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A receptor for monomeric IgG2b on rat macrophages

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SUMMARY

The binding to rat splenic and peritoneal macrophages of affinity-purified monoclonal rat IgGs, representing all IgG subclasses, was measured by the direct binding of ^{125}I-labelled proteins using an assay that did not require the removal of unbound Ig by washing. Only rat monomeric IgGs of the subclass IgG2b bound specifically and in large amounts to rat macrophages. The binding was temperature dependent and more IgG2b bound to the cells at 4° than at 37°. Spleen macrophages bound approximately 10 times more IgG2b than the same number of peritoneal macrophages, although the association constants (Kas) for the binding were similar for both types of macrophage. The calculated values for the Kas, which varied slightly with each experiment and increased with decrease in temperature, fell within the range $1{\cdot}3$–$5{\cdot}3 \times 10^8$ M^{-1}; the number of binding sites was estimated as about 10^5/splenic macrophage and 10^4/peritoneal macrophage. The binding of ^{125}I-IgG2b to splenic macrophages was inhibited only by unlabelled proteins of the IgG2b isotype and not by IgG_1, IgG2a and IgG2c proteins. Soluble IgG2b–antigen complexes also bound to the FcR for monomer but a soluble IgG2a–antigen complex did not inhibit the binding of monomeric IgG2b.

INTRODUCTION

A variety of cells with the ability to bind the Fc moiety of both homologous and heterologous IgG in either the monomeric or complexed state exists in many species (Burton, 1985). *In vivo* macrophage and monocyte receptors for antigen-bound IgG facilitate the clearance of soluble and particulate complexes by the reticulo-endothelial system and may have roles in the initiation and regulation of immune responses (Wen Chang, 1985). Binding of monomeric IgG may occur as a consequence of the presence of receptors for complexed antibodies, or be a function of specific receptors for monomer (Carter, Leslie & Reeves, 1982). The biological function of FcR for monomeric IgGs is not totally understood but their presence on mononuclear cells may mediate natural and antibody-dependent cellular cytotoxicity (Horwitz & Bakke, 1984) and be involved in macrophage-dependent Fc fragment-induced B-cell proliferation (Morgan & Weigle, 1980).

Our department has a current interest in the development of technologies for the use of rat monoclonal antibodies for the imaging of tumour localization (North & Dean, 1983). One of the problems encountered in the evaluation of monoclonal antibodies for tumour imaging in man is the non-specific localization of indicator antibodies as a result of their ability to bind to FcRs on normal cells (Buraggi *et al.*, 1985). Recent studies on the distribution and localization of rat monoclonal antibodies in tumour-bearing rats indicated that uptake by normal host tissue occurred most obviously when antibodies of the IgG2b isotype were used (S.A. Eccles, H.P. Purvies, J.M. Styles, S.M. Hobbs and C.J. Dean, manuscript in preparation). Although a large volume of work exists that is concerned with the binding of homologous and heterologous IgGs to mouse macrophage FcR (Unkeless, Fleit & Mellman, 1981; Burton, 1985) there are few studies on the binding of monomeric IgG to rat cells and no studies in which the direct binding of labelled antibodies has been attempted.

We have measured the binding of a number of radiolabelled monoclonal and some polyclonal rat antibodies, representing all the rat IgG subclasses, to macrophages from the spleens and peritoneal exudates of rats. We demonstrate here that rat spleen in particular contains a population of macrophages which binds high levels of monomeric IgG2b but does not bind any other IgG subclass above background control values.

Abbreviations: BSA, bovine serum albumin; FcR, receptor for the Fc fragment of immunoglobulin; HPLC, high-pressure liquid chromatography; HRP, horseradish peroxidase; PBS, phosphate-buffered saline; PEMϕ, peritoneal exudate macrophage; RT, room temperature; SRBC, sheep red blood cells; TBI, total body irradiation.

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MATERIALS AND METHODS

Animals

Inbred rats of CBH/Cbi (RT1^c) and Wistar (RT1^u) strains were used for immunizations; these strains and rnu/rnu (RT1^c) strain rats were used as tissue donors. CBH/Cbi ('Hooded') and rnu/rnu ('Nude') rats were taken from our own specific pathogen free colonies, whilst Wistar rats were provided by the National Institute for Medical Research, London.

Immunoglobulins (Igs)

Monoclonal antibodies to SRBC and to HRP were prepared by the authors. Spleen or mesenteric lymph nodes from rats immunized twice with the relevant antigen were fused with the Y3 Ag 1.2.3 rat myeloma (Galfre, Milstein & Wright, 1979). Hybridoma cells producing specific antibodies were cloned twice and then grown in bulk for the production of culture supernatants. Monoclonal antibodies raised against the syngeneic sarcomata HSN and MC24 were kindly provided by Dr C.J. Dean and his group, Institute of Cancer Research, Sutton, Surrey (Dean *et al.*, 1984; Styles *et al.*, 1984). Polyclonal antibodies to SRBC and Hooded rat alloantigens were obtained by hyperimmunization of Wistar rats with SRBC or Hooded spleen cells and bleeding the recipients 10 days after the final immunization. Concentrated hybridoma supernatants were fractionated on Ultrogel AcA34 (LKB, Surrey) to remove free light chain and further purified on a MARK-1 (Bazin *et al.*, 1984) affinity column. Polyclonal Igs were similarly affinity purified using subclass-specific rabbit anti-rat Ig reagents. Screening for subclasses of IgG was carried out using a solid phase radioimmunoassay with absorbed and affinity-purified rabbit antisera and ^{125}I-labelled sheep anti-rabbit F(ab′)$_2$ (as described by Rose, Peppard & Hobbs, 1984). Igs were iodinated using the chloramine T method; specific activities were in the order of 0·1–1·0 μCi/μg. Both ^{125}I-labelled and unlabelled IgGs were spun at 100,000 ***g*** for 30 min before use.

Preparation of soluble complexes

Use was made of a monoclonal IgG2a anti-idiotype antibody HIM/1/230, generated in response to the monoclonal anti-HSN tumour antibody 11/160 (IgG2b): F(ab′)$_2$ fragments of HIM/1/230 and intact 11/160 were added together or F(ab′)$_2$ fragments of 11/160 and intact HIM/1/230 were added together. Intact IgG and F(ab′)$_2$ fragments were added in equivalent molar amounts at a final protein concentration of 1 mg/ml and allowed to react for 72 hr at 4°. The mixture was then fractionated by HPLC on TSK 4000 (LKB) and free Ig discarded. Protein peaks in the range of 200,000–300,000 MW (probably 1:1 complex) and a larger MW fraction (containing molecules larger than dimer) were collected and concentrated in centricon tubes (Biorad, Richmond, CA) to an approximate final concentration of 100 μg/ml.

Preparation of cells

Spleens were cut into small fragments and digested with collagenase (Boehringer, Mannheim, FRG), 0·05%, in serum-free Dulbecco's MEM. Spleen cells were washed once after digestion and resuspended in MEM containing 1% BSA. Normal PEMϕ were washed out of the abdominal cavity with cold medium; activated PMNϕ were induced by injecting 3·0 mg *Corynebacterium parvum* (Welcome Research Laboratories, Beckenham, Kent) into the peritoneal cavity 7–10 days before their collection. Adherent cells were removed from spleen cell suspensions by incubation on plastic for 3 hr at 37° and phagocytic cells removed by treating the suspension with carbonyl iron (100 mg/20 ml cell suspension) for 30 min at 37° and allowing iron-containing cells to sediment in a magnetic field. Spleen cell suspensions were fractionated on discontinuous gradients of Percoll containing 50% specific gravity (SG: 1·058), 60% (SG: 1·067) and 70% (SG: 1·078) isotonic Percoll in MEM + 1% BSA. Irradiated spleen donors were exposed to 450R TBI from a ^{60}Co gamma source.

Binding assay

The assay method adopted here is a modified version of that described by Segal & Hurwitz (1977). Routinely, target cells were made to a concentration of 10^7/ml in MEM + 1% BSA, and 0·5 ml volumes placed in plastic tubes (Luckham, West Sussex). Immunoglobulins labelled with ^{125}I were added to the cells, the tubes stoppered and the contents mixed continually by rotation. After the allotted incubation period, routinely 3 hr at 4°, 1 hr at room temperature (RT;21–23°) and 30 min at 37°, the contents of the tubes were layered over a mixture of dibutyl phthalate and diocytyl phthalate (in a v/v ratio of 1:1·1) in Microfuge tubes, and spun in a Microfuge (Beckman Instruments, Fullerton, CA) for 1–2 min. The total aqueous supernatant was then removed, together with most of the organic gradient mixture, and the radioactivities of the unbound Ig in the supernatant and Ig bound in the cell pellet were measured separately. Counts bound to the cell pellet were expressed as a percentage of the total radioactivity added to each sample of cell suspension. Association constants were derived from binding curves in which the binding of a constant input of ^{125}I-labelled Ig was inhibited by increasing amounts of unlabelled Ig; μg of protein bound to the cells (r) and the molar concentration of unbound Ig (c) were calculated and r/c plotted against r (Scatchard, 1949). Specifically-bound Ig was calculated by subtracting the percentage of non-specifically bound Ig from the observed values. Non-specifically bound Ig, estimated from either amounts of ^{125}I-Ig bound to control (non-FcR-bearing) cells or from counts bound in the presence of a large excess of unlabelled Ig, amounted to 1·0–1·5% of the total input. The slopes of the plots indicate the values of Ka and the points at which the plots meet the intercept represent the maximum Ig bound and, therefore, the number of binding sites for n, the number of cells per sample. We have calculated the results using values of $1{\cdot}5 \times 10^5$ for the MW of IgG and 4×10^9 for molecules of IgG per ng. In these experiments we have assumed that the binding affinities of ^{125}I-labelled and unlabelled IgGs are similar.

RESULTS

Uptake of monomeric IgGs by rat spleen cells

Affinity-purified ^{125}I-IgGs representing all subclasses and having various specificities as antibodies were incubated at RT for 1 hr with unfractionated spleen cell suspensions from three rat strains. IgG2b IgGs only bound to spleen cells in amounts that exceeded the background, 1–2%, of radioactivity associated with non-FcR-bearing control cells or the binding of F(ab′)$_2$ fragments of IgG2b to spleen cells (Table 1). High levels of binding of IgG2bs by spleen cells were observed which, in some instances, represented more than 20% of the radioactive input. Binding was not inhibited by the presence of 0·1% sodium azide nor was it reduced if the incubation medium (MEM + 1% BSA) was replaced by PBS.

Removal of adherent of phagocytic cells from spleen-cell suspensions resulted in decreased binding of IgG2b proteins, whilst unfractionated cell suspensions of spleens from rats given TBI 24 hr or 3 days previously displayed higher binding than cells from normal rats (data not shown). The properties of adherence, phagocytosis and radio resistance are associated

Table 1. Binding of monomeric rat IgGs to rat spleen cells at RT

Antibody				% ^{125}I-labelled Ig bound to cells*				
				Spleen cells			Control cells	
Code	Specificity	IgG subclass		Wistar	Hooded	rnu/rnu	Thymocytes	YAC mouse lymphoma
1A4bH	SRBC	1	Mc	1·2±0·2†	1·2±0·2	1·7±0·7	0·8‡	
91b	HRP	1	MC	1·6±0·5				
	SRBC	1	Pc	1·9±0·6	2·2±0·5	2·9±0·5	1·8	
H6B5	SRBC	2a	Mc	1·1±0·3	1·5±0·3	1·6±0·3	1·1	1·1‡
ALN11/53	HSN	2a	Mc	0·9±0·3	0·8±0·03	1·7±0·5	0·4	0·9
	SRBC	2a	Pc	1·2±0·4	1·9±0·1	1·5±0·2	0·6	
W/H	CBH allo-ag	2a	Pc	0·6±0·1	—	—	0·5	
4B1F8	SRBC	2b	Mc	14·4±0·9	15·7±3·2	20·9±3·5	0·8	1·8
A2aC7	SRBC	2b	Mc	13·0±1·0	14·2±3·9	20·1±2·3	1·1	1·1
11/160	HSN	2b	Mc	8·9±2·1	8·3±1·2	16·6±4·7	0·9	
M1076	MC24	2b	Mc	8·6±1·1	6·7±0·9	19·1±2·1	1·1	0·9
W/H	CBH allo-ag	2b	Pc	5·6±1·2	—	—	1·2	
4B2b	SRBC	2c	Mc	1·7±0·2	2·1±0·1	2·5±0·4		
47d/3	HRP	2c	Mc	1·4±0·6	1·4±0·5	—		
M1076	MC24	$F(ab')_2$	2b	1·6±0·3	1·6±0·5	2·3		

* Cells incubated with antibody for 1 hr at RT; concentration of antibody: 100 ng/ml; concentration of cells: 10^7/ml.
† Figures represent mean of 6–9 measurements ± SD.
‡ Figures represent mean of triplicated measurements.
Mc: monoclonal; Pc: polyclonal

with macrophages and these results indicated that IgG2b-binding cells in spleen were a population of mature macrophages. Fractionation of spleen cells on discontinuous Percoll gradients confirmed that the cells with the highest capacity for binding IgG2b were located in the low-density (< 1·078 g/ml) fractions; 80–90% of the cells in these fractions were macrophage-like from their morphology in Geimsa-stained cytocentrifuge preparations. Cell suspensions made from the spleens of Nude rats contained a higher proportion of cells in the low-density Percoll fractions than splenocytes from euthymic rats.

Effects of temperature on the uptake of monomeric Ig by spleen macrophages

Macrophages were prepared from the spleens of rats that had been irradiated (400 rads TBI) 24 hr previously to reduce numbers of lymphocytes to a minimum; a cell suspension was obtained by collagenase digestion and macrophages separated from other spleen cells by isolation on 60% Percoll.

In the presence of a pulse of 100 ng/ml of ^{125}I-IgG2b, the percentage uptake of labelled monomer by any one spleen macrophage population was dependent on temperature, and decreased as the temperature rose. The kinetics of uptake, however, increased as the temperature increased: at 4° maximum binding was complete by 30 min, at RT within 15 min and at 37° by 5 min.

Association constants for the binding of the monoclonal protein A2aC7 (IgG2b) to splenic macrophages at different temperatures were calculated from the inhibition of the binding of a fixed amount of ^{125}I-A2aC7 in the presence of increasing concentrations of the unlabelled monomer. Scatchard plots of the data obtained for the binding of ^{125}I-A2aC7 to one spleen macrophage population at different temperatures are shown in Fig. 1. In this experiment Kas for the binding of A2aC7 to spleen macrophages at 4°, RT and 37° were calculated as $5{\cdot}3 \times 10^8$, $3{\cdot}3 \times 10^8$ and $1{\cdot}9 \times 10^8$ per M, respectively, and the maximum uptake as a percentage of the radiolabelled input was 38·7, 30·1 and 25·2 at these temperatures. However, estimates for the Ka of A2aC7 at each temperature varied with each macrophage population tested; Table 2 gives the range of Kas calculated for A2aC7 IgG2b at each temperature and the estimated numbers of FcR sites per macrophage. The source of variation between experiments was not clear but as each preparation of spleen cells represented a different batch of donor rats it is possible that variations in the natural states of immunological reactivity of the donors might be a contributory factor. However, spleen cells from three groups of rats treated with *C. parvum* 7–10 days before spleen harvest did not bind noticeably greater amounts of IgG2b than cells from untreated rats. Affinity data and site numbers for *C. parvum*-treated rats are included in Table 2. Variations in cell concentrations from assay to assay (as, for example, introduced by estimating cell numbers with a cell-counting chamber) also may have contributed to the variation in binding affinity estimated for A2aC7.

The affinities of monoclonal IgG2bs, other than A2aC7, for the rat spleen macrophage receptor were estimated, and the results are included in Table 2. Inhibition binding curves could not be obtained for IgG_1, IgG2a and IgG2c proteins on spleen macrophages, a result which confirmed that the low-level binding of these proteins to rat macrophages was entirely non-specific.

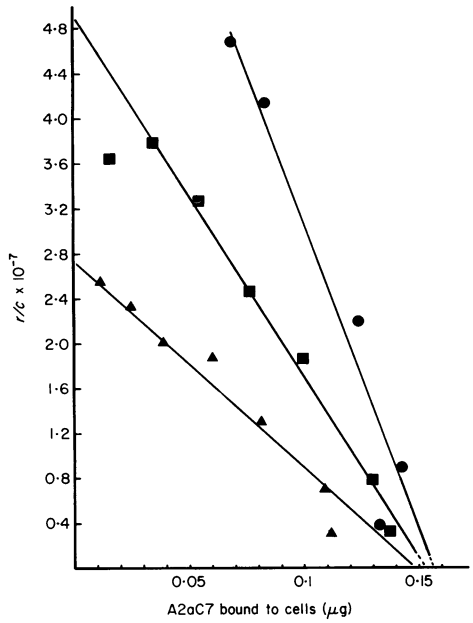

Figure 1. Scatchard plots of data obtained for the binding of A2aC7 (IgG2b) to spleen macrophages. Cells were incubated with a constant input of ^{125}I-A2aC7 (100 ng/ml) plus concentrations of unlabelled A2aC7 ranging from 100 ng/ml–10 μg/ml. Cell concentration: $5 \cdot 10^6/0 \cdot 5$ ml. Points represent the mean of triplicate estimates; *r*, μg A2aC7 bound to cells; *c*, unbound A2aC7 as moles/litre; ●, cells incubated at 4° for 3 hr; ■, cells incubated at RT for 1 hr; ▲, cells incubated at 37° for 30 min.

Table 2. Association constants for the binding of IgG2b to rat spleen macrophages

IgG2b protein	Temp.	No. exps	*C. parvum*-treated donors	Ka ($M^{-1} \times 10^8$)	No. sites per cell $\times 10^{-5}$
A2aC7	4°	4	—	2·8–5·3	0·8–1·3
A2aC7	RT	7	—	1·6–3·3	0·8–1·2
A2aC7	37°	3	—	1·3–1·5	0·9–1·1
A2aC7	RT	3	+	1·6–2·2	0·8–1·6
4B1	RT	2	—	2·7–3·5	0·5–0·7
11/160	RT	2	—	1·3–1·5	0·8–0·9

Experimental conditions were the same as those described in the legend to Fig. 1.

Binding of monomeric IgGs to rat peritoneal macrophages

Radiolabelled IgGs were incubated with peritoneal macrophages obtained from the spleen donors. At RT specific binding of IgG2b to peritoneal cells was very low, usually amounting to less than 3% of the labelled protein input; at 4° binding was higher but still very low compared with binding by equivalent numbers of spleen macrophages. These results are shown in Table 3. Kas estimated for the binding of A2aC7 to PE macrophages at 4° were in the same range as the values calculated for spleen macrophages, but the binding sites per cell were 10 times fewer. Again, we found the binding affinity of

Table 3. Binding of monomeric IgGs to rat peritoneal macrophages at 4°

IgG subclass	% ^{125}I-protein* bound to macrophage	Ka ($M^{-1} \times 10^8$)†	No. sites/cell $\times 10^{-4}$
IgG_1	1·5±0·7	—	—
IgG2a	0·7±0·2	—	—
IgG2b, A2aC7	3·8±1·2	1·4–2·4 (3)	1·0–2·0
IgG2b, A2aC7	5·7±1·4‡	1·8–2·5 (3)	1·2–1·6
IgG2b, 4B1	5·9±2·3	—	—
IgG2b, 4B1	8·1±2·2‡	—	—
IgG2b, 11/160	4·3±0·5	—	—
IgG2c	1·6±0·5	—	—

* Concentration of ^{125}I-Ig: 100ng/ml; concentration of cells: 10^7/ml. Figures represent the mean uptake±SD by cells from four to eight groups of rats.

† Kas calculated as described in the Materials and Methods. Figures in parentheses refer to the number of complete experiments performed; samples were assayed in triplicate.

‡ Binding to macrophages from rats treated with *C. parvum* 7–10 days before the assay.

IgG2b varied with each cell population tested and that pretreatment of the macrophage donors with *C. parvum* made no consistent difference to the uptake of IgG2b, although, on average, the amounts bound by cells from treated rats were higher than those bound by cells from untreated rats. IgG_1, IgG2a and IgG2c monoclonal proteins did not bind specifically to PE macrophages at 4°.

Properties of the rat macrophage FcR for IgG2b

Specificity. Splenic macrophages, isolated from 60% Percoll gradients, were incubated at RT with various amounts of unlabelled monomeric IgG_1, IgG2a, IgG2b or IgG2c followed after 10–15 min by a fixed amount of ^{125}I-labelled IgG2b (A2aC7). Competition for the binding of A2aC7 IgG2b to macrophages by monomeric IgGs of other subclasses is shown in Fig. 2; binding of A2aC7 was inhibited only by unlabelled A2aC7 or other IgG2bs. In a similar manner, soluble complexes of idiotype and anti-idiotype monoclonal antibodies (prepared as described in the Materials and Methods) were made to compete with the standard input of ^{125}I-A2aC7 for binding to spleen macrophages. In this experiment (Fig. 3) only complexes containing the IgG2b Fc moiety inhibited the binding of monomeric ^{125}I-A2aC7. Fifty per cent inhibition of binding of ^{125}I-A2aC7 at 100 ng/ml was achieved by 3 μg/ml of monomeric IgG2b (11/160), 1·5 μg/ml of the 1:1 complex (1 IgG2b molecule + 1 $F(ab')_2$ fragment of IgG2a) and 0·8 μg/ml of the larger complex. Complexes containing complete IgG2a molecules + $F(ab')_2$ of IgG2b did not inhibit the binding of monomeric IgG2b to splenic macrophages (Fig. 3).

Trypsin sensitivity. Splenic and peritoneal macrophages were incubated in 0·1% trypsin in PBS for 15 min at 37°. Subsequently, 0·05% sodium azide was added to the cell suspension (in MEM + 1% BSA) to prevent regeneration of cell-surface molecules removed by trypsin. This procedure was carried out five times on separate macrophage preparations but in no instance did trypsin treatment completely abolish ^{125}I-IgG2b

uptake. However, IgG2b binding was always diminished to some extent after trypsinisation; inhibition of normal levels of binding ranged from 5·0–50% and averaged 32·6% over five experiments. As IgG2b uptake was never completely abolished by trypsinisation and as the observed diminution in uptake may have been the result of reduced viability of the cells after enzyme treatment, we concluded that the FcR for IgG2b on rat macrophages was probably not trypsin sensitive.

DISCUSSION

Monomeric Igs, unlike multivalent complexes, bind relatively weakly to macrophages and have high rates of association and dissociation (Unkeless & Eisen, 1975). The binding assay procedure described by Segal & Hurwitz (1977) and adopted by us in this series of experiments allows low level binding to be measured and avoids the loss of bound material as a consequence of the need to wash unbound Ig from the cells. Our results show that rat macrophages bear a high affinity FcR for rat monomeric IgG2b but do not have a receptor for monomeric Igs of other IgG subclasses. The receptor is not exclusive for monomeric IgG2b but also binds soluble IgG2b complexes; it does not, however, bind soluble complexes of IgG2a. Whether complexes of IgG subclasses other than IgG2b and IgG2a bind to the FcR for IgG2b monomer is the subject of a current investigation.

The binding affinity of monomeric IgG2b increased as the temperature decreased, resembling the results of Unkeless & Eisen (1977) for the binding of mouse IgG2a monomer on mouse macrophages. Large amounts of IgG2b monomer (up to 30% of the Ig added) bound to splenic macrophages at RT or 37°, but at these temperatures the amounts bound to peritoneal macrophages were little above background levels. At 4°, however, sufficient IgG2b bound to peritoneal cells for the Ka to be measured, and it was apparent that the affinity of the Ig for peritoneal macrophages was as high as for spleen cells, although the amounts of Ig bound, in terms of molecules/cell, were 10 times lower. The levels of IgG2b bound by peritoneal macrophages may represent binding by a small number of receptors ($\sim 10^4$) on all cells or binding by a higher number of receptors ($\sim 10^5$) on a minority of cells but, either way, the results suggest that the peritoneal cell is functionally different from the spleen macrophage. The expression of macrophage FcRs for monomeric Igs can be modulated during macrophage activation (Ezekowitz, Bampton & Gordon, 1983) but the administration of *C. parvum*, a well known macrophage-activating agent (Halpern *et al.*, 1973) did not relate to consistent differences in the binding of IgG2b in our experiments. Inadequate stimulation of the macrophages may have been the reason for this result but an alternative consideration is that the spleen macrophage is *a priori* an 'active' cell. The abundant presence of active macrophages in the spleen would be consistent with the immunological functions of this organ, in particular the IgG-mediated clearance of foreign material (Schreiber & Frank, 1972) from the circulation.

Our results and conclusions differ from those published by Medgyesi *et al.* (1980) and Boltz-Nitulescu, Bazin & Spiegelberg (1981), who found evidence for IgG2a, IgG2c and IgG1/IgG2b receptors on rat peritoneal and alveolar macrophages, but our methods, also, differed radically from theirs. We used monoclonal antibodies affinity purified from culture supernatants and a direct binding assay. Medgyesi *et al.* (1980) and Boltz-Nitulescu *et al.* (1981), attempted to inhibit the binding of complexes (i.e. rosette formation) by the addition of excess amounts of myeloma proteins. The proteins, moreover, were isolated from sera or ascitic fluids and may have contained low

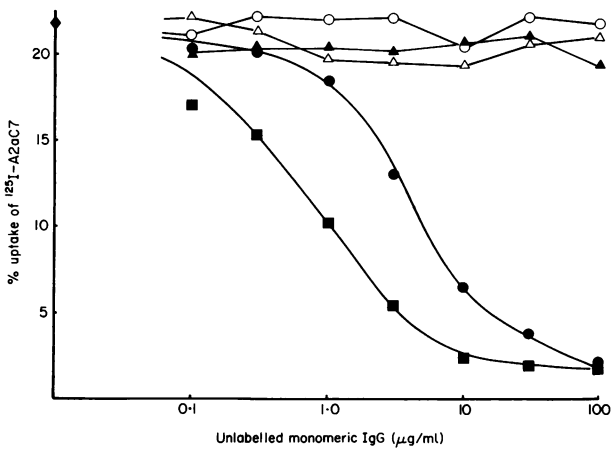

Figure 2. Inhibition of binding of A2aC7 (IgG2b) to rat spleen macrophages by IgGs of other subclasses. Concentration of ^{125}I-A2aC7: 100 ng/ml; concentration of cells: 5×10^6/0·5 ml. Percentage uptake of ^{125}I-A2aC7 in the absence of inhibitor, ♦; in the presence of stated concentrations of: unlabelled A2aC7, ■; unlabelled 11/160 (IgG2b), ●; unlabelled IgG_1, ○; unlabelled IgG2a ▲; unlabelled IgG2c, △. Points represent the mean of duplicate or triplicate measurements.

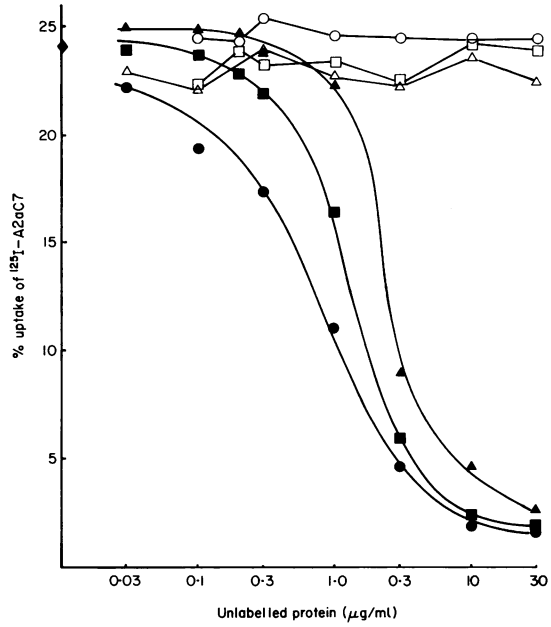

Figure 3. Inhibition of the binding of A2aC7 (IgG2b) to rat spleen macrophages by soluble complexes. Concentration of ^{125}I-A2aC7: 100 ng/ml; concentration of cells: 5×10^6/0·5 ml. Percentage uptake of ^{125}I-A2aC7 in the absence of inhibitor, ♦; in the presence of the stated concentrations of monomeric 11/160 (IgG2b), ▲; monomeric HIM/1/230 (IgG2a), ○; $F(ab')_2$ HIM/1/230+11/160 at 1:1, ■; $F(ab')_2$ HIM/1/230+11/160 complex at high MW, ●; $F(ab')_2$ 11/160+HIM/1/230 at 1:1, □; monomeric $F(ab')_2$ 11/160, △. Points represent the mean of triplicate measurements.

levels of contaminating IgG subclasses. In normal adult rats IgG2b is a major serum component and may account for half of the total serum IgG (Peppard, 1981).

In mice, a trypsin-sensitive receptor (FcRI) on macrophages binds monomeric IgG2a with an affinity constant of 2×10^7 M^{-1}, and a trypsin-resistant receptor (FcRII) binds monomeric IgG2b and IgG$_1$ with lower affinity (Unkeless & Eisen, 1975). In our experiments, trypsin treatment reduced but never abolished IgG2b binding to rat macrophages and the Kas calculated for rat IgG2b were much higher than the Ka for mouse IgG2a on mouse cells. Hence, despite their phylogenic relationship, there are species differences for the major receptors for monomeric IgG on the macrophages of rats and mice. Monomeric mouse IgG2a is reported as having a much higher affinity for FcR on human monocytes than for its homologous receptors (Leatherbarrow *et al.*, 1985). No figures are available for the affinity of rat monomeric IgG2b for human FcR, but rat IgG2b monoclonal antibodies specific for human cell-surface determinants have been shown to be far more effective than rat antibodies of other IgG subclasses in mediating ADCC reactions in man (Hale, Clark & Waldmann, 1985). The potential use of rat monoclonal antibodies as agents for serotherapy or tumour localization warrants further investigation of their physiology in homologous systems.

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