that when yeast and PMN were incubated with serum in the fluid phase, C3 fragments appeared to promote adhesion, whilst the IgG triggered ingestion. Yeast treated with high concentrations (100 mg/ml) of normal serum IgG were phagocytosed by PMN, but not unopsonized yeast or yeast opsonized with C3 alone. PMN were able to phagocytose unopsonized yeast only if placed in close contact, for example by centrifugation. This phagocytosis of unopsonized yeast has been suggested to be due to recognition of carbohydrate structures on the yeast cell surface by lectin-like molecules: either glucan, by a receptor on the PMN surface which is probably the same as the type 3 C3 (CR3) receptor (Ross, Cain & Lachmann, 1985); or mannan, by a mannose specific receptor (Sharon, 1984).

Most of the yeast opsonizing activity of normal serum is EDTA sensitive and destroyed by heating at 56°C. It is therefore considered to be complement-dependent. Normal serum has in fact very little detectable heat-stable opsonizing activity for any micro-organism, although hyperimmu-

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nized subjects can show considerable activity which can be attributed to specific IgG antibodies (Quie, 1972; Young & Armstrong, 1972). The low levels of IgG anti-yeast antibodies detectable in normal serum are presumed to be insufficient for opsonization in the absence of complement (Kemp & Turner, 1986; Kozel, Highison & Stratton, 1984).

In a previous study (Kerr *et al.*, 1983) we showed that whilst 38 pathological sera with low C3 levels exhibited defective yeast opsonization, three sera with very low C3 levels had apparently normal opsonic activity. The activity was heat stable but not due to IgG. Since two of these patients showed severe liver damage, we have now investigated the yeast opsonizing activity of a large number of sera from patients with chronic liver disease. In this paper we show that the heat stable yeast opsonin identified in three of the sera is specific anti-yeast mannan IgA. We believe this is the first report of a role for serum IgA in the stimulation of phagocytosis of a micro-organism by PMN.

METHODS

PMN were separated from normal heparinized blood by a modification of the method of English & Anderson (1974) as described previously (Kerr *et al.*, 1983). The yeast cell wall preparation used for phagocytosis studies was made from baker's yeast (*Saccharomyces cerevisiae*). The yeast was killed by boiling and then reduced and alkylated essentially as described by Lachmann & Hobart (1978). Yeast cells, 10^9, were iodinated with 1 mCi^{125}I in a final volume of 2 ml 0·3 M sodium phosphate pH 7·3 using chloramine T (1 mg/ml.) After extensive washing to remove all unbound radioactivity, the yeast was resuspended in 1 ml RPMI 1640 medium.

C3, C4, total IgG, and IgA were measured by radial immunodiffusion; circulating immune complexes were measured by the method of Burton-Kee, Morgan-Kapner & Mowbray (1980).

Sera were taken from 72 patients with liver disease (12 primary biliary cirrhosis; four chronic active hepatitis; seven alcoholic hepatitis; 39 alcoholic cirrhosis; and 10 alcoholic portal fibrosis). The sera were assayed for opsonic activity immediately and then stored at −30°C.

Assay of opsonization. The assay is described in detail elsewhere (Yeaman & Kerr, submitted for publication). Briefly, 150 μl of a suspension of purified peripheral blood PMN in RPMI 1640 ($7·5 \times 10^6$ cells/ml), 10 μl test serum and 50 μl ^{125}I yeast suspension ($3·0 \times 10^7$ cells/ml), were incubated at 37°C for 15 min. Three 50 μl aliquots were removed and layered onto 200 μl of 60% Percoll (Pharmacia) in 0·4 ml polyethylene microfuge tubes. After centrifugation at 10,000 ***g*** for 1 min the bottom of the tubes containing the pelleted yeast was cut off and the radioactivity determined. The PMN and phagocytosed yeast remained at the Percoll/buffer interface. Control tubes contained heat treated normal serum or mixtures without incubation. Results were expressed as % uptake:

$$\frac{\text{total ct/min in yeast sample} - \text{ct/min pelleted yeast} \times 100}{\text{total ct/min in yeast sample}}$$

ELISA for the detection of anti-yeast mannan antibodies. Microtitre wells were coated with yeast mannan (Sigma), 0·5 mg/ml in 0·1 M carbonate buffer, pH 9·6, and incubated overnight at 40°C. The plates were then washed in PBS/0·05% Tween 20, and sera diluted 1 : 100 with PBS were added and incubated for 1·5 h at 37°C. After washing, the plates were developed using affinity-purified, class-specific, alkaline phosphatase-conjugated antibodies (Sigma) with *p*-nitrophenol phosphate as substrate. The release of *p*-nitrophenol which was proportional to the amount of anti-mannan antibody bound was determined from the increase in A_{405} after incubation for 15–30 min.

Fractionation of sera. For the determination of the apparent molecular weight of the heat stable opsonin and the demonstration of monomeric and dimeric IgA anti-yeast mannan activity, sera (10–100 μl) were fractionated using the Fast Protein Liquid Chromatography apparatus (Pharmacia) with a Superose 6 column (HR 10/30) equilibrated in phosphate-buffered saline, pH 7·1, Fractions of 0·5 ml were collected and the protein content of the eluant was monitored continuously by the A_{280}.

For the purification of the heat stable opsonin, a serum sample from patient G.E. (1 ml) was diluted to 5 ml with water and then fractionated by the addition of solid ammonium sulphate (1·45 g) to give a 50% saturated solution. The material precipitated after incubation at 4°C for 1 h was

collected by centrifugation at 23,000 *g* for 30 min. The precipitate was resuspended in 1 ml of 50 mM Tris-HCl, pH 8·0, and the sample passed through a column of Sephadex G-25 (5 ml) equilibrated in the buffer. The protein fraction from the column was then loaded in four 0·5 ml aliquots onto an FPLC monoQ column (HR5/5) equilibrated in the same buffer and after extensive washing of the column the bound proteins were eluted with a linear salt gradient 0–0·5 M NaCl, 50 mM Tris-HCl, pH 8·0. Thirty 1·0 ml fractions were collected.

RESULTS

Yeast opsonization by sera from patients with liver disease. Yeast opsonization by sera from 72 patients with liver disease is shown in Fig. 1. Seven sera showed markedly lower opsonic activity than the majority although they had C3 levels within the normal range. When assayed at the same time, three out of 52 sera from an unselected population of healthy young adults showed this lower level of activity (less than 50% uptake of the added yeast in 15 min). These results are consistent with other reports on the frequency of defective opsonization in the normal population (Soothill & Harvey, 1976; Turner *et al.*, 1978) and the increased incidence of defective yeast opsonization in patients with liver disease (Wyke, Rajkovic & Williams, 1983).

On treatment at 56°C for 1 h the opsonic activity of most of the sera was markedly decreased. However, two sera showed little or no decrease on heat treatment: one (open squares) was from patient M.C., who was described in our previous study (Kerr *et al.*, 1983); the other (open circles) was from patient G.E. Sera from these patients showed similar levels of heat-stable opsonizing activity in all samples available, which in the case of G.E. covered a period of 2 years. These sera were the source of the opsonic protein characterized below. Similar results have also been obtained for the opsonin from patient C.A. from our original study (lupoid hepatitis); serum from the third patient D.R. was no longer available.

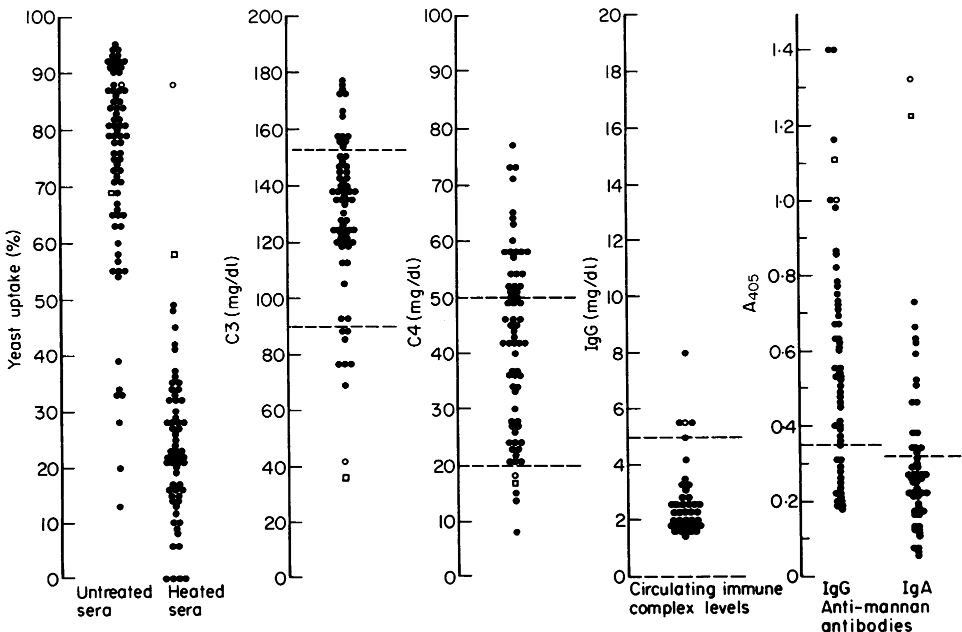

Fig. 1. Immunological parameters for 72 patients with chronic liver disease. Yeast opsonizing activity was measured before and after treatment of the sera at 56°C for 1 h. Sera from patients M.C. (□) and G.E. (○) were used as the source of the heat stable opsonin. Normal levels established in the laboratory are shown by a dashed line.

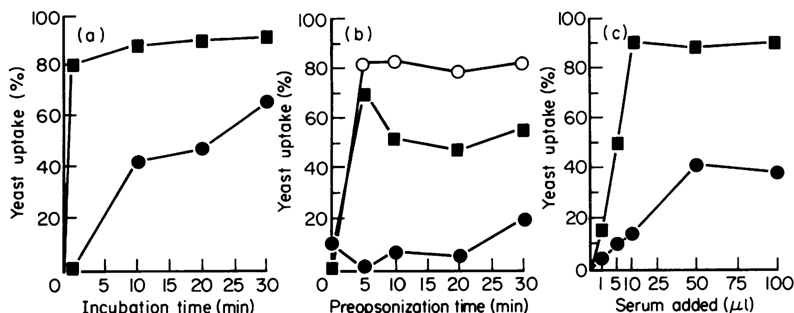

Fig. 2. Time course for uptake by PMN of yeast in the presence of unheated normal serum (■) or serum G.E. (●) heated at 56°C for 1 h. (b) Effect of varying times of preopsonization by normal serum (○), heat treated normal serum (●) or heat treated serum G.E. (■) on the uptake of yeast by PMN in 15 min. (c) Effect of preopsonization by different concentrations of normal serum (■) or heat-treated GE serum (●) on the uptake of yeast by PMN in 15 min.

Sera from patients G.E. and M.C. had very low levels of C3 and C4 and elevated levels of circulating immune complexes. Both patients had severe alcoholic cirrhosis.

Properties of the heat-stable opsonic activity. In the standard assay, the uptake of yeast opsonized with heat-treated sera from patients G.E. or M.C. was slower than yeast opsonized by normal serum, although both eventually reached similar levels (Fig. 2a). Yeast incubated with heat treated normal human sera showed little uptake by PMN in 15 min but on further incubation, when the cells began to settle, some uptake was observed. Microscopic observation of the yeast associated with PMN in all these assays showed that most of the yeast had been internalized and were not merely adherent.

When yeast were preopsonized with normal sera or heat stable opsonic sera before the addition of PMN and further incubation, it was found that in both cases opsonization was optimal in less

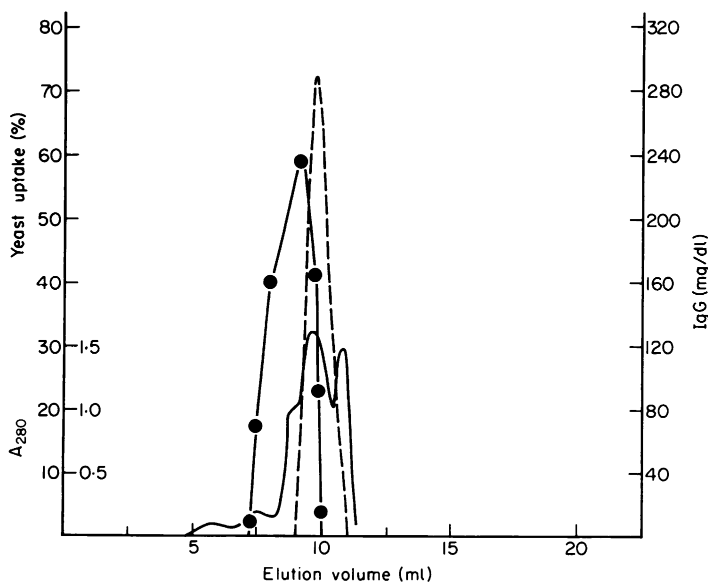

Fig. 3. Gel filtration of a sample (100 μl) of serum G.E. on a column of Superose 6 equilibrated in phosphate buffered saline, pH 7·1. Heat stable opsonic activity (●); IgG (– – – –) and protein, A_{280} concentrations (—) were determined for every fraction.

than 5 min (Fig. 2b). Optimal opsonization with serum G.E. was found at a concentration of approximately 25%, much higher than the optimal 5% observed for the complement-dependent opsonic activity of most normal sera (Fig. 2c).

Molecular characterization of the heat stable opsonin. On gel filtration of serum MC on Sepharose CL-6B or FPLC-Superose 6, opsonic activity eluted as a broad peak with a maximum corresponding to a molecular weight 300–400 kD. Activity tailed into the fractions containing proteins of around 200 kD mol. wt. but only minimal activity was associated with the IgG (150 kD) containing fractions (Kerr *et al.*, 1983). Serum from patient G.E. gel filtered on Superose 6 showed activity in the same fractions but peak activity was associated with the later fractions (app. mol. wt. 180 kD) (Fig. 3).

The heat stable opsonin was precipitated from serum at 50% saturation with ammonium sulphate. When this precipitate was subjected to FPLC ion-exchange chromatography on a MonoQ column, the activity eluted in those fractions containing IgA. The peak of opsonic activity corresponded to those fractions containing most IgA measured by radial immunodiffusion (Fig. 4). Analysis of these fractions by SDS polyacrylamide gel electrophoresis showed them to contain IgA, a trace of IgG and a higher molecular weight protein (Fig. 5). No activity was associated with the fractions, L1-4, containing most of the IgG which passed unretarded through the column.

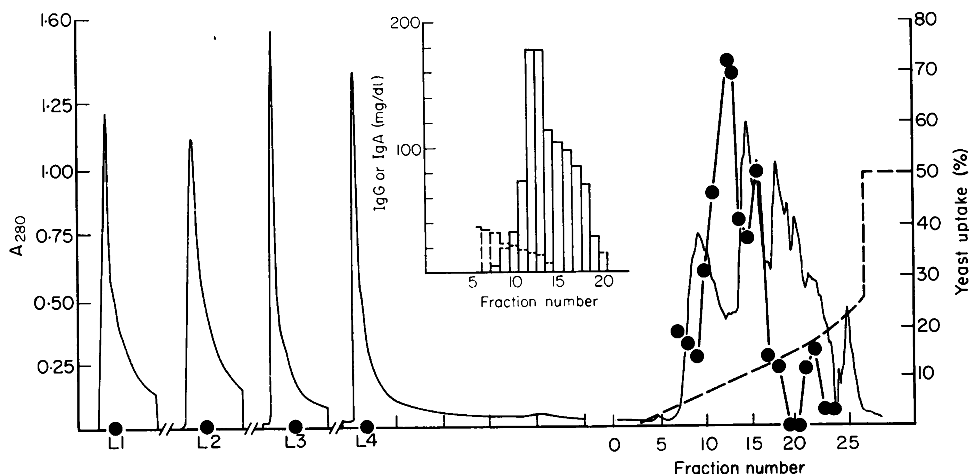

Fig. 4. Ion exchange FPLC-chromatography on MonoQ resin of the proteins precipitable with 50% saturated ammonium sulphate from the serum of patient G.E. The sample and column were equilibrated in 50 mM Tris-HCl, pH 8·0. The sample was loaded in four 0·5 ml aliquots (L1–4) and then, after washing with equilibration buffer, the column was developed with a NaCl gradient from 0–1·0 M (- - - -). The heat stable opsonic activity (●) and protein content, A_{280} (—) of each fraction was determined. The histogram, shows the total IgG (dashed lines) and IgA concentrations (solid lines) in the fractions eluted by the salt gradient.

When the fractions containing most opsonic activity were subjected to gel filtration on a Superose 6 column, the peak protein fractions were shown by SDS polyacrylamide gel electrophoresis to contain only IgA. This IgA preparation was iodinated by the chloramine T method and the radiolabelled protein subjected to autoradiography after gel electrophoresis. Radioactivity was found to be associated only with the alpha and light chains of IgA. When the radiolabelled IgA preparation was incubated with yeast, binding of radioactivity was rapid and proportional to the amount of IgA added. In the presence of an excess of yeast, around 1·5% of total radioactivity would bind. Both the bound and unbound material showed the same distributon of radioactivity on autoradiography of polyacrylamide gels. The binding of radiolabelled IgA was not affected when the assay was carried out in the presence of normal human serum.

Correlation of heat stable opsonic activity with IgA anti-mannan activity. In preliminary experiments using an ELISA to detect binding of different classes of immunoglobulin to whole

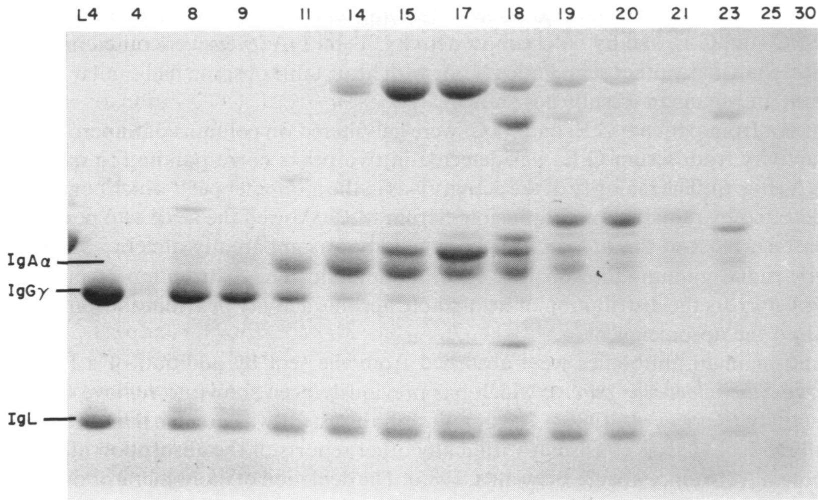

Fig. 5. SDS-polyacrylamide gels (Laemmli, 1974) of individual fractions of proteins in Fig. 4.

yeast, it was observed that sera with highest levels of heat stable opsonin had very high levels of IgA anti-yeast antibodies. Although IgG anti-yeast antibodies were raised in these sera, the levels were lower than those of other sera showing no heat stable opsonic activity.

A solid-phase ELISA assay for the detection of anti-yeast mannan antibodies was developed and used to screen a large number of sera. The results for the 72 sera from patients with liver disease are shown in Fig. 1. The levels of IgG anti-mannan antibodies were raised in most sera relative to

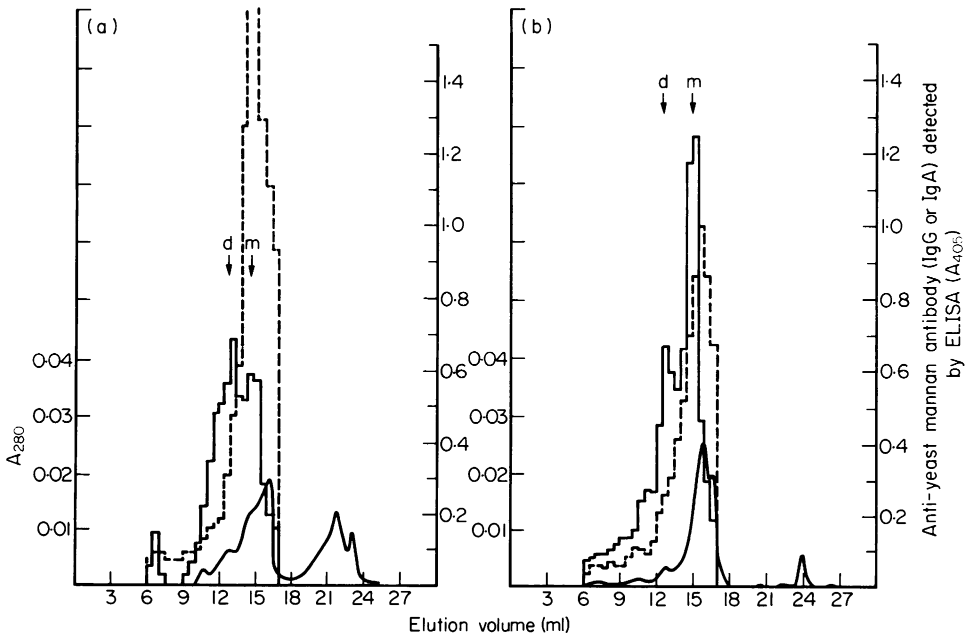

Fig. 6. Gel filtration of samples (10 μl) of sera from patients (a) M.C. and (b) G.E. on columns of Superose 6 equilibrated in phosphate-buffered saline pH 7·1. The column profiles show total protein concentrations in each fraction (—) and the levels of monomeric (m) and dimeric (d) IgA (solid histogram) and IgG (dashed histogram), anti-yeast mannan antibody detected by ELISA (A_{405}) using class specific, alkaline phosphatase-conjugated antibodies.

normal. Several sera showed elevated levels of IgA anti-mannan antibodies, but two sera, those of patients M.C. and G.E. had by far the most activity. Total IgA levels were raised in both patients but were less than many other sera. Patient C.A. with heat stable opsonin had similar elevated levels of IgA yeast anti-mannan (results not shown).

When sera from patients G.E. and M.C. were gel-filtered on columns of Superose 6, IgA anti-mannan activity from serum G.E. was detected in two peaks corresponding to monomeric and dimeric IgA (Fig. 6). The majority of the activity was in the monomer peak which contained most of the IgA detected by radial immunodiffusion. Serum M.C. showed the same two peaks of activity, but in contrast, most of the IgA anti-mannan activity was apparently dimeric. Most of the IgA detected by radial immunodiffuson was again monomeric. The distribution of monomeric and dimeric IgA mirrors the distribution of heat-stable opsonic activity providing strong evidence that IgA is indeed the opsonic agent.

IgA anti-mannan antibodies were absorbed from the sera by addition of a large excess of immobilized *Staphylococcus aureus*, which has previously been shown to remove the heat-stable opsonic activity (Kerr *et al.*, 1983). The amount of absorbent was greater than that necessary to remove all the IgG. This is consistent with many other reports of the absorption of human serum IgA by protein A (Bruin, Faber & Biewenga, 1985). The depletion of monomeric or dimeric IgA and IgG anti-mannan antibodies with protein A and the effects on opsonc activity is currently under more detailed investigation. Anti-mannan antibodies were not absorbed by chromatography on gelatin-Sepharose. Although we had previously suggested that this removed opsonic activity, the loss of activity appears to be due to dilution of the opsonin below the level detectable in the phagocytosis assay, rather than its absorption.

DISCUSSION

Despite its relative abundance, no clearly defined function has been attributed to serum IgA. Selective deficiency of IgA is one of the most frequent immunodeficiencies with a reported frequency as high as 1 in 300 (Clark *et al.*, 1983). Although IgA deficiency has been associated with a range of autoimmune diseases, recurrent infections and atopy, not all deficients are symptomatic (Anman & Hong, 1971). IgA is a poor activator of the complement system and has been shown to block complement dependent immune functions (Griffiss & Goroff, 1983).

Several studies have shown that IgA does not opsonize micro-organisms for phagocytosis by PMN. However, these studies have used either animal serum IgA, human myeloma IgA or human colostral secretory IgA (Wilson, 1972; Zipursky, Brown & Bienenstock, 1973; Magnusson *et al.*, 1979.). We are unaware of reports of similar experiments using specific human serum IgA recognizing yeast or bacterial antigens, reflecting the difficulty of obtaining such antibodies.

The polyclonal increase in serum levels of IgG and IgA is a well-documented feature of alcoholic liver disease. It has been suggested that this increase may be due, in part, to the production of antibodies against food antigens and gut bacteria which are present in the circulation because of abnormal intestinal permeability and the failure of the reticuloendothelial system in the liver (reviewed in MacSween & Anthony, 1981). The results of Nolan *et al.* (1986), who demonstrated raised levels of IgA antibodies against bovine serum albumin and against lipid A, the core lipid of many enteric bacteria, and our own data showing raised levels of IgG and IgA anti-yeast mannan antibodies, are consistent with this hypothesis. These sera are a unique source of serum IgA antibodies of known specificity.

Using such sera we have shown that the heat stable opsonic activity, previously identified in certain pathological sera, is due to serum IgA. Those patients with the highest IgA anti-mannan antibody levels showed highest heat stable opsonic activity. The activity copurified with IgA, even to the extent that serum from patient M.C. with a higher percentage of dimeric IgA anti-mannan showed most opsonic activity in the mol. wt. range 300–400 kD, whereas serum from patient G.E. with a greater proportion of monomeric IgA anti-mannan had opsonin of mol. wt. 180 kD.

In view of the well-documented role of IgG as a heat stable opsonin, it is important to rule out the possibility of IgG contaminating the fractions. In our assay, serum from patients with

considerably higher IgG anti-mannan antibody levels than M.C. or G.E. did not exhibit heat stable opsonic activity. Furthermore, neither the IgG containing fractions from gel filtration of the two sera nor IgG purified by ion exchange chromatography from the sera showed opsonic activity. When the purified IgG was radiolabelled with ^{125}I, binding to yeast was detectable. Although it is difficult to rule out the possibility of a small trace of IgG in the IgA preparations, it is most unlikely that it is a major factor in the observed activity.

Several reports have shown IgA to affect PMN function. Van Epps, Strickland & Williams (1975) demonstrated an inhibitor of PMN chemotaxis in the serum of patients with liver disease, which was subsequently identified as polymeric IgA. The same group (Van Epps & Williams, 1976; Van Epps & Brown, 1981) demonstrated that several human IgA myeloma proteins inhibited the chemotaxis and chemotactic peptide-induced chemiluminescence of PMN. This inhibition was mediated by the Fc part of the molecule and was potentiated by heating and aggregation.

Wilton (1978) reported that both normal serum IgA and secretory IgA inhibited the binding to PMN and phagocytosis of *Candida* opsonized with IgG and complement, but not the binding of *Candida* opsonized with complement alone. This inhibition which was also enhanced by heat aggregation, was apparently due to the binding of the IgA to the PMN, since surface IgA could be detected by immunofluorescence using FITC labelled anti-IgA: binding of IgA to the yeast could not be detected. Although the biological significance of these inhibition reactions is unknown, they do suggest the presence of specific receptors for IgA on the PMN surface.

Fanger and colleagues (Fanger *et al.*, 1980; Fanger, Pugh & Bernier, 1981; Fanger, Golstine & Shen, 1983) have identified Fcα-receptors on PMN and monocytes by cytofluorographic analysis of the binding FITC-labelled IgA myeloma protein and by rosette formation using rabbit secretory IgA-sensitized ox erythrocytes. Similar results using human myeloma IgA coated red cells were obtained by Walsh & Kay (1986). Fanger *et al.* (1983) also reported that PMN from the oral cavity expressed more Fcα-receptors than blood PMN and that only the former were capable of phagocytosing target cells coated with IgA alone. IgA mediated phagocytosis was also shown by Henson, Johnson & Spiegelberg (1972) who demonstrated that the rate of uptake of aggregated IgA myeloma proteins and subsequent release of granule enzymes was similar to that of IgG aggregates.

Our present data suggest a possible role for serum IgA in the removal of micro-organisms by PMN phagocytosis indicating the need for a more detailed study of the interaction of serum IgA with PMN. The results suggest that species specificity and a need for Fc portion unbound to secretory piece might be prerequisite for opsonic activity. The possibility that this phenomena is specific for yeast, which can bind to and be phagocytosed by PMN in the absence of opsonin, cannot be overlooked.

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REFERENCES

ANMAN, A.J. & HONG, R. (1971) Selective IgA deficiency: presentation of 30 cases and review of the literature. *Medicine* **50**, 223.

BRUIN, G., FABER, A. & BIEWENGA, J. (1985) Binding of human IgA fragments to Protein A-Sepharose studied by an ELISA method. *Scand. J. Immunol.* **21**, 49.

BURTON-KEE, J., MORGAN-KAPNER, P. & MOWBRAY, J.F. (1980) Nature of circulating immune complexes in infective endocarditis. *J. clin. Pathol.* **33**, 653.

CLARK, J.A., CALLICOAT, P.A., BRENNER, N.A., BRADLEY, C.A. & SMITH, D.M. (1983) Selective IgA deficiency in blood donors. *Am. J. Clin. Pathol.* **80**, 210.

ENGLISH, D. & ANDERSON, B.R. (1974) Single-step separation of red blood cells, granulocytes and mononuclear leukocytes on discontinuous density gradients of Ficoll-Hypaque. *J. immunol. Meth.* **5**, 249.

FANGER, M.W., SHEN, L., PUGH, J. & BERNIER, G.M. (1980) Subpopulations of human peripheral granulocytes and monocytes express receptors for IgA. *Proc. natn Acad. Sci. USA* **77**, 3640.

FANGER, M.W., GOLDSTINE, S.N. & SHEN, L. (1983) Cytofluorographic analysis of receptors for IgA on human polymorphonuclear cells and monocytes and the correlation of receptor expression with phagocytosis. *Mol. Immunol.* **20**, 1019.

FANGER, M.W., PUGH, J. & BERNIER, G.M. (1981) The

specificity of receptors for IgA on human peripheral polymorphonuclear cells and monocytes. *Cell Immunol.* **60,** 324.

GRIFISS, J.M. & GOROFF, D.K. (1983) IgA blocks IgM and IgG initiated immune lysis by separate molecular mechanisms. *J. Immunol.* **130,** 2882.

HENSON, P.M., JOHNSON, H.B. & SPIEGELBERG, H.L. (1972) The release of granule enzymes from human neutrophils stimulated by aggregated immunoglobulin of different classes and sublcasses. *J. Immunol.* **109,** 1182.

KEMP, A.S. & TURNER, M.W. (1986) The role of opsonins in vacuolar sealing and the ingestion of zymosan by human neutrophils. *Immunology* **59,** 69.

KERR, M.A., FALCONER, J.S., BASHEY, A. & SWANSON BECK J. (1983) The effect of C3 levels on yeast opsonization by normal and pathological sera: identification of a complement independent opsonin. *Clin. exp. Immunol.* **54,** 793.

KOZEL, T.R., HIGHISON, B. & STRATTON, C.J. (1984) Localization on encapsulated *Cryptococcus neoformans* of serum components opsonic for phagocytosis by macrophages and neutrophils. *Infect. Immun.* **43,** 574.

LACHMANN, P.J. & HOBART, M.J. (1978) Complement technology. In *Handbook of Experimental Immunology* 3rd. edn (Ed. by D.M. Weir) Vol. 1, p. 5.8, Blackwell Scientific, Oxford.

LAEMMLI, U.K. (1974) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* **227,** 680.

MACSWEEN, R.N.M. & ANTHONY, R.S. (1985) Immune mechanisms in alcoholic liver disease. In *Alcoholic Liver Disease: Pathobiology, Epidemiology and Clinical Aspects.* (ed. P. Hall) p. 68 Edward Arnold, London.

MAGNUSSON, K.-E, STENDAHL, O., STJERNSTROM, I. & EDEBO L. (1979) Reduction of phagocytosis, surface hydrophobicity and charge of *Salmonella typhimurium* 395 MR10 by reaction with secretory IgA (SIgA) *Immunology* **36,** 439.

NEWMAN, S.L. & JOHNSTON JR. R.B. (1979) Role of binding through C3b and IgG in polymorphonuclear neutrophil function; studies with trypsin generated C3b. *J. Immunol.* **123,** 1839.

NOLAN, J.P., DELISSIO, M.G., CAMARA, D.S., FIEND, D.M. & GAGLIARDI, N.C. (1986) IgA antibody to lipid A in alcoholic liver disease. *Lancet* **i,** 176.

QUIE, P.G. (1972) Bactericidal function of human polymorphonuclear leukocytes. *Pediatrics* **50,** 264.

ROOS, D., BOT, A.A.M., VAN SCHAIK, M.L.J., DE BOER, M. & DAHA, M.R. (1981) Interaction between human neutrophils and zymosan particles: the role of opsonins and divalent cations. *J. Immunol.* **126,** 433.

ROSS, G.D., CAIN, J.A. & LACHMANN, P.J. (1985) Membrane complement receptor type three (CR3) has lectin like properties analogous to bovine conglutinin and functions as a receptor for zymosan and rabbit erythrocytes as well as a receptor for iC3b. *J. Immunol.* **134,** 3307.

SCRIBNER, D.J. & FAHRNEY, D. (1976) Neutrophil receptors for IgG and complement: their roles in attachment and ingestion phases of phagocytosis. *J. Immunol.* **116,** 892.

SHARON, N. (1984) Surface carbohydrates and surface lectins are determinants in phagocytosis. *Immunology Today,* **5,** 143.

SOOTHILL, J.F. & HARVEY, B.A.M. (1976) Defective opsonization. A common immunity defficiency. *Arch. dis. Child* **51,** 91.

TURNER, M.W., MOWBRAY, J.F., HARVEY, B.A.M., BROSTOFF, J., WELLS, R.S. & SOOTHILL, J.F. (1978) Defective yeast opsonization and C2 deficiency in atopic patients. *Clin. exp. Immunol,* **34,** 253.

VAN EPPS, D.E., STRICKLAND, R.G. & WILLIAMS, R.C. JR. (1975) Inhibition of leukocyte chemotaxis in alcoholic liver disease. *Am. J. Med.* **59,** 200.

VAN EPPS, D.E. & WILLIAMS, R.C. JR. (1976) Suppression of leukocyte chemotaxis by human IgA myeloma components. *J. exp. Med.* **144,** 127.

VAN EPPS, D.E. & BROWN, S.L. (1981) Inhibition of formylmethionylleucylphenylalanine-stimulated neutrophil chemiluminescence by human immunoglobulin A paraproteins. *Infect. Immun.* **34,** 864.

WALSH, G.M. & KAY, A.B. (1986) Binding of immunoglobulin classes and subclasses to human neutrophils and eosinophils. *Clin. exp. Immunol.* **63,** 466.

WILSON, I.D. (1972) Studies on the opsonic activity of human secretory IgA using in vitro phagocytosis system. *J. Immunol.* **108,** 726.

WILTON, J.M.A. (1978) Suppression by IgA of IgG mediated phagocytosis by human polymorphonuclear leucocytes. *Clin. exp. Immunol.* **34,** 423.

WYKE, R.J., RAJKOVIC, I.A. & WILLIAMS, R. (1983) Impaired opsonization by serum from patients with chronic liver disease. *Clin. exp. Immunol.* **51,** 91.

YEAMAN, G.R. & KERR, M.A. (1986) A radiometric assay for the measurement of yeast opsonization and phagocytosis by polymorphonuclear leucocytes. *J. immunol. Meth.* Submitted for publication.

YOUNG, L.S. & ARMSTRONG, D. (1972) Human immunity to *Pseudomonas aeruginosa* 1. In vitro interaction of bacteria, polymorphonuclear leukocytes and serum factors. *J. infect. Dis.* **126,** 257.

ZIPURSKY, A., BROWN, E.J. & BIENENSTOCK, J. (1973) Lack of opsonization potential of 11S human secretory IgA. *Proc. Soc. exp. Biol.* **142,** 181.