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Binding of human IgA1 to rat peritoneal macrophages

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SUMMARY

In the present study we have investigated whether bovine erythrocytes (E^b) specifically sensitized with human polyclonal IgA1 (E^b-IgA1) are able to bind to resident adherent rat peritoneal cells (PMϕ). Rat PMϕ formed rosettes with E^b-IgA1 at room temperature and at 37°. The formation of these rosettes could be blocked completely by excess human serum IgA or myeloma IgA1. In contrast, human IgG or rat IgG did not inhibit the formation of rosettes, whereas human polymeric myeloma IgA2 only partially inhibited rosette formation. Complete inhibition of rosette formation was also induced by rat monomeric and polymeric myeloma IgA, suggesting species interchangeability. Furthermore, rosette formation could be completely blocked in the presence of excess asialofetuin or D-galactose, while excess ovalbumin or D-mannose had no effect. These results suggest that the oligosaccharides in the hinge region of human IgA1 are involved in the binding of E^b-IgA1 to rat PMϕ.

INTRODUCTION

Receptors for IgA (FcRα) have been demonstrated on B and T cells and phagocytes of various species (reviewed by Mestecky & McGee, 1987). FcRα on lymphocytes are thought to play a role in the regulation of the IgA isotype-specific immune response, and evidence for the presence of such an immune-regulatory function has been presented by Müller & Hoover (1985) in mice.

On the other hand, FcRα on phagocytes are thought to be involved in ingestion of IgA-coated particles or IgA-immune complexes and antibody-dependent cellular cytotoxicity. In support of this latter view are experimental studies by others (Lowell *et al.*, 1980; Fanger, Goldstine & Shen, 1983; Clark *et al.*, 1984; Maliszewski, Shen & Fanger, 1985) and ourselves (Gorter *et al.*, 1987), suggesting that particles opsonized with IgA are able to induce phagocytosis and are capable of triggering intracellular processes. In addition, IgA-containing material has been demonstrated in monocytes and granulocytes of patients with IgA nephropathy (Sato *et al.*, 1983; Roccatello, Coppo & Piccoli, 1984), suggesting a mechanism for internalization of complexes containing IgA.

Abbreviations: BSA, bovine serum albumin; DEAE, diethyl aminoethyl; E^b, bovine erythrocytes; E^b-IgA1, bovine erythrocytes sensitized with human IgA1; ELISA, enzyme-linked immunosorbent assay; FcRα, receptor for the Fc portion of IgA; GVB, VBS containing 0·1% gelatin; PBS, phosphate-buffered saline; PMϕ, peritoneal macrophages; RPMI-BSA, RPMI-1640 containing 0·5% BSA; VBS, veronal-buffered saline.

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In order to detect the interactions between human FcRα and human IgA most authors use a rosette technique. But due to the obvious practical problems in obtaining erythrocytes specifically sensitized with human IgA, ox erythrocytes sensitized with either rabbit or mouse IgA have been used (Gupta, Platsoucas & Good, 1979; Fanger, Pugh & Bernier, 1981; Fanger *et al.*, 1983). In the present study human polyclonal IgA1, which reacted specifically with bovine erythrocytes (E^b; Tamura, Kano & Milgram, 1984), was used to prepare indicator cells. These indicator cells are demonstrated to bind to resident rat peritoneal macrophages (PMϕ), and the binding characteristics and the specificity of binding of these E^b sensitized with IgA1 are reported.

MATERIALS AND METHODS

Materials

Asialofetuin, D-mannose, D-galactose, bovine serum albumin (BSA), diethyl aminoethyl (DEAE) Sephacel (Sigma Chemical Company, St Louis, MO), chicken egg albumin (ovalbumin, Koch Light Laboratories Ltd, Haverhill, U.K.), RPMI-1640 (Seromed, Biochrom KG, Berlin, FRG), Sepharose 4B, Sephacryl S-300, (Pharmacia, Woerden), gelatin (Difco Laboratories, Detroit, MI), Na ^{125}iodine (the Radiochemical Centre, Amersham, Bucks, U.K.), iodobeads (Pierce Chemical Company, Rockford, IL) and Dowex 1 × 8 (Serva, Heidelberg, FRG) were purchased as indicated. Monoclonal rat monomeric IgA, polymeric IgA (Rits *et al.*, 1986) and monoclonal rat IgM were a generous gift of Professor H. Bazin and Dr M. Rits (Catholic University of Louvain, Brussels, Belgium). Human polymeric myeloma IgA2 was kindly provided by Professor J. P. Vaerman (Catholic University of Louvain, Brussels).

Isolation of proteins
Polyclonal IgA from pooled normal human serum and IgA1 from serum of a myeloma patient were isolated as described previously (Hiemstra *et al.*, 1987). Human and rat IgG were isolated from pooled normal human or rat serum, respectively, by subsequent $(NH_4)_2SO_4$ precipitation, anion exchange chromatography on DEAE–Sephacel and rechromatography on DEAE–Sephacel to remove residual contaminating IgA. The IgG preparations were shown to contain less than 0·05% IgA on a weight basis by enzyme-linked immunosorbent assay (ELISA) methods. Human IgM was isolated from plasma of a myeloma patient essentially as described by Kijlstra *et al.* (1978). By ELISA methods the IgM preparations were shown to contain less than 1·0% IgA on a weight basis.

Isolation of IgA1 from sera of infectious mononucleosis patients
Human IgA1 was isolated from pools of sera of infectious mononucleosis patients, which had a positive score in the Paul–Bunnell test (titre $\geqq 1:64$). A pool of serum was heat inactivated for 30 min at 56° and, after cooling to room temperature, IgA was precipitated by $(NH_4)_2SO_4$ (final concentration 2 M). The solubilized precipitate enriched for IgA was subjected to gel filtration on Sephacryl S-300. IgA-containing fractions were pooled and dialysed against veronal-buffered saline (VBS) containing 1 mM $MgCl_2$, 1 mM $CaCl_2$ and 1 mM $MnCl_2$, and subjected to affinity chromatography on a jacalin–Sepharose 4B column (Roque-Barreira & Campos-Neto, 1985). Bound IgA1 was eluted with VBS containing 0·8 M D-galactose and 0·5 M NaCl. Protein-containing fractions were pooled, extensively dialysed against phosphate-buffered saline (PBS) and concentrated to a protein content of 1–5 mg/ml. Finally the IgA1 preparation was aliquoted and stored at −20° until use.

Opsonization of bovine erythrocytes with IgA1
Bovine erythrocytes (E^b; 4×10^8/ml) and IgA1 isolated from sera of infectious mononucleosis patients (in a subagglutinating dose) were incubated in VBS containing 0·1% gelatin (GVB) for 30 min at 37° and then kept for 30 min at 4°. Unbound IgA1 was removed by washing with GVB and a second wash with a mixture of equal volumes of GVB and RPMI-1640 containing 0·5% BSA (RPMI-BSA). E^b sensitized with IgA1 (E^b-IgA1) were finally resuspended in RPMI-BSA at a concentration of 4×10^7/ml and held at 4° until use.

Isolation of resident rat peritoneal cells
Resident peritoneal cells were obtained from young Wistar rats (raised in our institution) (180–220 g) by lavaging the peritoneal cavity with 20 ml PBS. The peritoneal cells were subsequently centrifuged for 10 min at 110 ***g*** and resuspended in RPMI-BSA at a concentration of 2×10^6 cells/ml and kept on ice until use.

Rosette assay
To flat-bottomed tubes each containing a lightly siliconized coverslip (12 mm in diameter), 1×10^5 peritoneal cells in 3 ml PBS were added. After centrifugation for 10 min at 110 ***g*** the supernatant was removed and 0·5 ml E^b-IgA1 (4×10^7/ml) or E^b (as a control) in RPMI-BSA added. Depending on the type of experiment the tubes were either incubated for 45 min at room temperature or at 37° in a moist 5% CO_2, 95% air mixture. After this period the coverslips were washed twice with RPMI-BSA to remove the excess E^b-IgA1 or E^b and non-adherent cells. Nucleated cells were stained with a drop of 10 μg/ml acridine orange in PBS and the percentage of adherent cells which had bound three or more bovine erythrocytes was determined, using a fluorescence microscope (Leitz, Wetzlar, FRG). At least 400 cells were counted by two investigators. All experiments were performed at least twice.

Phagocytosis of latex particles
Phagocytosis was determined essentially as described by Toh *et al.* (1979). After an incubation period of 45 min at 37°, the coverslips were washed with RPMI-BSA and the adherent cells were fixed with methanol, stained with Giemsa and the percentage of adherent cells that had phagocytized the latex particles determined.

^{125}I-labelling of IgA1
Purified IgA1 was radiolabelled with 3·7 MBq ^{125}iodine in the presence of an iodobead for 20 min at room temperature to a specific activity of approximately 10 MBq/mg protein. Protein-bound iodine was separated from free iodine by chromatography on Dowex 1×8.

Determination of the number of IgA1 molecules per E^b
A varying dose of unlabelled IgA1, to which a constant trace amount of ^{125}I-IgA1 was added (both containing IgA1 specific for E^b) and E^b (4×10^8/ml) were incubated in GVB for 30 min at 37° and then kept for 30 min at 4°. Unbound IgA1 and ^{125}I-IgA1 were removed by washing with GVB. Next, the E^b-IgA1 were transferred to new tubes and after a second wash with a mixture of equal volumes GVB and RPMI-BSA the amount of ^{125}I-IgA1 bound to the E^b was determined. The fraction of ^{125}I-IgA1 bound to the E^b was used to calculate the number of molecules per E^b.

RESULTS

Effect of temperature on the ability of adherent rat peritoneal cells to form rosettes with E^b-IgA1

The characteristics and the specificity of binding of E^b sensitized with human IgA1 to rat adherent peritoneal cells were examined. Initial experiments revealed that adherent peritoneal cells were able to form rosettes with E^b-IgA1 when incubated for 45 min at 37° (Fig. 1). To investigate whether rosette formation was temperature dependent, E^b-IgA1 or unopsonized E^b and rat peritoneal cells were incubated for 45 min at a ratio of 200:1 at 0°, room temperature or 37°, respectively. The results shown in Fig. 2 demonstrate that almost no binding of E^b-IgA1 at 0° occurred. In contrast, at room temperature and at 37°, 32% and 37% of the adherent peritoneal cells were able to form rosettes with E^b-IgA1, respectively. Unopsonized E^b did not form rosettes with adherent peritoneal cells at all temperatures tested. Because E^b-IgA1 were phagocytosed at 37°, rosette experiments were performed routinely at room temperature.

Phagocytosis of opsonized latex by adherent peritoneal cells

In order to determine the percentage of adherent peritoneal cells that were able to ingest opsonized latex beads, peritoneal cells were incubated with latex beads in the presence of 20%

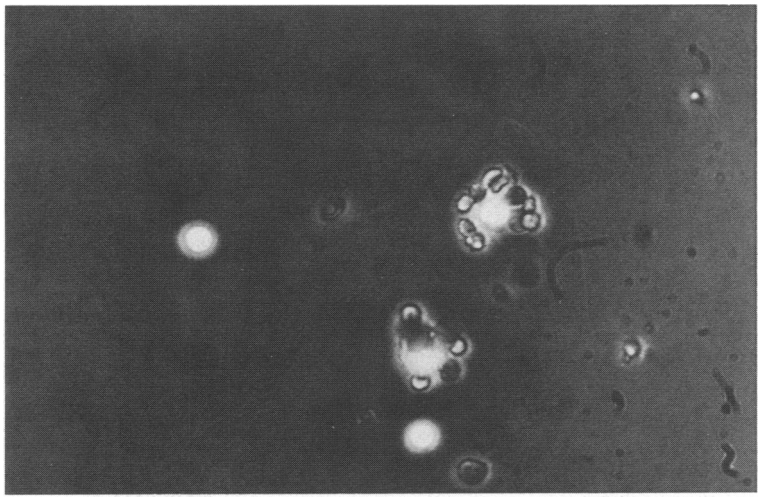

Figure 1. Adhesion of E^b-IgA1 to rat PMϕ. Rat peritoneal cells were incubated with E^b-IgA1 (ratio 1:200) for 45 min at 37° and assessed for rosette formation (magnification × 240).

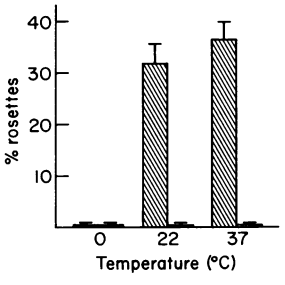

Figure 2. Effect of temperature on the binding of E^b-IgA1 to rat PMϕ. Rat peritoneal cells were incubated for 45 min at different temperatures with E^b-IgA1 (ratio 1:200). After washing the percentage of PMϕ forming rosettes with E^b-IgA1 was assessed. Open bars represent the percentage of PMϕ forming rosettes with unopsonized E^b, hatched bars represent the percentage of PMϕ forming rosettes with E^b-IgA1. The results are expressed as the mean ± SD.

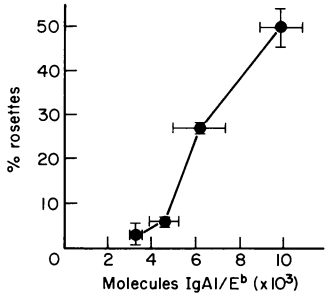

Figure 3. Effect of the number of IgA1 molecules per E^b on the fraction of PMϕ-forming rosettes. Rat PMϕ were incubated for 45 min at room temperature with E^b-IgA1 bearing known amounts of IgA1 on their surface (ratio 1:200). After washing the fraction of PMϕ forming rosettes with E^b-IgA1 was assessed. The results are expressed as the mean ± SD.

autologous serum for 45 min at 37°. The results of these experiments revealed that at least 98% of the adherent cells was able to ingest these opsonized particles. Therefore, in the following part the adherent peritoneal cell population is referred to as peritoneal macrophages (PMϕ).

Dependence of rosette formation on the number of IgA1 molecules per E^b

In the following set of experiments we tested whether the percentage of PMϕ-forming rosettes with E^b-IgA1 was dependent on the number of IgA1 molecules per E^b. Therefore PMϕ were incubated with E^b-IgA1 bearing different numbers of IgA1 molecules on their surface. As demonstrated in Fig. 3, an increase in the number of IgA1 molecules per E^b resulted in an increase in the number of PMϕ which formed rosettes.

Specificity of rosette formation

In order to determine the specificity of the interaction between PMϕ and E^b-IgA1, we performed rosette experiments in the presence of excess (1 mg/ml) human serum IgA, human IgG and human IgM. No inhibition of rosette formation was observed in the presence of IgG (5 ± 12%) and partial inhibition in the presence of IgM (35 ± 13%). In contrast serum IgA could fully inhibit rosette formation (Table 1).

Effect of human IgA subclasses on rosette formation

Since human serum IgA contains mainly IgA1 (80%; Delacroix *et al.*, 1982), we tested whether both subclasses were equally effective in inhibiting rosette formation. As demonstrated in Table 1 human myeloma IgA1 and human serum IgA (used at a concentration of 1 mg/ml) were able to block rosette formation completely, whereas polymeric myeloma IgA2 exhibited only partial inhibition. When tested at different concentrations, human serum IgA inhibited the formation of rosettes efficiently in a dose-dependent manner, while human polymeric myeloma IgA2 failed to demonstrate effective inhibition of rosette formation (Fig. 4).

Table 1. Effect of human immunoglobulins on rosette formation

Inhibitor	% inhibition
IgG	5±12
IgM	35±13
IgA	95±1
IgA1	100±2
IgA2	30±3

Rat peritoneal cells were incubated with E^b-IgA1 (ratio 1:200) in the presence of 1 mg/ml immunoglobulin for 45 min at room temperature. After removal of non-adherent cells the percentage of PMϕ-forming rosettes was assessed. Results are expressed as the mean±SD.

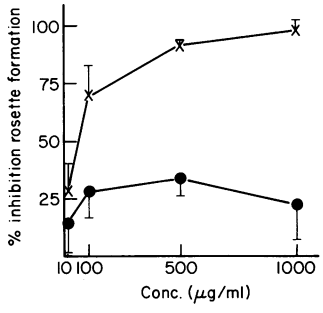

Figure 4. Effect of human serum IgA and human polymeric myeloma IgA2 on rosette formation. Rat peritoneal cells were incubated with E^b-IgA1 (ratio 1:200) for 45 min at room temperature in the presence of various concentrations of human serum IgA (x) or polymeric myeloma IgA2 (●). After washing the fraction of PMϕ forming rosettes with E^b-IgA1 was assessed. The results are expressed as the mean±SD.

Inhibition of rosette formation by rat immunoglobulins

Since human IgA inhibited rosette formation efficiently, we tested whether the receptors involved in the binding of E^b-IgA1 to PMϕ were specific for human IgA. Therefore we investigated whether purified rat immunoglobulins could block rosette formation. Concordant with the results observed for human IgG and IgM, rat IgG was not able to block rosette formation, whereas partial inhibition was observed in the presence of rat IgM (Table 2). Experiments performed in the presence of various concentrations of either monomeric or polymeric monoclonal rat IgA revealed that both monomeric and polymeric rat IgA were able to inhibit rosette formation in a dose-dependent manner (Fig. 5). However, inhibition of rosette formation by polymeric IgA was more efficient.

Effect of glycoproteins and saccharides on rosette formation

The major structural differences between human IgA1 and IgA2 are found in the hinge region. IgA2 molecules have a 13 amino

Table 2. Effect of rat immunoglobulins on rosette formation

Inhibitor	% inhibition
IgG	8±14
IgM	56±13
Monomeric IgA	100±1
Polymeric IgA	100±1

Rat peritoneal cells were incubated with E^b-IgA1 (ratio 1:200) in the presence of 1 mg/ml rat immunoglobulin for 45 min at room temperature. After removal of non-adherent cells the percentage of PMϕ-forming rosettes was assessed. Results are expressed as the mean±SD.

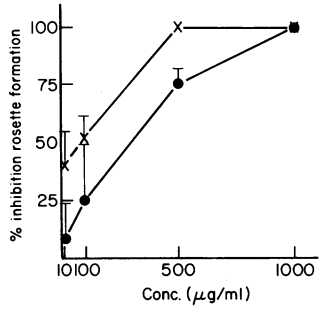

Figure 5. Inhibition of rosette formation by monoclonal rat monomeric and polymeric IgA. PMϕ were incubated with E^b-IgA1 (ratio 1:200) for 45 min at room temperature in the presence of various concentrations of monoclonal rat monomeric (●) or polymeric IgA (x). After washing the fraction of PMϕ forming rosettes with E^b-IgA1 was assessed. The results are expressed as the mean±SD.

Table 3. Effect of glycoproteins and saccharides on rosette formation

Inhibitor	Concentration (mg/ml)	% inhibition
Asialofetuin	1	90±1
Ovalbumin	1	21±9
D-galactose	9	100±1
D-mannose	9	13±11

Rat peritoneal cells were incubated with E^b-IgA1 (ratio 1:200) in the presence of various inhibitors for 45 min at room temperature. After removal of non-adherent cells the percentage of PMϕ-forming rosettes was assessed. Results are expressed as the mean±SD.

acid deletion, including the absence of five galactosamine oligosaccharides compared with IgA1 molecules (Tsuzukida, Wang & Putnam, 1979). Since oligosaccharide receptors are present on rat macrophages and rat Kupffer cells (Stahl *et al.*, 1978; Kolb *et al.*, 1980; Müller *et al.*, 1983), we investigated whether sugar residues expressed in the hinge region of human IgA1 might be involved in the binding of E^b-IgA1 to rat PMϕ. Therefore PMϕ were incubated with E^b-IgA1 in the presence or absence of asialofetuin or ovalbumin. These proteins were chosen because others have shown that asialofetuin and ovalbumin are able to prevent binding of glycoproteins containing galactose and mannose-terminal residues to their receptors, respectively (Stahl *et al.*, 1978; Brown, Russel & Mestecky, 1982). As demonstrated in Table 3, ovalbumin at a concentration of 1 mg/ml caused only $21 \pm 9\%$ inhibition, while asialofetuin at the same concentration prevented the formation of rosettes completely. Next we tested whether the free sugars by themselves were able to inhibit rosette formation. As shown in Table 3 only D-galactose was able to inhibit rosette formation.

DISCUSSION

In the present paper we studied the characteristics and specificity of binding of E^b sensitized with specific human polyclonal IgA1 to rat PMϕ. We have demonstrated that the binding of these E^b-IgA1 to rat PMϕ was temperature dependent. Only at room temperature and at 37° was rosette formation evident, while at 0° no measurable rosette formation occurred. The temperature dependence of rosette formation observed in the present study might explain the lack of rosette formation observed by Boltz-Nitulescu, Bazin & Spiegelberg (1981) who performed the binding experiments at 4°. The absence of rosette formation in our experiments was not due to pre-occupation by rat IgA of receptors for IgA, because no rat IgA could be detected by ELISA (with a detection limit of 25 ng/ml rat IgA) in the supernatant of one million peritoneal cells which had been incubated in 300 μl RPMI-BSA for 45 min at room temperature. The inability to detect rosettes at 0° might be explained by the presence of a low number of receptors for IgA expressed by PMϕ, which therefore need to be clustered to form rosettes with E^b-IgA1. This receptor clustering probably does not occur at 0°. The use of different rat strains might also explain the inability to detect rosettes in the study of Boltz-Nitulescu *et al.* (1981). An alternative explanation might be that in their study indicator cells were prepared by chemical cross-linking of human myeloma IgA to trypsinized ox red blood cells. When this method is applied the orientation of the IgA molecules on the erythrocytes is most probably random, while in the present study using specific IgA1 the molecules are properly orientated for ligand–receptor interactions. Binding of E^b-IgA1 to rat PMϕ was not due to complement component C3 produced by the PMϕ, because E^b-IgA1 did not lyse in the presence of high and low concentrations of normal rat serum.

Whether receptors for the constant part of IgA (FcRα) are present on rat phagocytes is still unclear. Boltz-Nitulescu *et al.* (1981) could not detect FcRα on rat alveolar and peritoneal macrophages using a rosette assay. In contrast, the studies reported by Johnson *et al.* (1984, 1986; Warren *et al.*, 1987) using preformed IgA immune complexes suggested that receptors for IgA are present on rat alveolar macrophages and activated neutrophils. However, in these studies the specificity of the receptor for IgA was not investigated. In the present study the specificity of the receptor on the PMϕ involved in binding E^b-IgA1 was assessed by adding purified immunoglobulins to the assay system. When purified human immunoglobulins were tested for their capacity to block the binding of E^b-IgA1 to rat PMϕ, only human serum IgA was able to block rosette formation in a dose-dependent manner. Human IgM partially inhibited rosette formation but when the amount of IgM was varied no relationship between inhibition of rosette formation and the dose of IgM was observed. At present it is not clear why IgM exhibits this partial inhibition. Not only human IgA, but also rat monomeric and polymeric IgA were able to block rosette formation in a dose-dependent manner, thus illustrating that this receptor for IgA is not specific for human IgA. These results are in agreement with the previous findings of Fanger *et al.* (1981) who demonstrated lack of species specificity for human FcRα. Because human IgA consists of two subclasses, namely IgA1 and IgA2, we tested their capacity to block rosette formation. IgA1 was able to block rosette formation completely, whereas polymeric myeloma IgA2 induced only partial inhibition (approximately 30%). However, as previously observed for IgM, no dose-dependent relationship was found.

The main difference between IgA1 and IgA2 is a deletion of 13 amino acids and the absence of five oligosaccharides in the hinge region of the IgA2 molecule compared with the IgA1 molecule (Tsuzukida *et al.*, 1979). The presence of these galactose terminal oligosaccharides in the hinge region of IgA1 molecules (Kornfeld & Kornfeld, 1976) and the presence of oligosaccharide receptors specific for galactose terminal glycoproteins on rat Kupffer cells, peritoneal macrophages and hepatocytes (Kolb *et al.*, 1980; Müller *et al.*, 1983) pointed to a role of these oligosaccharides on the IgA1 molecules in the binding of E^b-IgA1 to rat PMϕ. Furthermore, *in vitro* binding of human polymeric IgA to purified rat hepatic binding protein, a receptor specific for galactose residues, has been demonstrated (Stockert *et al.*, 1982). Asialofetuin, a glycoprotein with terminal galactose residues, was able to block rosette formation completely, whereas ovalbumin, a glycoprotein with terminal mannose residues, only marginally blocked rosette formation. Consistent with these findings, D-galactose could inhibit rosette formation completely whereas D-mannose had no effect. These results suggest that the terminal D-galactose residues in the hinge region of the IgA1 molecules are involved in the binding of E^b-IgA1 to rat PMϕ.

Only a few authors who have studied the interaction between IgA-coated particles and cells *in vitro*, have tested whether the oligosaccharides on the IgA molecules interact with oligosaccharide receptors on the cells. Sancho, Gonzalez & Egido (1986) have investigated the binding of immunobeads coated with human myeloma IgA to mouse Kupffer cells and hepatocytes. In this study no significant inhibitory effect of galactose or mannose on rosette formation was found. This lack of inhibition might be due to high affinity interactions of the IgA-coated immunobeads with the cells. For human FcRα–IgA interactions, blocking studies were performed with human myeloma IgA1 and myeloma IgA2 (Fanger *et al.*, 1981). In this study IgA1 and IgA2 were equally effective in their ability to block rosette formation, although a specificity of the FcRα for sugar residues present on the IgA molecules was not investigated. Thus on mouse Kupffer cells, hepatocytes and human phagocytes it seems that for the interaction between human IgA and

FcRα the oligosaccharides in the hinge region of human IgA1 do not play a role.

In conclusion, the present study demonstrates that rat PMϕ are able to bind E^b-IgA1. Furthermore, the binding of E^b-IgA1 to rat PMϕ is probably due to the presence of receptors for galactose on the rat PMϕ. Finally, one should be reminded that interactions between the oligosaccharides on the IgA molecules and oligosaccharide receptors might also be involved in the binding of IgA-coated particles.

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