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IgA antibodies in the bile of rats

II. EVIDENCE FOR IMMUNOLOGICAL MEMORY IN SECRETORY IMMUNITY

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Summary. The Peyer's patches of Wistar rats were injected with suspensions of either sheep red blood cells (SRBC) or killed *Brucella abortus* organisms in doses that were insufficient to induce the appearance of biliary antibodies. The rats were challenged after periods ranging from 1 week to 1 year with the same dose of antigen given by the same route, and their bile was monitored for the appearance of specific antibodies. The test animals produced biliary antibodies to a much higher titre, and usually more rapidly, than control rats which had received the total dose of antigen as a single injection. As in primary responses, the biliary antibodies produced by challenging the primed rats were predominantly of the IgA class.

The ability to mount substantial biliary responses to suboptimal doses of antigen could be transferred from primed donor rats to unimmunized recipients by thoracic duct lymphocytes, but not humoral factors, collected between 3 weeks and 5 months after priming. γ-Irradiation of the lymphocytes abolished this effect.

These results suggest strongly that immunological memory exists in the IgA system and that it is mediated by circulating lymphocytes.

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INTRODUCTION

The existence of immunological memory within the secretory immune system is controversial. Attempts to demonstrate increased responsiveness after local immunization in humans (Ogra & Karzon, 1969) and in pigs (Porter, Kenworthy, Noakes & Allen, 1974) failed to do so, and studies of the splenic response in mice, measured by enumerating plaque-forming cells, after repeated oral immunization showed no evidence of a secondary IgA response (André, Bazin & Heremans, 1973). These studies have been interpreted by many to indicate that the secretory immune system lacks immunological memory. On the other hand, increased numbers of IgA antibody-containing cells have been found in the lamina propria of the small intestine after repeated intragastric immunization of mice with sheep red blood cells (SRBC; André, André, Druget & Fargier, 1978) and after intraduodenal challenge of parenterally primed rats (Pierce & Gowans, 1975; Husband & Gowans, 1978) and dogs (Pierce, Sack & Sircar, 1977), or intraduodenally primed rats (Pierce, 1978) with cholera toxoid. The results of these studies are consistent with the existence of a secondary secretory IgA (sIgA) response to local stimuli, but this has not been shown by the direct measurement of humoral antibody in secretions.

The recent demonstration that humoral responses involving the production of sIgA can be monitored by titrating the specific IgA antibodies that appear in the

bile of appropriately immunized rats (Lemaître-Coelho, Jackson & Vaerman, 1978; Hall, Orlans, Reynolds, Dean, Peppard, Gyure & Hobbs, 1979) has provided another approach to this problem. In the experiments reported here the production of IgA antibodies during secondary responses was studied by measuring specific agglutinating antibodies in the bile of rats that had been primed and challenged by the injection of antigens into the Peyer's patches.

MATERIALS AND METHODS

General plan

Two means of demonstrating immunological memory were essayed. Firstly, a dose of antigen just insufficient to provoke the appearance of biliary antibody (chosen on the basis of our previous study of the effect of primary injection of antigens) was given as a priming dose into the Peyer's patches of rats. At various times thereafter the same, sub-threshold, dose of antigen was injected into the Peyer's patches of the primed rats and the subsequent appearance of substantial titres of specific biliary antibodies was taken as evidence of secondary responsiveness.

Secondly, thoracic duct lymphocytes were collected from primed rats and adoptively transferred by intravenous injection into unimmunized, syngeneic recipients, which were challenged later with the same dose of antigen and their bile monitored for the appearance of specific antibodies.

Detailed methods were the same as in the previous paper.

RESULTS

Response to a second injection of antigen

Six Wistar rats were primed by injecting a dose of 5×10^5 *Brucella abortus* into the Peyer's patches; this dose was shown previously to cause no detectable antibody to appear in the bile, although a little appeared in the serum. Three weeks later the rats were challenged with the same dose of *B. abortus*; substantial titres of agglutinating antibodies occurred in the bile on day 4 and reached a peak the following day (Fig. 1). This peak occurred 2–3 days earlier than that which occurred after the optimal, primary doses of the same antigen that we used in our previous study. Rats challenged 7 days to 1 year after priming all showed this rapid and substantial production of biliary antibody (Table 1), although in rats challenged 8 weeks or more after priming the peak occurred between days 5 and 7. Six control animals injected in the Peyer's patches with saline in place of either the first or second injection of *B. abortus* produced no detectable specific agglutinins. Similarly, a further six control animals

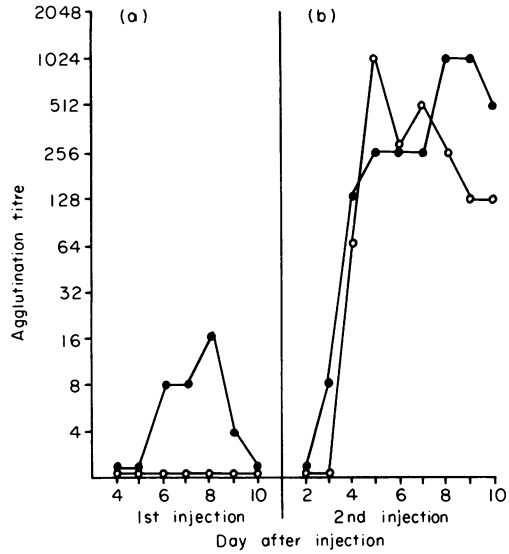

Figure 1. The titres of antibody in the bile (o——o) and blood serum (●——●) of Wistar rats that had had killed *B. abortus* organisms onjected into their Peyer's patches: (a) after a single injection of 5×10^5, (b) after a second injection of 5×10^5 given 21 days later. Each point records the result obtained by pooling equal volumes of either bile or blood serum from six rats.

Table 1. Maximum antibody titres in the bile and serum of Wistar rats given a second injection of 5×10^5 organisms of *B. abortus* into the Peyer's patches at different times after the first injection at the same site with the same number of *B. abortus*

Time interval between 1st and 2nd injections	No. of rats in group	Bile		Serum	
		Peak titre	Day	Peak titre	Day
7 days	4	512	5	512	7
14 days	6	512	5	1024	6
21 days	6	1024	5	1024	8
8 weeks	6	512	7	2048	7
3 months	3	4096	5	4096	9
6 months	3	1024	6	4096	7
12 months	4	1024	7	1024	8

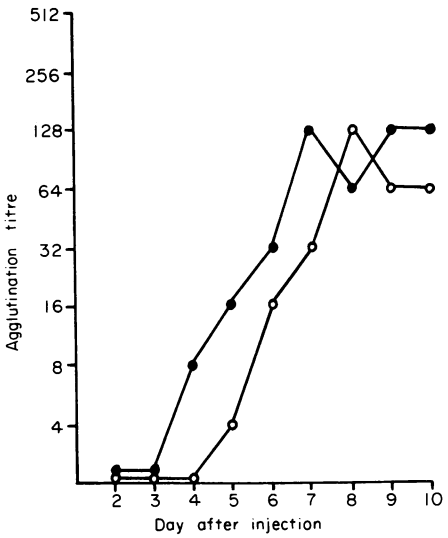

Figure 2. The titres of antibody in the bile (o——o) and blood serum (●——●) of Wistar rats injected in the Peyer's patches, on a single occasion, with a total dose of 10^6 killed *B. abortus* organisms. Each point records the result obtained by pooling equal volumes of either bile or blood serum from six rats.

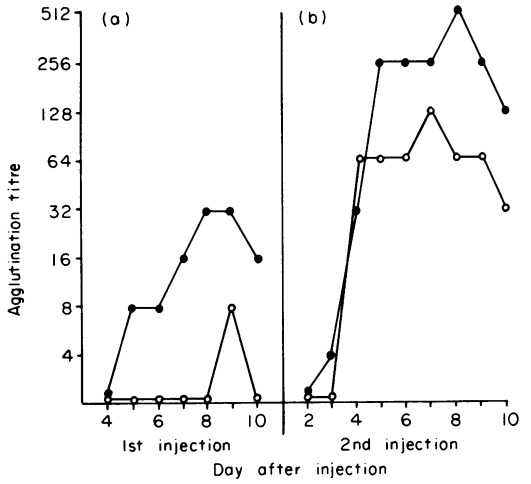

Figure 3. The titres of antibody in the bile (o——o) and blood serum (●——●) of Wistar rats that had had SRBC injected into their Peyer's patches; (a) after a single injection of 10^5, (b) after a second injection of 10^5 given 21 days later. Each point records the result obtained by pooling equal volumes of either bile or blood serum from six rats.

which were primed with *B. abortus* and challenged with *Salmonella typhi O* produced no anti-brucella agglutinins.

B. abortus has been shown to persist for several weeks in host tissue and it was thus just possible that two sub-threshold doses might have summated to produce an immunogenic stimulus. However, Fig. 2 shows that rats given the total dose (10^6 organisms) as a single injection into Peyer's patches, produced biliary responses which were much lower than those produced after secondary challenge.

Secondary responses to SRBC

In order to exclude the possibility that the apparent secondary responsiveness was restricted to the somatic antigens of bacteria, we repeated the experiment using SRBC, a more thymus-dependent antigen. Figure 3 shows the responses of six Wistar rats to priming and challenge doses of 10^5 SRBC given 21 days apart. The results were generally similar to those obtained with *B. abortus*.

Isotype of biliary and blood serum antibodies collected during secondary responses

The distribution of antibody activity amongst the isotypes of immunoglobulins was determined by radioimmunoassay using affinity-purified antiglobulins labelled with ^{125}I and is shown in Table 2. It can be seen that, as in primary responses, the antibody activity was associated predominantly with IgA, which was the principal immunoglobulin in all the specimens of rat bile that we have examined. The antibody activity in the blood serum was associated mainly with IgM, the remainder with IgG.

Adoptive transfer of the secondary IgA response with small lymphocytes from thoracic duct lymph

An important characteristic of immunological memory is that it can be transferred to an unprimed, syngeneic recipient with small lymphocytes from the thoracic duct but not with humoral factors (Gowans & Uhr, 1966; Hunt, Ellis & Gowans, 1972; Mason, 1976).

The ability of thoracic duct lymphocytes from donors primed by injecting antigens into the Peyer's patches, to transfer to unprimed recipients a secondary biliary IgA response is shown in Table 3. A secondary humoral response occurred also in the blood serum of the recipients.

Thoracic duct lymphocytes collected 5 months after priming were as competent at transferring a secondary

Table 2. The distribution of antibody isotypes to *B. abortus* in bile and serum 6 days after the second of two injections each of 5×10^5 organisms of *B. abortus* into the Peyer's patches of Wistar rats

Bacteria treated with	Antibody titre of sample	Dilution of sample	c.p.m. $\times 10^{-3}$/tube after addition of ^{125}I-antibody* to		
			α	γ	μ
Medium	—	—	0·6	0·5	0·2
Normal bile	0	1:10	0·8	0·7	0·4
Normal serum	0	1:10	0·9	1·0	0·9
Immune bile	1000	1:10	36·1	0·9	1·1
Immune bile	1000	1:40	13·0	0·6	0·5
Immune serum	1000	1:10	1·5	1·9	7·0
Immune serum	1000	1:40	0·8	0·8	1·3

* $1{\cdot}68 \times 10^5$ c.p.m./tube added.

biliary response as those collected after 3 weeks. Even at this earliest time immunoblast responses in the donors' lymph were over so that less than 2% of the lymph cells were immunoblasts.

The fact that the rats that received irradiated cells responded as they would during a normal primary response to the same dose of bacteria (Fig. 1) shows that these results cannot be attributed to the inadvertent transfer of antigen, or of antibody-producing immunoblasts which, unlike small lymphocytes, are very radioresistant (Denham, Wrathmell & Alexander, 1975).

Control experiments were carried out to ensure that humoral factors could not transfer a secondary biliary

Table 3. The adoptive transfer of thoracic duct lymphocytes from Wistar rats (primed with a single injection into the Peyer's patches of 5×10^5 organisms of *B. abortus*) to unimmunized recipient rats. One day after the cell transfer the recipients were injected with 5×10^5 organisms of *B. abortus* into the Peyer's patches

Rat	No. TDL injected ($\times 10^{-9}$)	Pretreatment of TDL*	Time Interval†	Antibody titre‡			
				Bile		Serum	
				Peak titre	Day	Peak titre	Day
A	1·1	—	21 days	128	7	512	6
B	1·1	—	21 days	256	7	256	9
C	12·0	—	22 days	256	7	64	7
D	0·9	—	26 days	256	8	2048	7
E	1·3	—	5 months	128	6	32	6
F	1·2	40 Gray	5 months	0		8	7
G	0·75	40 Gray	5 months	0		16	8
H	saline	—		0		16	8

* Two recipient rats received cells which had been exposed to 40 Gray of γ-irradiation to stop cell division and differentiation.

† Time between the injection of the donor rats and the collection of TDL for adoptive transfer.

‡ Maximum antibody titres in the bile and serum of the recipient rats.

response. Four rats which had been primed as had the lymph donors were exsanguinated a month after priming. A dose of 5 ml of blood serum, which contained no antibody detectable by agglutination, was injected intravenously (i.v.) into each of four recipients which received a challenge dose of *B. abortus* into the Peyer's patches a day later. The ensuing responses were of the primary pattern, i.e. specific agglutinins appeared in the blood in low titre but none appeared in the bile. Similarly, the transfer of serum from hyperimmune donors with a titre of 1:16,000 did not induce a biliary response to a primary challenge of 5×10^5 organisms given into the Peyer's patches of the recipients.

DISCUSSION

In attempting to explain previous failures to detect enhanced secondary sIgA responses the nature of the immunization, the type of antigen and its route of administration, must be taken into consideration. In many of the earlier studies antigens were administered orally (André *et al.*, 1973; Porter *et al.*, 1974) and usually serial immunization was necessary to produce any detectable antibodies in either the secretions or the blood serum. Thus the primary and secondary responses may have arisen consecutively and appeared only as a prolonged primary response. We have found oral feeding to be an inefficient method of immunization which produced responses that varied greatly between individual rats. Presumably, such factors as the rate of peristalsis and the presence of food, mucus, bile salts and enzymes in the gastrointestinal tract affected the amount of antigen that reached the gut-associated lymphoid tissues. However, antigens such as cholera toxin or toxoid which are capable of binding to cell membranes are better at eliciting local responses than those which do not (Pierce, 1978) and hence the use of such antigens may obviate the need for serial immunizations. That this is so was demonstrated by Pierce & Gowans (1975) and Husband & Gowans (1978) who detected an enhanced cellular response in the intestinal lamina propria to a secondary challenge of parenterally primed rats with a single intraduodenal dose of cholera toxoid. The experiments reported here complement these findings and show that after a secondary challenge into the Peyer's patches of Peyer's patch-primed rats a rapid and increased production of biliary antibodies was produced, and furthermore that this secondary response in bile was, like the primary response, almost exclusively of the IgA class. Taken together with the ability of this heightened responsiveness to be transferred with thoracic duct lymphocytes but not with serum from primed donor rats, these results suggest strongly the existence of immunological memory in the IgA system. During the secondary response to *B. abortus* serum antibodies were also produced and these were predominantly of the IgM class which is consistent with the findings of other workers (e.g. Mond, Caporale & Thorbecke, 1974) using this antigen. The cellular details of the priming process remain to be elucidated; special sets of T cells (Elson, Heck & Strober, 1979) and B cells (Fuhrman & Cebra, 1981) are believed to be necessary, and our experiments say nothing about this aspect. However, the overall features of IgA responses are turning out to be generally similar to those involving other Ig classes, except that until recently they have not been as easy to study.

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