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Comparison of *in vitro* binding to enterocyte brush borders of rat IgG subclasses and their transmission *in vivo* in the rat

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Summary. The nature of the Fc receptors for IgG subclasses displayed on the epithelium of the small intestine in neonatal rats was examined by comparing results gained from ^{125}I-IgG binding studies using isolated enterocyte brush borders, and from experiments where *in vivo* transmission from the gut to the bloodstream was quantified for each subclass. These measurements were made in two strains of rat, Cob Wistar and CB Hooded Nu/+. Subclasses of IgG were transported from gut to blood in different amounts, and the *in vitro* binding also varied according to isotype. Comparison of the results between rat strains and between isotypes suggested that at least two IgG Fc receptors are present on the enterocytes of the young rat.

INTRODUCTION

The transport of IgG across the epithelial cells lining the proximal small intestine of the neonatal rat provides an interesting system for investigating the transcellular transport of macromolecules. Studies (Wallace & Rees, 1980; Mackenzie, Morris & Morris, 1983a) have shown that IgG binds first to Fc receptors displayed on the brush border membranes of the epithelial cells: an important facet of this binding is that it is pH dependent, taking place at pH 5·0–6·5 but not at pH 7·5 (Mackenzie, Morris & Morris, 1983b). Uptake of the IgG onto the cell membrane is followed by endocytosis via pits and accumulation into endosome-like compartments (Rodewald, 1973; Peppard *et al.*, 1984) where sorting from other receptor/ligand complexes (Geuze *et al.*, 1984) presumably occurs. The IgG still bound to its receptor is then transported, probably in vesicles which would be specifically protected from fusion with lysosomes, to the abluminal surface of the cell. Fusion of the vesicle membrane with that of the cell exposes the IgG/receptor complexes to a more neutral pH, which is believed to cause dissociation and release of the immunoglobulin ligand into the interstitial plasma. The vacated IgG receptor is thought to be recycled back to the luminal surface (Rodewald, 1980) by an unknown route. These events take place only during the first 20 days of life (i.e. before the time that digestive enzymes are produced in the small intestine of the young rat), and it is thought that the appearance of such enzymes and the associated changes in environmental pH may be the cause of the inactivation of IgG receptors on the enterocytes (Borthistle *et al.*, 1977).

Most experiments on this system have been carried out using unspecified mixtures of IgG subclasses. Some work using mouse IgG isotypes, one using rat epithelia (Borthistle *et al.*, 1977) and the others mouse (Guyer *et al.*, 1976; Mackenzie & Keeler, 1984) have shown that binding of the individual subclasses to epithelial cells was not identical. These differences may

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either be due to differential affinities of the IgG subclasses for a common Fc receptor molecule (Guyer *et al.*, 1976), or there may be more than one receptor involved (Mackenzie & Keeler, 1984). These subclass differences may be of importance in the protection of the young animal against disease. Also, previous experiments (Peppard *et al.*, 1984) showed that antigen in combination with rat IgG antibody may be transported intact from the gut lumen to the bloodstream, and indicated that the dose of antigen gaining access to the systemic circulation could be dependent on the binding affinity of the IgG involved.

The present study was thus initiated between two groups to compare the degree of binding *in vitro* of the rat IgG subclasses to rat enterocyte brush borders with the amount actually transported *in vivo* from the gut to blood. We provide results demonstrating a genetic polymorphism in the level of Fc receptor expression in the neonatal rat, indicating the existence of (at least) two separate Fc receptors in operation on the neonatal rat enterocyte which show specificity in terms of the subclass of rat IgG they bind.

MATERIALS AND METHODS

Animals

Rats at 12 days old of the Wistar Cobs strain were obtained from the National Institute for Medical Research. The breeding colony at ICR, Sutton, Surrey, provided CB Hooded/rnu strain hairy heterozygote (nu/+) rats of the same age. All rats were separated from their mothers for about 4 hr before use.

Purification of IgG subclasses

The production of IgG-secreting rat/rat hybridomas with specificity for horseradish peroxide (HRP) has been described previously (Dean *et al.*, 1984). Two cell lines secreting IgG1 (C8 and E11) and two secreting IgG2c (C11 and 3j) were used. The IgG anti-HRP was specifically purified from culture supernatants using HRP linked to AH-Sepharose (Pharmacia Ltd, Milton Keynes, Bucks) and fractionated on Ultrogel AcA34 (LKB Ltd, Croydon, Surrey) to prepare monomeric IgG as already described (Peppard *et al.*, 1984).

Another monoclonal IgG2b (E9) with unknown antibody specificity was derived from similar fusions. This was purified from culture supernatants by repeated gel filtration through Ultrogel AcA34 and AcA22.

Polyclonal IgG2a was prepared from pooled normal serum from several rat strains using DEAE, and absorbed of contaminating IgG2b using an immunoabsorbent column of rabbit anti-rat γ2b, detailed in Rose, Peppard & Hobbs 1984). Its purity, tested by radioimmunoassay, was greater than 99%.

Iodination of immunglobulins

All immunoglobulins were centrifuged to remove aggregates, labelled with ^{125}I with iodogen to about 0·5 μCi/μg, and dialysed against phosphate-buffered saline (pH 6) as already described (Peppard *et al.*, 1984).

Measurement of immunoglobulin transmission in vivo

Radiolabelled immunoglobulins (approximately 100 μg) of predetermined TCA-precipitability were injected into tied-off loops of the small intestine of 12-day-old rats. Blood was collected after the requisite time period, and the TCA-precipitable radioactivity in the serum measured so that the total recovery in the bloodstream could be estimated. These procedures are more fully described by Peppard *et al.* (1984).

For the estimation of intravenous catabolism of ^{125}I-labelled immunoglobulins, anaesthetised rats were injected *via* the jugular vein. The level of circulating ^{125}I-IgG was followed in individual rats over several hours by passing the tail of the sleeping rat through a small hole drilled through a 1-mm lead sheet and down into one counting chamber of a Hydragamma-16 (Innotron, Oxford). Radioactivity was estimated for 5 min every hour.

Isolation of intestinal brush borders and estimation of immunoglobulin binding in vitro

Brush borders were isolated from the jejunum of rats as described in Mackenzie *et al.* (1983a). Radiolabelled immunoglobulins (10 nM) were added to vials containing approximately 10^6 brush borders per ml. The vials were incubated at 37° for 1 hr, and the amount of radiolabelled protein bound to the membranes was determined by the method of Mackenzie (1984). Non-specific binding was measured by including a large excess (50 μM) of the equivalent unlabelled immunoglobulin in a duplicate vial.

RESULTS

Intravenous catabolism of IgG1, IgG2a, IgG2b and IgG2c

Over the 5 min–4 hr time period studied, no significant decrease in the circulating radioactivity was detected

for any of the four radiolabelled IgG subclasses after intravenous injection into 12-day-old rats.

Comparison of *in vivo* transport with *in vitro* binding in two different strains of rats

The amounts of ^{125}I-labelled IgG of different subclasses transported from gut lumen to bloodstream by 2 hr after injection was compared for the Wistar and the CB Hooded nu/+ rats (Table 1). For both strains, the recovery of IgG2a was always highest and much greater than the other subclasses. There was also a quantitative difference between the amounts transported across the small intestine of the two rat strains: calculation of the ratio of the recoveries in the bloodstream between nu/+ and Wistar rats (Table 1) showed that these were identical for IgG1, IgG2a and IgG2c, but a different value was obtained for IgG2b.

In order to extend these observations, time-courses were done where the appearance of each subclass in the bloodstream was measured after killing groups of rats at 1, 2 and 3 hr. For comparison, the IgG2b time-course was followed for both strains of rat. The results are illustrated in Fig. 1. The higher rate of recovery of IgG2a and the close similarity between the patterns of recovery of IgG1 and IgG2c in the nu/+ strain rats can be seen clearly.

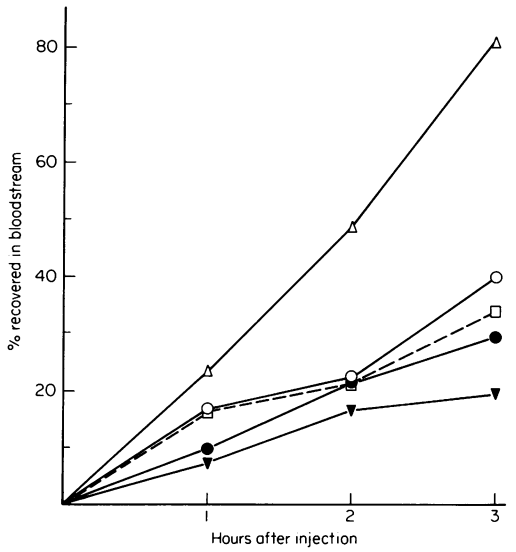

Figure 1. Time-course of the recoveries (●——●) of radiolabelled IgG1 (○——○), IgG2a (△——△) IgG2c (□——□) and IgG2b (●——●) in Nu/+ rats and IgG2b in Wistar rats (▼——▼). Radiolabelled IgG was injected into the lumen of the small intestine and the total percent recovered in the bloodstream at 1, 2 and 3 hr measured. All injected doses and the recoveries were corrected for TCA precipitability, and each point represents at least four animals.

Table 1. Percentage of dose recovered in the bloodstream 2 hr after injection into the gut of 12-day-old rats

Isotype injected	% recovery ± SE (no. rats) Wistar	Nu/+	Ratio of Nu/+ : Wistar
IgG1	16·2 ± 1·2 (12)	22·1 ± 1·3 (9)	1·36
IgG2a	35·9 ± 6·8 (6)	48·5 ± 2·4 (6)	1·35
IgG2b	20·4 ± 1·7 (7)	21·0 ± 2·4 (6)	1·03
IgG2c	15·5 ± 3·2 (6)	21·1 ± 2·3 (6)	1·36

All results are corrected for the % TCA precipitability of the injected dose and the amount recovered in the bloodstream.

Where *in vitro* binding was tested (Table 2), a similar order for the IgG subclasses was seen, except that in this case less IgG1 was bound than IgG2c. Again, there was a considerable quantitative difference between rat strains and, as before, the ratios calculated were not the same for every subclass.

To compare the *in vitro* recoveries and *in vitro* binding data more easily, Table 3 shows the results of Tables 1 and 2 recalculated, so that the value for IgG2a for either rat strain is set as 100% and the other subclasses are compared as a percentage of the IgG2a

Table 2. The binding of ^{125}I-IgG subclasses to isolated brush borders

Isotype	Mean specific binding of IgG ± SE Wistar	Nu/+	Ratio of Nu/+ : Wistar
IgG1	0·16 ± 0·02	0·33 ± 0·07	2·06
IgG2a	1·22 ± 0·07	2·07 ± 0·05	1·70
IgG2b	0·51 ± 0·09	0·93 ± 0·04	1·82
IgG2c	0·22 ± 0·06	0·43 ± 0·02	1·95

Each group contained four rats. Binding is expressed as picomoles of IgG bound per million brush borders.

Table 3. Comparison of *in vivo* transmission with *in vitro* binding of IgG subclasses

	Binding *in vitro*		Transmission *in vivo*	
Isotype	Wistar	Nu/+	Wistar	Nu/+
IgG1	13·1	15·9	45·1	45·6
IgG2a	100·0	100·0	100·00	100·00
IgG2b	41·8	44·9	56·8	43·3
IgG2c	18·0	20·8	43·2	43·5

IgG2a was set as 100% in each vertical set and the other subclasses were calculated as a percentage of the IgG2a figure.

binding or transmission. In relation to IgG2a, binding or transmission of IgG2b was roughly the same, whereas for IgG1 and IgG2c, transmission was much higher than binding.

DISCUSSION

We have compared the *in vitro* binding to isolated brush borders of radiolabelled IgG subclasses with the amounts transmitted *in vivo* by the small intestine to the bloodstream, in two different strains of rats. We have shown that the Fc receptor in the gut of the neonatal rat displays isotype specificity similar to that shown on the mouse small intestine (Mackenzie & Keeler, 1984). In both rat strains, IgG2a showed both the greatest *in vivo* transmission (Table 1) and *in vitro* binding (Table 2), and nu/+ rats bound and transmitted all subclasses in greater amounts than Wistar rats. These data could suggest that IgG2a binds to a common Fc receptor molecule with a higher affinity than the other three subclasses. However, if the results from the two strains of rat are compared, it is clear that, both *in vivo* and *in vitro*, the nu/+ rat gut is more 'active' than that of the Wistar rat. If this were just a question of a greater number of a common IgG receptor being displayed on the gut of the nu/+ rat, then the ratio of the values obtained for the two strains should be identical for all subclasses. This is not the case for *in vitro* binding (Table 2), where IgG1 and IgG2c are shown to have similar ratios but IgG2a and IgG2b are different. Also, for *in vivo* transmission (Table 1), IgG1, IgG2a and IgG2c yields the same ratio but IgG2b was different, suggesting that it binds to a separate receptor from the other three subclasses.

Calculation of these ratios allows a comparison between the two strains of rat, but to compare the *in vitro* and *in vivo* performance of each subclass the results in each case were related to the behaviour of IgG2a (Table 3). This showed that *in vivo*, the amount of IgG2a transported into the bloodstream from the gut lumen over 2 hr was twice the amount of IgG1, IgG2b or IgG2c. In terms of *in vivo* transmission, IgG2b behaved much as it did *in vitro* in relation to IgG2a, but IgG1 and IgG2c binding was very much lower. These discrepancies between subclasses again suggest that separate receptors are in operation, which perhaps respond differently to the method of preparation of brush borders or are variably accessible to IgG after such treatment.

Our data demonstrate, therefore, the presence of at least two distinct Fc receptors on rat enterocytes, either or both of which have differential affinities for the H chains that they recognize. Similarly, two receptor molecules have been identified on both rat (Bolz-Nitulescu, Bazin & Spiegelberg, 1981) and mouse (Lane *et al.*, 1980) macrophages which bind to homologous IgG2a or IgG2b/IgG1. The interplay between different IgG subclasses in the immune response is an area which is, as yet, largely unknown. Rat IgG subclasses have different half-lives in the circulation (Peppard & Olans, 1980), varying susceptibilities to different enzymes (Rousseaux, Biserte & Bazin, 1980), and they interact differently with Protein A and the complement system (Medgyesi *et al.*, 1978). The presence in separate cell populations of surface receptor molecules which differentiate between IgG isotypes, and not always in the same way, is a further indicator of the complexity which is likely to be uncovered in such a system.

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