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The specificity of the IgA receptor purified from human neutrophils

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A receptor for IgA was purified from human polymorphonuclear neutrophils (PMN) by affinity chromatography on human serum IgA–Sepharose. The receptor appeared on SDS/polyacrylamide gels as a diffuse band with an apparent molecular mass of 50–70 kDa, whether reduced or non-reduced. During purification, the protein showed remarkable stability to proteolytic digestion by endogenous PMN proteinases. Purified radioiodinated receptor re-bound to IgA–Sepharose, but not to IgG–Sepharose or BSA–Sepharose. The binding of the receptor to IgA–Sepharose was inhibited in a dose-dependent manner by human serum IgA1 or IgA2 or secretory IgA1 or IgA2, but not by IgG or IgM. Binding of receptor to IgA–Sepharose was also inhibited by the Fc fragment of IgA, but not by the Fab fragment. An IgA fragment produced by digestion with pepsin which lacks the CH3 domain also inhibited binding, but to a more limited extent than did the whole IgA molecule.

INTRODUCTION

Polymorphonuclear neutrophils (PMN) have Fc receptors for immunoglobulins on their surface. These receptors enable the PMN to ingest foreign particles coated with specific immunoglobulins. Three different receptors for IgG have been purified from human leucocytes. FcγRI (CD64; 72 kDa) is a high-affinity (5×10^8 M^{-1}) receptor found on monocytes, and it is inducible on PMN following treatment with γ-interferon [1]. FcγRII (CD32; 40 kDa) is a medium-affinity (approx. 10^6 M^{-1}) receptor found on PMN, macrophages, B cells and eosinophils [2,3]. FcγRIII (CD16; 50–70 kDa) is a low-affinity receptor which binds poorly to monomer IgG but, on the cell surface, binds well to aggregated IgG. It is found on PMN, macrophages and natural killer cells [4]. Each of these receptors has been cloned and studied extensively [5]. High-affinity receptors for IgE are found on mast cells and basophils, but not on neutrophils. An unrelated, low-affinity IgE receptor (CD23) is found on B cells, activated macrophages and eosinophils, but not on neutrophils. Both of these receptors have also been studied extensively [6,7]. Fc receptors for IgA have received less attention [8,9].

IgA receptors on neutrophils were first demonstrated by Fanger and colleagues by rosetting with IgA-coated erythrocytes [10,11]. These techniques allowed study of the cellular distribution and specificity of interaction of IgA with PMN [12]. They also showed that the expression of IgA receptor on peripheral blood neutrophils was apparently enhanced by overnight incubation with IgA [13] and that oral neutrophils expressed more IgA receptor per cell than did blood neutrophils, and were capable of phagocytosing target cells coated with IgA alone. In functional studies using blood PMN, IgA receptors appeared to co-operate with receptors for IgG in enhancing the phagocytosis of target cells coated with IgG and IgA [13]. Other studies have also shown that the expression of IgA receptors can be controlled by external factors. For example, granulocyte/monocyte colony-stimulating factor and granulocyte colony-stimulating factor, but not interleukin 3, have been shown to induce a change from low- to high-affinity receptors on PMN within 30 min, a change associated with the development of IgA-mediated phagocytosis [14]. Receptors for IgA have also been detected on monocytes [10,15], macrophages [16] and lymphocytes [17].

The function of the IgA receptor on human PMN remains unclear. There has been conflicting evidence concerning the ability of IgA to stimulate phagocytosis, although more recent papers do suggest that IgA can act as an opsonin. Our own studies [18] have shown that zymosan particles coated with specific IgA antibodies purified from the sera of patients with liver or inflammatory bowel disease were phagocytosed as efficiently as zymosan coated with IgG purified from the same sera. Aggregated IgA has been shown to cause degranulation of neutrophils and to inhibit chemotaxis [19,20]. Recently, a monoclonal antibody of the IgM type which blocks the IgA receptor on monocytes has been reported [21].

We have previously reported the isolation of a radioiodinated IgA receptor from solubilized neutrophil membranes by affinity chromatography on IgA–Sepharose [22]. When the membrane extract was chromatographed on IgG–Sepharose under the same conditions, FcγRII was isolated [22]. The IgA receptor appeared on SDS/polyacrylamide gels as a broad band of apparent molecular mass 50–70 kDa. This finding has recently been confirmed by Monteiro *et al.* [23], who also demonstrated the presence of the same receptor on monocytes and U937 cells. In the present study, we have investigated further the purification of the IgA receptor (FcRα) by this technique. We have used an assay depending on inhibition of the binding of the purified receptor to IgA–Sepharose to study the specificity of the receptor.

MATERIALS AND METHODS

Preparation of PMN

PMN were isolated from the peripheral venous blood of normal donors by a modification of the method of English & Anderson [24] as previously described [22]. Between 1×10^6 and 1.5×10^6 PMN were obtained per ml of blood, with less than 1 %

Abbreviations used: CD, cluster of differentiation antigens defined by International Leucocyte Workshops; DFP, di-isopropyl fluorophosphate; PBS, phosphate-buffered saline (8.5 mM-sodium phosphate/0.15 M-NaCl, pH 7.1); PMSF, phenylmethanesulphonyl fluoride; PMN, polymorphonuclear neutrophils; RID, radial immunodiffusion.

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red blood cell contamination. PMN were then resuspended in phosphate-buffered saline (PBS).

Cell-surface radioiodination

PMN were surface-labelled at 20 °C using the lactoperoxidase/glucose oxidase method as described by Kulczycki *et al.* [25], except that 1 mCi of ^{125}I was used per 1×10^8 PMN. Washed radiolabelled PMN were solubilized at 4 °C in PBS containing 1% (v/v) Nonidet-40 and, unless indicated, 20 mM-phenylmethanesulphonyl fluoride (PMSF). The solubilized PMN were centrifuged at 25000 *g* for 30 min and the Nonidet P-40 extract was removed for receptor purification.

Purification of immunoglobulins

Unless stated otherwise, all procedures were carried out at 4 °C. Immunoglobulins were purified from normal human serum by precipitation with ammonium sulphate at 50% saturation. The precipitated proteins were pelleted by centrifugation at 25000 *g* for 1 h and the pellet was resuspended in a minimal volume of distilled water. The proteins were then subjected to gel filtration on Sepharose 6B (Pharmacia, Milton Keynes, U.K.) equilibrated in 50 mM-Tris/HCl, pH 8.0. IgM-containing fractions, as determined by radial immunodiffusion assay (RID), were concentrated by ultrafiltration and purified further by gel filtration on a Superose 6 HR 10/30 f.p.l.c. column (Pharmacia). IgA- and IgG-containing fractions from the first gel filtration column were pooled and subjected to ion-exchange chromatography on a DEAE-Sephacel (Pharmacia) column (10 cm × 4.4 cm) equilibrated in 50 mM-Tris/HCl, pH 8.0. The material which did not bind to the DEAE-Sephacel column was found to be pure IgG, as determined by SDS/PAGE and RID. The DEAE-Sephacel column was washed thoroughly with 50 mM-Tris/HCl, pH 8.0, and the bound material was eluted with a linear salt gradient (0–0.5 M-NaCl) in the same buffer. IgA-containing fractions, eluting between 0.1 and 0.2 M-NaCl, were pooled and applied to a column (7.5 cm × 2.5 cm) of Jacalin–Agarose (Vector Laboratories, Peterborough, U.K.) equilibrated in PBS.

The IgA1 bound to the Jacalin–Agarose column was eluted with PBS containing 1 M-D-galactose and was found to be homogeneous by SDS/PAGE. The material which did not bind to Jacalin–Agarose contained many proteins, including IgA2 and a small amount of IgG which was removed by passage through a column (10 cm × 1 cm) of Protein G–Sepharose (Pharmacia). The unbound proteins were equilibrated by gel filtration into 50 mM-sodium acetate buffer, pH 4.5, and then subjected to cation-exchange chromatography on an HR 5/5 f.p.l.c. Mono S column (Pharmacia) equilibrated in the same buffer. Bound proteins were eluted with a linear gradient from 0 to 0.5 M-NaCl in the same buffer. The IgA2-containing fractions which eluted between 0.03 and 0.05 M-NaCl were found to contain only minor contaminants. These earlier fractions were pooled and further purified to homogeneity (as judged by SDS/PAGE) by gel filtration on an f.p.l.c. Superose 6 HR 10/30 column (Pharmacia). The identities of serum IgA1 and IgA2 were confirmed by immunodot-blotting using human isotype-specific mouse monoclonal antibodies: 2D7 [anti-(A1 and A2)], NIF2 (anti-A1) and 2E2 (anti-A2), all of which were kindly donated by Dr. R. Jefferis, University of Birmingham, Birmingham, U.K.

Secretory IgA1 and secretory IgA2 were purified from human colostrum as previously described [26]. Briefly, human colostrum was centrifuged at 30000 *g* for 2 h to remove fat, and then acidified and centrifuged at 30 000 *g* for 1 h to remove casein. The supernatant was subjected to gel filtration on Sepharose 6B and then anion-exchange chromatography on DEAE-Sephacel, as described above for serum IgA. Pure IgA, as judged by SDS/PAGE, was eluted between 0.15 and 0.2 M-NaCl on the linear salt gradient. Secretory IgA1 and IgA2 were then separated by affinity chromatography on Jacalin–Agarose and the identities of the two IgA subtypes were confirmed by immunodot-blotting as described above for serum IgA.

Preparation of fragments of IgA1

Serum IgA1 Fc and Fab fragments were prepared by cleaving purified IgA1 with a crude IgA1 proteinase preparation from *Neisseria gonorrhoeae*, obtained as described previously [27]. IgA1 proteinase (0.2 ml) was incubated with 3 mg of serum IgA1 in 1 ml of 50 mM-Tris/HCl, pH 8.0, at 37 °C for 72 h. Fc and Fab fragments were then separated by gel filtration on f.p.l.c. columns of Superose 6 (HR 10/30) and Superose 12 (HR 10/30) columns (Pharmacia) arranged in series. The identities of the Fc and of Fab fragments were confirmed by RID using rabbit anti-(human IgAα) chain-specific antibodies (Dakopatts, Copenhagen, Denmark) and rabbit anti-(human immunoglobulin light chain) antibodies (Sigma, Poole, Dorset, U.K.).

$F(abc')_2$ fragment was prepared by pepsin digestion of serum IgA1 as described by Mestecky & Kilian [28]. Briefly, 3 mg of serum IgA1 was incubated in 1 ml of 0.1 M-sodium acetate buffer, pH 4.5, with 2 mg of porcine pepsin (Koch–Light, Haverhill, Suffolk, U.K.) at 37 °C for 16 h. The $F(abc')_2$ fragment was purified by gel filtration on an f.p.l.c. Superose 6 HR 10/30 column equilibrated in 50 mM-Tris/HCl, pH 8.0. The peak fraction when subjected to SDS/PAGE showed a single protein (140 kDa), in agreement with the original report [28].

Preparation of immunoadsorbents and other protein adsorbents

IgA and IgG were each coupled to Sepharose 4B (Pharmacia) at 10 mg of protein/ml of Sepharose. BSA (Fraction V; Sigma) was also coupled at 10 mg/ml of Sepharose using the cyanogen bromide method, essentially as described by Kulczycki [29]. For the preparation of IgA–Sepharose, IgA was equilibrated in 0.2 M-$NaHCO_3$ (pH 8.5)/0.5 M-NaCl before coupling.

Purification of the IgA receptor

Unless otherwise stated, all steps were carried out at 4 °C. The IgA receptor was purified by affinity chromatography on an IgA–Sepharose column using a modification of a method described previously [22]. Briefly, Nonidet P-40 extracts of radioiodinated PMN were precleared by incubation with 0.2 ml of BSA–Sepharose in a Microfuge tube for 30 min. The mixture was then transferred to a small plastic column, the effluent was collected and then the column was washed with 1 ml of PBS containing 1% (v/v) Nonidet P-40. The effluent and wash were then pooled and incubated with 0.2 ml of IgA–Sepharose on a rotating shaker for 16 h. The incubation mixture was then transferred to a small plastic column (2.5 cm × 1 cm), and washed with 20 ml of 0.2 M-$NaHCO_3$ buffer, pH 8.5, containing 0.5 M-NaCl and 1% Nonidet P-40. The receptor was eluted in successive 1 ml portions of 0.5 M-acetic acid containing 1% Nonidet P-40 and each eluate was rapidly neutralized with 0.36 ml of 2 M-Tris/HCl, pH 8.7/1% Nonidet P-40. IgG receptors were isolated by a similar technique using IgG–Sepharose as the affinity resin.

Assessing the ligand-binding specificity of the IgA receptor

IgA–Sepharose or any other protein–Sepharose used in these experiments was first incubated in 5 vol. of a 10 mg/ml solution of BSA in 0.2 M-$NaHCO_3$ (pH 8.5)/0.5 M-NaCl/1% Nonidet P-40 for 30 min, and then washed with 15 vol. of the same buffer. The IgA–Sepharose was then divided into 10 μl portions and mixed with 40 μl of underivatized Sepharose 4B to give a visible pellet. Purified radioiodinated IgA receptor was preincubated

with or without potential inhibitors diluted in the above buffer containing 3 mg of BSA/ml for 2 h after adjusting the final volume to 500 μl and the BSA blocking protein concentration to 0.3 mg/ml. The mixture was Microfuged for 30 s at 2800 *g* and the supernatant was removed. The resin was then washed twice with bicarbonate/Nonidet P-40 buffer and the three supernatants were pooled and the radioactivity determined.

Immunoprecipitation of FcγRIII from PMN

A Nonidet P-40 extract of radioiodinated PMN, precleared with BSA–Sepharose as described above, was incubated with 15 μl of an anti-FcγRIII monoclonal antibody for 16 h at 4 °C. (The monoclonal antibody was clone BW 209/2, anti-CD16, provided by the 4th International Leucocyte Workshop.) Goat anti-(mouse IgG)–Sepharose (0.2 ml) pre-equilibrated in PBS containing 0.2% Nonidet P-40 was then added to the incubation mixture. [The goat anti-(mouse IgG) was purchased from Sigma and coupled to Sepharose 4B as described above.] Incubation was continued for a further 2 h on a rotary shaker at 4 °C. The incubation mixture was then transferred to small plastic column and the column effluent was removed. The goat anti-(mouse IgG)–Sepharose was washed first with 10 ml of PBS containing 0.2% Nonidet P-40, then with 1 ml of 20 mM-sodium phosphate (pH 7.4)/0.2% Nonidet P-40/0.75 M-NaCl, and finally with 5 ml of PBS/0.2% Nonidet P-40. The column was eluted with five successive portions (263 μl) of 0.5 M-acetic acid containing 0.2% Nonidet P-40 and each elution was neutralized with 188 μl of 1 M-Tris (pH 8.7)/0.2% Nonidet P-40.

SDS/polyacrylamide-gel electrophoresis

SDS/PAGE was carried out by the method of Laemmli [30] as previously reported [22]. Unless otherwise stated, gels were 5–15% linear gradient slab gels. Samples were denatured in sample buffer containing 0.1 M-Tris, 8 M-urea and 2% SDS, pH 8.0, and either 80 mM-dithiothreitol (reduced samples) or 40 mM-iodoacetamide (non-reduced samples). Gels were dried and subjected to autoradiography as previously described [26]; typically $(0.5–3) \times 10^3$ c.p.m. were loaded on to each lane. Samples requiring concentration were first precipitated with 20% trichloroacetic acid and washed with ice-cold acetone before preparation for electrophoresis. The following molecular mass markers (Sigma) were used: myosin, 205 kDa; β-galactosidase, 116 kDa; phosphorylase *b*, 97.4 kDa; BSA, 66 kDa; egg albumin, 45 kDa; carbonic anhydrase, 29 kDa.

RESULTS

Affinity purification of IgA receptor

PMN (6×10^7) were surface-labelled as described in the Materials and methods section. After solubilizing the PMN in 1 ml of PBS/1% Nonidet P-40/20 mM-PMSF, the Nonidet P-40 extract was divided into two equal portions. One portion was subjected to affinity chromatography on IgA–Sepharose and the other on IgG–Sepharose. When analysed by SDS/PAGE and autoradiography, the purified IgA receptor appeared as a single diffuse band with a molecular mass of between 50 and 70 kDa (Fig. 1, lane 1) as previously reported [22]. The IgG–Sepharose (Fig. 1, lane 2) bound predominantly one protein (34 kDa) corresponding to the proteolytic fragment of FcγRII reported by Kulczycki [31] and demonstrated previously in this laboratory [22]. There was no evidence of the FcγRIII, probably due to the low affinity of this receptor. It was observed that the yield, in terms of radioactivity, of the IgA receptor was similar to that of FcγRII. The apparent molecular mass of the IgA receptor was the same when run either reduced or non-reduced on SDS/PAGE (Fig. 1, lanes 3 and 4).

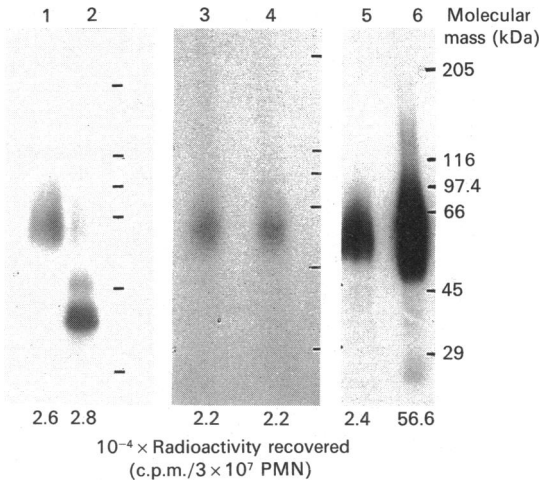

Fig. 1. SDS/polyacrylamide gels of purified IgA and IgG receptors

Radiolabelled PMN membrane proteins were solubilized in PBS/1% Nonidet P-40/20 mM-PMSF, and after preclearing with BSA–Sepharose, were incubated at 4 °C for 16 h with IgA–Sepharose (lanes 1, 3, 4 and 5), IgG–Sepharose (lane 2) or monoclonal anti-FcRγIII followed by goat anti-(mouse IgG)–Sepharose (lane 6). After washing, the columns were eluted with 0.5 M-acetic acid/1.0% Nonidet P-40 and the eluates were counted for radioactivity and aliquots were analysed by SDS/PAGE and autoradiography. Lane 4 was run under non-reducing conditions; other lanes were under reducing conditions. Molecular mass markers are defined in the Materials and methods section.

In a separate experiment, 6×10^7 radioiodinated PMN were solubilized in 1% Nonidet P-40 and divided into two equal portions. The IgA receptor was affinity-purified on IgA–Sepharose from one portion and FcγRIII was immunoprecipitated with an anti-CD16 monoclonal antibody from the other portion. The radioactivity of the IgA receptor and in the FcγRIII immunoprecipitate was counted and the proteins were analysed by SDS/PAGE and autoradiography. Although both the IgA receptor and FcγRIII appeared as broad bands of similar mobility, FcγRIII was consistently a broader band when similar amounts of radioactivity were loaded on to the gel. Furthermore, the amount of radioactivity recovered for the IgA receptor was over 20-fold less than for FcγRIII.

Effects of different proteinase inhibitors on purification of the IgA receptor

Radioiodinated PMN were solubilized in 1% Nonidet P-40 in the absence or presence of different proteinase inhibitors, added either singly or in combination. When the IgA receptor was affinity-purified from these extracts it was observed that the mobility of the receptor on SDS/PAGE changed marginally, from that of a protein with a molecular mass of approx. 50 kDa when no proteinase inhibitors were added, to around 55 kDa where one or more proteinase inhibitors were added (Fig. 2, lanes 1–4). When IgG receptors were affinity-isolated under the same conditions, FcγRII was seen to change in mobility on SDS/PAGE (Fig. 2, lanes 5–8) from a protein of 31 kDa (no proteinase inhibitors added) to one of 34 kDa (one or more proteinase inhibitors added), consistent with published results [31]. Remarkably, the highest yield of each receptor was obtained in the absence of any proteinase inhibitors. In the presence of iodoacetamide, a minor contaminant protein of 40 kDa was consistently detected in the IgA receptor preparation. These results suggest that the IgA receptor is highly stable to proteolysis by endogenous neutrophil proteinases.

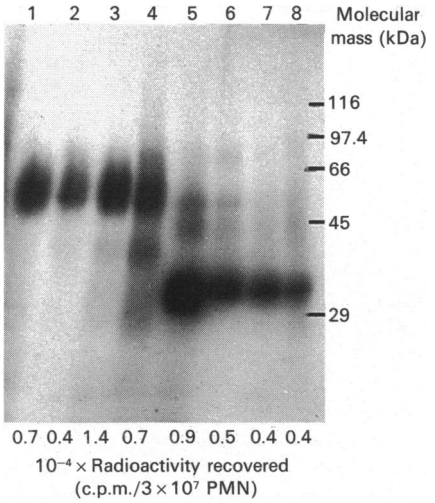

Fig. 2. Effects of different proteinase inhibitors on purification of IgA receptor and FcγRII by affinity chromatography

Autoradiographs of SDS/PAGE of IgA receptor (lanes 1–4) or FcγRII (lanes 5–8) purified by affinity chromatography on IgA–Sepharose or IgG–Sepharose respectively from ^{125}I-surface-labelled PMN. The PMN were solubilized in PBS/1% Nonidet P-40 in the presence of no proteinase inhibitors (lanes 1 and 5); 20 mM-PMSF (lanes 2 and 6); 10 mM-DFP plus 20 mM-PMSF (lanes 3 and 7); or 10 mM-DFP plus 20 mM-PMSF plus 100 mM-iodoacetamide (lanes 4 and 8).

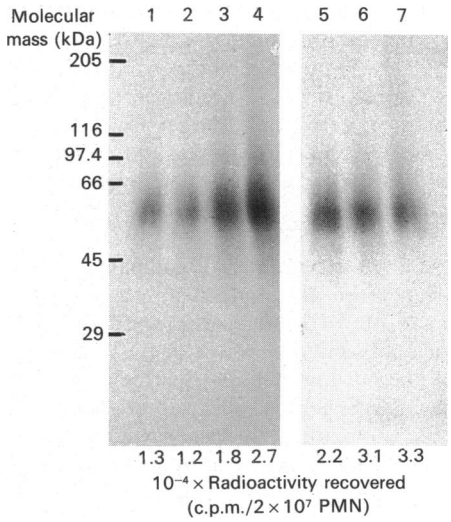

Fig. 3. Effects of time and temperature of incubation of PMN Nonidet P-40 extract with IgA–Sepharose on the recovery of IgA receptor

^{125}I-surface radiolabelled PMN (2×10^7), solubilized and precleared on BSA–Sepharose as described in the Materials and methods section, were each incubated with 0.2 ml of IgA–Sepharose for 2 h (lane 1), 4 h (lane 2), 8 h (lane 3) or 16 h (lanes 4–7) at 4 °C (lanes 1–5), 23 °C (lane 6) or 37 °C (lane 7). Acid eluates from each incubation were counted for radioactivity and then analysed by SDS/PAGE and autoradiography.

Table 1. Determination of the ligand-binding specificity of purified radiolabelled IgA receptor

For measurement of radioactivity added and radioactivity bound, each experiment was done in triplicate using 0.02 ml of resin. Specific binding was calculated after subtraction of the radioactivity which bound to BSA–Sepharose. Receptor binding (%) is expressed relative to the radioactivity bound in the absence of competing immunoglobulin. Abbreviations: S, Sepharose; h.a., heat-aggregated.

Resin used	Inhibitor used	Radioactivity added (counts/5 min)	Radioactivity bound (counts/5 min)	Radioactivity bound (%) (means ± S.D.)	Specific radioactivity bound (%)	Receptor binding (%)
BSA–S	None	5185	0	0.9 ± 1.5	0.0	
		5695	150			
		5605	0			
IgG–S	None	6170	150	2.5 ± 0.5	1.6	
		6010	180			
		5180	110			
IgA–S	None	5945	2146	36.2 ± 0.4	35.3	100.0
		6145	2255			
		6105	2190			
IgA–S	IgA1 (0.1 mg)	5240	765	13.8 ± 3.4	12.9	36.6
		5505	950			
		5115	510			
IgA–S	IgA1 (1.0 mg)	5265	325	5.3 ± 0.9	4.4	12.5
		4850	195			
		5045	305			
IgA–S	IgA1 (3.0 mg)	5415	180	2.5 ± 0.8	1.6	4.6
		5025	125			
		4680	80			
IgA–S	IgA1 (3.0 mg) (h.a.)	4997	180	4.3 ± 1.1	3.5	9.9
		5570	306			
		5210	193			
IgA–S	IgG (3.0 mg)	5785	2100	38.0 ± 1.4	36.5	100.0
		5515	2125			
		5150	2005			
IgA–S	IgG (3.0 mg) (h.a.)	5260	1936	38.0 ± 1.1	36.5	100.0
		5112	1994			
		5348	2054			

Effects of different conditions on the yield of IgA receptor

When solubilized PMN were incubated with IgA–Sepharose at 4 °C for various times, the yield of IgA receptor was seen to increase with increasing length of incubation time, routinely reaching a plateau at around 16–18 h. The results of one such experiment are shown in Fig. 3 (lanes 1–4). For this reason, the incubation time for routine receptor purification was increased to 18 h, giving improved yields compared with our previously reported method [22].

When the packed volume of IgA–Sepharose was progressively increased from 0.025 ml to 0.40 ml, there appeared to be no corresponding increase in the yield of IgA receptors that could be purified from 1×10^7 radiolabelled cells, suggesting that all of the receptor had been bound even at the lowest amount of IgA–Sepharose used. Similarly, when ^{125}I-surface-labelled PMN were solubilized in Nonidet P-40 in the presence of PMSF and di-isopropyl fluorophosphate (DFP) and the IgA receptor was affinity-purified at three different temperatures (4 °C, 23 °C and 37 °C), no difference either in yield of receptor or its mobility on SDS/PAGE gels was observed (Fig. 3, lanes 5–7). These results confirm the marked stability of the IgA receptor and suggest that a single incubation of membrane extract with IgA–Sepharose under these conditions is sufficient to bind all of the receptor.

Typical pure receptor preparations from 6×10^7 PMN radiolabelled with 1 mCi of ^{125}I yielded 50000 c.p.m., of which 40–50% was precipitable in trichloroacetic acid. In 30 experiments using six receptor preparations, 39.5 ± 3.1% (mean ± S.D.) of the radiolabel re-bound to IgA–Sepharose, whereas only 4.2 ± 3.2% bound to BSA–Sepharose. SDS/PAGE and autoradiography demonstrated that all of the receptor was re-bound to IgA–Sepharose.

Assessing the ligand-binding specificity of the purified radiolabelled IgA receptor

The results of typical re-binding experiments are shown in Tables 1 and 2. Here, when purified radiolabelled IgA receptor was incubated with fresh IgA–Sepharose, more than 90% of trichloroacetic acid-precipitable radioactivity re-bound. In contrast, less than 2% bound to the same amount of IgG–Sepharose or BSA–Sepharose. Experiments using inputs of between 5000 c.p.m. and 1000 c.p.m. of receptor gave similar results. In spite of this low input, the assay proved to be highly reproducible, allowing 30–50 measurements to be made using the same receptor preparation.

A series of experiments was carried out to determine the ligand-binding specificity of the IgA receptor by studying inhibition of the binding of purified receptor to IgA–Sepharose by IgA, other immunoglobulins and IgA fragments. Table 1 shows the results obtained. It is clear that soluble IgA inhibits re-binding by purified receptor to IgA–Sepharose in a dose-dependent manner. IgA aggregated by heating at 60 °C was slightly less effective at inhibiting binding than was untreated IgA. Soluble IgG, either heat-aggregated or untreated, failed to inhibit IgA receptor re-binding to IgA–Sepharose. Inhibition of binding of 50% was achieved consistently in three separate competition assays at a mean concentration of serum IgA1 inhibitor of 0.077 mg/ml, suggesting a K_i of 4.8×10^{-7} M (S.D. = 1.30×10^{-7} M). When serum IgA1 and IgA2 or secretory IgA1 and IgA2 were used in similar competition assays, it was observed that both subclasses of IgA, irrespective of source, inhibited purified IgA receptor re-binding to IgA–Sepharose (Fig. 4). Weight for weight, serum IgA1 and IgA2 were more potent inhibitors than secretory IgA1 and IgA2, with 50% inhibition of binding of IgA receptor to IgA–Sepharose by secretory IgA being achieved at a concentration of about 0.2 mg/ml, again

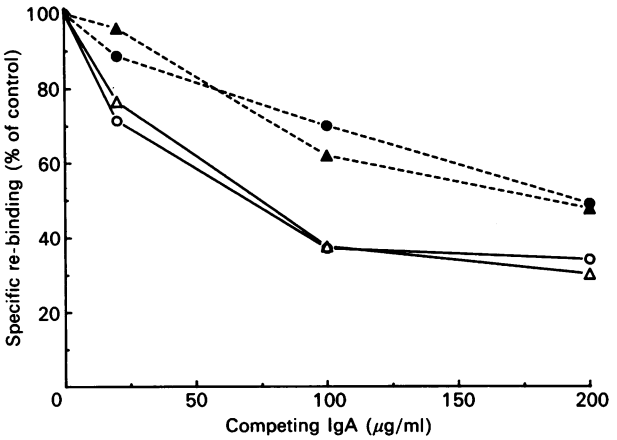

Fig. 4. Inhibition of the binding of purified radiolabelled IgA receptor to IgA–Sepharose in the presence of increasing concentrations of serum or secretory IgA1 or IgA2

Purified ^{125}I-radiolabelled IgA receptor was preincubated with or without competing immunoglobulin [serum IgA1 (△), serum IgA2 (○), secretory IgA1 (▲), or secretory IgA2 (●)] at 4 °C for 1 h. The samples were then added to 50 μl of packed IgA–Sepharose and specific binding was determined as described in the Materials and methods section.

suggesting a K_i value of around 5×10^7 M^{-1}. IgG and IgM failed to inhibit IgA receptor re-binding; in a separate experiment, the specific re-binding of the receptor to IgA–Sepharose in the presence of IgG, IgM or serum IgA1 (0.2 mg/ml) was 101, 100 and 30% respectively of control.

IgA1 fragments were used in competition assays in an attempt to determine which part of the IgA molecule bound to the IgA receptor. The fragments used were a 140 kDa F(abc')$_2$ fragment generated by pepsin cleavage (this fragment lacks the CH3 domain), and Fc and Fab fragments generated by an IgA1-degrading proteinase secreted by *Neisseria gonorrhoeae*. This enzyme cleaves the hinge region of IgA1, yielding monomeric Fab fragments and an intact Fc fragment containing CH2 and CH3 domains. The results of the competition experiment are shown in Table 2. They indicated that rebinding of purified radiolabelled receptor to IgA–Sepharose was inhibited by the Fc fragment of IgA, but there was very little inhibition by the Fab fragment. The F(abc')$_2$ fragment also inhibited receptor re-binding to IgA–Sepharose, but to a more limited extent than did either intact IgA or IgA Fc fragment.

DISCUSSION

In a previous study [22] we reported the first isolation of ^{125}I-IgA receptor from human PMN. While purifying the IgA receptor on IgA–Sepharose, we routinely isolated FcγRII on IgG–Sepharose under identical conditions. In our experiments, the low affinity of FcγRIII appeared to prevent purification of this receptor on IgG–Sepharose. This suggested that the affinity of the IgA receptor for IgA was higher than that of FcγRIII for IgG, but of a similar order of magnitude to that of FcγRII for IgG. Our present results demonstrate that the affinity of the IgA receptor for IgA is around 5×10^7 M^{-1}, which is therefore much higher than the affinity of FcγRII for IgG. Kulczycki *et al.* [4], using a similar assay for PMN IgG receptors, showed 50% inhibition of binding by 3 mg of IgG/ml, suggesting a K_i of 2×10^5 M^{-1}.

The present study has shown that the IgA receptor has an apparent molecular mass of 50–70 kDa on both reducing and

Table 2. Competition between the binding of purified IgA receptor to IgA–Sepharose and proteolytic fragments of serum IgA1

For each experiment, 0.2 mg of inhibitor/ml and 0.02 ml of resin were used. Each experiment was done in triplicate; mean values ± S.D. are shown. Specific binding was calculated after subtraction of the radioactivity which bound to BSA–Sepharose. Receptor binding (%) is expressed relative to the radioactivity bound in the absence of competing immunoglobulin. Abbreviation: S, Sepharose.

Resin	Inhibitor	Radioactivity added (counts/5 min)	Radioactivity bound (counts/5 min)	Radioactivity bound (%) (means ± S.D.)	Specific radioactivity bound (%)	Receptor bound (%)
BSA–S	None	3435	58	4.3 ± 2.7	0.0	
		3580	252			
		3780	163			
IgA–S	None	3723	1633	44.2 ± 1.6	39.8	100.0
		4270	1962			
		4743	2042			
IgA–S	IgA1 whole molecule	2845	593	19.6 ± 1.9	14.8	37.2
		3020	515			
		2875	565			
IgA–S	IgA1 $F(abc')_2$ fragment	3606	1227	34.8 ± 0.8	30.4	76.4
		3532	1232			
		4300	1532			
IgA–S	IgA1 Fc fragment	4245	533	12.9 ± 1.3	8.6	21.6
		3585	515			
		4185	500			
IgA–S	IgA1 Fab fragment	3333	1520	41.3 ± 4.1	36.7	92.5
		4275	1698			
		4295	1623			

non-reducing SDS/PAGE and that the receptor appears on these gels as a diffuse band, characteristic of heavily glycosylated proteins. Although the appearance of the IgA receptor on gels is similar to that of FcγRIII, the latter consistently shows an even broader band on gels [4,32]. Furthermore, although our results show that IgA–Sepharose is able to bind all of the IgA receptor in a single extended incubation, immunoprecipitation of FcγRIII using a specific monoclonal antibody shows that more than 20 times as much radioactivity is associated with FcγRIII in these preparations. These results suggest that the two receptors are distinct. In a recent publication, Monteiro *et al.* [23] have precipitated an IgA receptor from PMN using IgA–anti-idiotype complexes. This receptor, which had similar properties to those reported here, was shown to differ from FcγRIII in that it was not attached to the cell membrane by a glycosyl-phosphatidyl-inositol linkage. Furthermore, the two receptors showed different mobilities on SDS/PAGE following *N*-glycanase treatment [23].

In contrast with the results with FcγRIII, the yield of radioactivity associated with FcγRII, purified by affinity chromatography, was always similar to that of the IgA receptor. This is consistent with the results of Teteroo *et al.* [32], who showed that immunoprecipitation of FcγRII from radiolabelled neutrophils using monoclonal antibodies resulted in recovery of much less radioactivity than immunoprecipitation of FcγRIII. Cytofluorimetric analysis has shown the existence of 7500–15000 molecules of FcγRII and 135000–200000 molecules of FcγRIII per neutrophil [33,34]. These results suggest that the IgA receptor and FcγRII are both present at about 5% of the concentration of FcγRIII.

The present study has confirmed the remarkable stability of the IgA receptor. The resistance to proteolysis by endogenous proteinases may be important in keeping the receptor intact on PMN under conditions where these cells have been triggered to degranulate. We have also shown that the IgA receptor retains ligand-binding activity, with a specificity for IgA, even after acid elution during purification. This allowed study of the specificity of ligand–receptor interaction using an assay measuring the binding of purified radioiodinated receptor to IgA immobilized on Sepharose beads. The specificity of the receptor for IgA was found to be consistent with an earlier study in which a rosetting assay was used to show that IgA, but not IgG nor IgM, inhibited binding of IgA-coated red cells to PMN [12]. In the same study IgA1 and IgA2 paraproteins were shown to be equally efficient at inhibiting rosetting. We have now confirmed these findings using purified receptor.

We have also shown that secretory IgA of either subclass was as efficient, in molar terms, as serum IgA at inhibiting re-binding of purified receptor to IgA–Sepharose. This is consistent with the data from functional studies using IgA from different sources. Henson & Oades [19] showed that aggregated IgA myeloma proteins of either subclass stimulated the release of granule enzymes from PMN, and Walsh & Kay [35] demonstrated the rosetting of PMN with erythrocytes coated with IgA myeloma proteins of either subclass. Fanger *et al.* [13] showed that red blood cells opsonized with secretory IgA could be phagocytosed by PMN. Our own results [36] suggest that serum and secretory IgA of either subclass are able to induce a respiratory burst from human neutrophils of a magnitude similar to that produced by IgG when they are coated on to microtitre wells.

The location of the receptor-binding site on the IgA molecule was examined using IgA fragments generated by proteolytic enzymes. When Fab and Fc fragments generated using a bacterial IgA1 proteinase were tested as potential inhibitors of receptor binding to IgA–Sepharose, only the Fc fragment caused significant inhibition, indicating that the binding site was located in the Fc portion of the IgA molecule. These results have been confirmed by a recent study by Monteiro *et al.* [23]. We have shown that a pepsin-derived $F(abc')_2$ fragment of IgA lacking the CH3 domain was able to inhibit receptor binding to IgA–Sepharose significantly, but to a limited extent. A previous study [11], using an erythrocyte rosetting assay, found that an abnormal IgA (Wal), consisting of one heavy/light chain pair and lacking the CH3 domain, was an even more effective inhibitor of receptor binding than was normal IgA. This would suggest that the receptor-binding site resides in the CH2 domain.

It is possible that the CH3 domain interacts with the CH2

domain to provide the correct molecular configuration for its optimal binding of the PMN. Similar observations have been made for IgG, where the CH3 domains influence the binding site for FCγRII, which is located on CH2. Since the affinities of serum and secretory IgA for this receptor are the same, it appears that the Fc region of secretory IgA is not extensively covered by secretory component, as some models suggest.

Taken together, these results demonstrate the existence of a single IgA receptor on PMN. They indicate that the receptor described here can indeed be classified as an FcαR. The receptor is of higher affinity than the IgG Fc receptors found on unstimulated PMN. IgA binding to the receptor appears to be able to stimulate a number of neutrophil functions, such as phagocytosis, degranulation and induction of a respiratory burst. In each case the response is similar to that elicited by IgG binding to its own receptor(s). It would therefore appear that IgA, which is the most abundant immunoglobulin, may play a more active role in immunity than was previously considered. The observation that secretory IgA is able to interact with neutrophils in this manner might have particular significance, since complement levels in secretions are usually very low, and destruction of micro-organisms by mechanisms which are solely immunoglobulin-dependent might play an important role.

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