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Studies of the transport of polyclonal IgA antibody from blood to bile in rats

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Summary. Bile or thoracic duct lymph, collected from rats 7–9 days after suspensions of *B. abortus, S. typhi* or SRBC had been injected into the Peyer's patches, contained high titres of specific agglutinins. Samples of these fluids were injected i.v. into unimmunized, syngeneic recipients and the partitioning between blood and bile of the injected antibodies was studied and found to depend on the source and class of the antibody. IgA antibodies from lymph plasma disappeared rapidly from the recipients' blood and half of the dose was recovered in the bile within 2 h of its injection. IgA antibodies which had been collected from bile and so had previously traversed the liver and acquired secretory component, appeared in the recipients' bile much less rapidly so that less than half of the dose entered the bile over a period of 40 h. Passively administered IgG antibodies did not enter the recipients' bile to any significant extent and specific haemolysins never appeared in the bile after either passive or active immunization.

INTRODUCTION

Rat bile is a rich source of IgA (Lemaitre-Coelho, Jackson & Vaerman, 1977) because this immunoglobulin is selectively and actively transported from blood to bile (Orlans, Peppard, Reynolds & Hall, 1978; Jackson, Lemaitre-Coelho, Vaerman, Bazin & Beckers, 1978) by the hepatocytes (Birbeck, Cartwright, Hall, Orlans & Peppard, 1979). It seems likely that the process is initiated by polymeric IgA in the blood uniting with secretory component (SC) which is displayed on the sinusoidal surfaces of the hepatocytes (Orlans, Peppard, Fry, Hinton & Mullock, 1979; Fisher, Nagy, Bazin & Underdown, 1979). All these experiments were based on the use of monoclonal myeloma IgA and although there is circumstantial evidence that polyclonal, IgA antibodies produced by the gut-associated lymphoid tissue (GALT) have similar properties (Hall, Orlans, Reynolds, Dean, Peppard, Gyure & Hobbs, 1979) we sought to verify this by direct, passive transfer experiments.

It would be predicted from the above outline that polymeric IgA antibodies that had not yet combined with SC (e.g. those in intestinal and thus thoracic duct lymph) would be transported across the liver much more rapidly than similar antibodies that had already united with SC (e.g. those in bile), and it was this difference that was tested by experiments *in vivo*.

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MATERIALS AND METHODS

General plan

Under general anaesthesia, cannulae were inserted

into the common bile duct and one femoral vein of the recipient rats which were then placed in Bollman restraining cages. When the rats had recovered fully from the anaesthesia, the test antibody present in either lymph plasma or bile, was injected into the venous cannula in a total volume of up to 1·5 ml. Thereafter, samples of blood and bile were collected at appropriate intervals, the titre of the specific antibody being measured by agglutination and its class by the binding of ^{125}I-labelled, class-specific antiglobulin reagents.

Animals and surgical techniques

Male, inbred Wistar rats between 10 and 12 weeks of age were taken from our own barrier-maintained colony as required. The details of the barbiturate anaesthesia and bile duct cannulation have been published (Hall *et al.*, 1979); thoracic duct lymph was obtained from cannulated animals after the method of Bollman, Cain & Grindlay, (1948).

Donor antisera

Rats were immunized by injecting the Peyer's patches with suspensions of *B. abortus* or *S. Typhi 'O'* (Wellcome Research Laboratories, Beckenham, Kent) or washed sheep red cells (SRBC) obtained by percutaneous venepuncture, as described previously (Hall *et al.*, 1979). Between 7 and 9 days later bile or thoracic duct lymph plasma, together with blood serum, was collected from the immunized animals and stored frozen pending assay and injection into recipient animals.

Antibody assay

Agglutinating antibodies to bacterial or SRBC were titrated by standard methods using doubling dilutions in 50 μl volumes of phosphate-buffered saline in round-bottomed microtitre plates.

The class of immunoglobulin binding to the target antigen was determined by using ^{125}I-labelled, affinity-purified antiglobulin reagents as detailed previously (Hall *et al.*, 1979). The methods for immunodiffusion and immunoelectrophoresis applicable to bile and lymph, and the preparation of myeloma IgA have been described (Orlans *et al.*, 1978).

Detection of rat secretory component

The isolation from bile of normal polymeric IgA and of free SC, together with the preparation and properties of a rabbit antiserum to rat SC have been described by Orlans *et al.* (1979) who also showed that normal biliary IgA contained SC.

RESULTS

Forty recipient rats and a similar number of donors were used in the experiments. In order to make direct comparisons easier, the results shown in the graphs below all came from experiments in which antibodies directed against the somatic antigens of killed *B. abortus* were measured.

Passive transfer of antibodies contained in thoracic duct lymph plasma

Although thoracic duct lymph plasma from either normal or actively immunized rats contained abundant IgA (Orlans *et al.*, 1978), it never reacted with the anti-SC serum in immunodiffusion tests; bile, on the other hand, always did. Lymph collected between 7

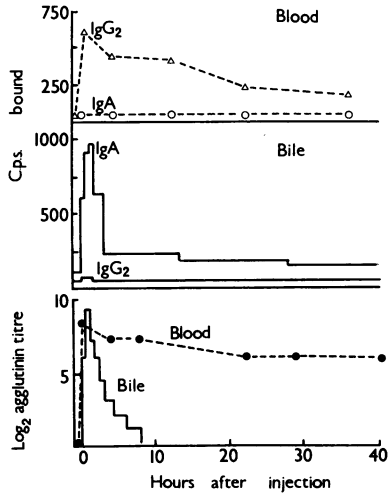

Figure 1. The partitioning of antibodies to *B. abortus* between the blood and bile of a recipient rat after passive transfer (i.v.) at time zero of thoracic duct lymph plasma from an actively immunized donor. The distribution of IgG2 and IgA antibodies is shown in the upper graph. The isotypes were determined by absorbing the antibodies on to *B. abortus* organisms and allowing them to react with class-specific ^{125}I-labelled antiglobulin reagents. The results are expressed in terms of the amounts of radioactivity (counts per second, c.p.s.) that were bound by the washed, antibody-coated bacteria. The lower graph shows the titres of agglutinins that appeared in the blood and bile of the recipient. IgA antibodies never achieved a substantial level in the blood, being conveyed rapidly to the bile at the same time as high titres of agglutinating activity. IgG_2 antibodies did not appear in the bile in significant amounts, they remained in the blood and presumably accounted for the agglutinating activity of the serum.

and 10 days after antigens had been injected into the Peyer's patches contained high titres (up to 1:64,000) of specific agglutinins. The partitioning between blood and bile of such material after intravenous injection is shown in Fig. 1. Agglutinating activity started to appear in the bile almost immediately and within 2 h reached peak levels which declined to insignificant levels by 5 h. By pooling the bile that had been collected quantitatively during this 5 h period and comparing its agglutinating titre with that of the injected material (diluted to the same volume) it was possible to estimate the proportion of the injected dose of agglutinating activity that had been recovered in the bile. In different experiments the recovery varied between 25 and 100% of the injected dose. Allowing for the variance implicit in titration by doubling dilutions, this suggest that half or more of the injected antibody was transported rapidly to the bile. As in active immune responses (Hall *et al.*, 1979), all the agglutinins that appeared in the bile were shown by antiglobulin tests to be of the IgA class, while the agglutinins that persisted in the blood could be accounted for by the IgG2 antibodies which, unlike those of the IgA class, remained in that compartment.

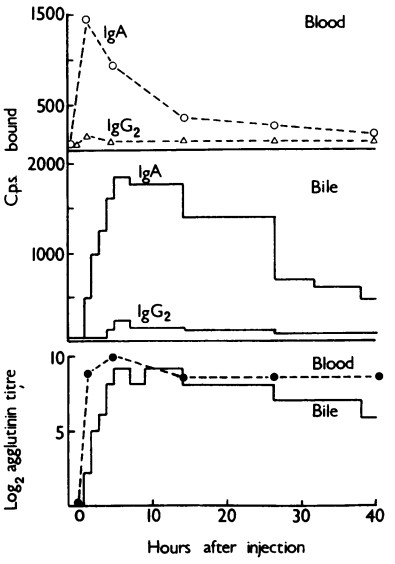

Figure 2. The partitioning of antibodies to *B. abortus* between blood and bile after the passive transfer (i.v.) of bile from an actively immunized donor. As in Fig. 1 the isotype distribution is shown in the upper graph and the antibody titres are shown in the lower one. Although the antibody activity was virtually confined to immunoglobulin of the IgA class it did not leave the blood and appear in the bile nearly as quickly as did the SC-free IgA antibody from thoracic duct lymph, shown in Fig. 1.

Passive transfer of antibodies contained in bile

When bile containing agglutinating antibodies was injected intravenously into unimmunized recipients, the antibody activity partitioned between blood and bile as shown in Fig. 2. Although the injected antibodies were of the IgA class, they did not appear in the bile as rapidly as IgA antibodies from thoracic duct lymph; maximum levels were not attained until the fifth hour and this level did not decline for 30–40 h, by which time less than half of the injected dose of agglutinating activity had been recovered.

An examination of the isotype distribution of the agglutinins in the blood showed that the level of IgA antibodies declined relatively slowly. It was evident that the IgA antibodies that had previously undergone secretion in the actively immunized donor, where they would inevitably have acquired SC, were transported from the blood to the bile of a passive recipient much less rapidly than their SC-free analogues from the thoracic duct lymph.

Control of non-specific effects of the intravenous injection of bile

The results of the above experiments are consistent with the view that IgA antibodies undergo secretion because they unite with SC on hepatocytes (or enterocytes) and are inhibited from doing so if they have previously combined with SC elsewhere. Nonetheless, there are alternative possibilities; for example, the intravenous injection of a bolus of raw bile might damage hepatic function and so delay the transport of antibody into the bile. In order to exclude this possibility samples of thoracic duct lymph with high agglutinin titres were mixed with normal bile before injection. This type of experiment presents difficulties; rat bile contains free SC (Lemaitre-Coelho *et al.*, 1978), and this might unite spontaneously with IgA and so perhaps delay its secretion. In order to elucidate the situation, three different preparations of antibody-rich thoracic duct lymph were injected. The first comprised 0·5 ml of lymph and 1·0 ml of saline; the second contained 0·5 ml of lymph mixed with 1·0 ml of normal bile which had previously been incubated for 6 h at 20° with approximately 20 mg of polymeric myeloma IgA; and the third contained 0·5 ml of lymph mixed with 1·0 ml of bile which had been similarly incubated with 20

mg of normal rat serum proteins. Each mixture was injected intravenously into a rat and the appearance of agglutinins in the recipients' bile was monitored and the results are shown in Fig. 3. The antibody activity in the thoracic duct lymph mixed with saline or with bile treated with an excess of myeloma IgA behaved normally, i.e. it was rapidly secreted in the recipients' bile. Conversely, the antibodies in thoracic duct lymph which had been mixed with bile in the presence of normal serum (which contains little IgA) did not attain a substantial titre in the recipient's bile during the 5 h of the experiment. It was concluded that the myeloma IgA that had been added to the bile had combined with all the free SC so that the IgA antibodies in the lymph reached the liver in their native state and were able to unite with the SC on the hepatocytes. In the absence of excess IgA, i.e. when only normal serum was added to the bile, the free SC united with the lymph antibodies and blocked their attachment to the hepatocytes, and so prevented their rapid transport to the bile.

Experiments with other antigens.

Donor rats immunized with *S. typhi 'O'* or SRBC yielded body fluids containing agglutinins which behaved like those to *B. abortus* in passive transfer experiments. Although high titres of agglutinins to SRBC appeared in the bile of both actively and passively immunized rats, specific haemolysins were never detected in this fluid even though they were present in the blood. This result was not caused by the inhibition by bile of complement-mediated lysis. The pre-incubation with bile for 1 h at 38° of either the immune serum or the normal rat serum that was used as a source of complement made no significant difference to the haemolysin titre. Presumably, this result reflects the fact that IgA antibodies are unable to activate complement (Heremans, 1974). Significant amounts of antibody activity associated with IgM were not found in any of the experiments.

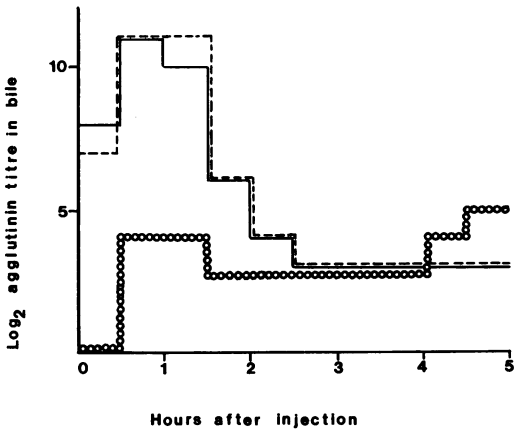

Figure 3. Agglutinin titres to *B. abortus* in the bile collected at intervals of 0·5 h for 5 h from rats which had received an i.v. injection at time zero of a preparation of antibody-rich thoracic duct lymph. One rat (——) received lymph mixed with normal saline; one rat (- - -) received lymph mixed with bile that had been pre-incubated with myeloma IgA, and one rat (ooo) received lymph mixed with bile that had been pre-incubated with normal serum. Antibody activity in lymph mixed with either saline or bile treated with excess IgA was transported to the bile in the normal, rapid way shown in Fig. 1. Antibody activity in lymph mixed with normal serum (which contained insufficient IgA to bind all the free secretory component in the bile with which it was incubated) was inhibited from entering the recipient's bile.

DISCUSSION

In contrast to the IgG and IgM antibodies, which are included inevitably in the ultrafiltrate of blood which is the basic material for lymph formation, the intestinal (and thus thoracic duct) lymph contains IgA in much higher concentrations than the blood (Orlans *et al.*, 1978). This IgA is produced in the GALT, and that proportion of it that does not unite immediately with the SC on the enterocytes must necessarily enter the intestinal lymph (Hall, Orlans, Peppard & Reynolds, 1978). Our present results confirmed that the lymph-borne IgA antibodies are not associated with SC, and as soon as the blood conveyed them to the liver they were able to undergo rapid active transport into the bile, presumably because they united with the SC on the surfaces of the hepatocytes. Thus, there is now a considerable body of direct evidence that both monoclonal and polyclonal IgA molecules behave in this way in rats, and this mechanism is in general accord with the concepts of IgA transport as recently reviewed by Brown (1978). However, the quantitative significance of biliary IgA secretion in relation to the total amount of IgA that enters the gut is not known precisely. Certainly, in rats, the bile can deliver at least 12 mg of IgA to the gut every day (Hall *et al.*, 1979), and if one takes the simple view that the amount of IgA transported is related directly to the mass of cells capable of doing so, then the liver must secrete more than the gut. On the other hand, the concentration of IgA is lower in the blood than in the interstitial fluid of the gut (Orlans *et al.*, 1978; Hall *et al.*, 1978) and so the hepatocytes may have less opportunity for transporting IgA than

the enterocytes that overlie the plasma cells of the lamina propria. The problem is not easy to resolve by simple experiments; labelled IgA that is injected intravenously passes through the fenestrated endothelium of the portal sinusoids so quickly that it is available to the hepatocytes virtually immediately (Birbeck *et al.*, 1979) and is thus removed from the circulation before it has a chance to diffuse into the extravascular compartment of the gut mucosa and reach the enterocytes. On the basis of our current experiments we believe that, in rats, bile is the major pathway for the delivery of IgA antibodies to the gut. However, this may not be true of other species, particularly those where the bile contains significant amounts of antibodies of the IgG classes; we have found this to be the case in sheep and similar findings have been reported in man (Chodirker & Tomasi, 1963) and primates (Keclik, Wolf, Felsenfeld & Smetana, 1970). Although it seems likely that bile may be an important pathway for the delivery of immunoglobulins to the gut in many animals, only further experiment will reveal the facts.

ACKNOWLEDGMENTS

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