Immunology 1985 **54** 601

Neutralization of cholera toxin by rat bile secretory IgA antibodies

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Accepted for publication 29 October 1984

Summary. IgA-antibody (AB) activities have been elicited in rat bile against several antigens such as bacteria, erythrocytes, tumour cells, haptens and proteins (Lemaître-Coelho, Jackson & Vaerman, 1978; Hall *et al.*, 1979; Montgomery, Lemaître-Coelho & Vaerman, 1980; Peppard *et al.*, 1982). However, their biological significance, except for plasma clearance of immune complexes (Peppard *et al.*, 1982) and bacterial agglutination, remains conjectural, despite their possible major contribution to rat intestinal immunity. The importance of local intestinal immunity in protection against cholera is today widely admitted (Jertborn, Svennerholm & Holmgren, 1984).

Intraintestinally given cholera toxin (CT) is a potent immunogen in rats whose intestinal mucosa then harbours numerous anti-CT IgA plasma cells (Pierce, 1978). Since bile IgA in rats is largely, but not entirely, derived from intestinal synthesis (Vaerman, Lemaître-Coelho & Jackson, 1978; Manning *et al.*, 1984), rats intestinally immunized with CT could display high levels of anti-CT IgA AB in their bile, and these AB might neutralize CT in the biologically relevant intestinal loop assay (Lange & Holmgren, 1978).

Four rats were twice immunized intraduodenally (2 weeks apart) with 20 μg of pure CT (Behring, La Jolla, CA) in 0·2 ml of phosphate-buffered saline, pH 7·4 (PBS) containing 0·02% (w/v) gelatin (PBSG). Bile was collected 4 days after boosting for 4–5 days. Individual bile samples were analysed for the anti-CT AB content of their IgA, IgG and IgM by autoradiography of anti-α, anti-γ and anti-μ Mancini plates incubated with ^{125}I-CT The bile of one rat had high CT-binding IgA, with virtually no IgM and weak IgG anti-CT AB (Fig. 1A). The corresponding serum contained small amounts of IgA with relatively high CT-binding AB and larger amounts of IgM and IgG with low CT-binding AB (not shown). Control rat biles and serum showed no CT-binding by any Ig class. The CT-neutralization capacity of this bile, and of its purified (Acosta-Altamirano *et al.*, 1980) sIgA fraction (Fig. 1B) was assayed. At first, rats were not used because their intestinal contents 'neutralized' the CT, requiring a 6-day long preparation and thorough pre-washing of their small intestine before lower ileal loop assays were consistent (Aziz *et al.*, 1968). Therefore, CT-neutralization was first assayed in fasted (24 hr) mouse (C57B1/6) upper jejunal loops (5–9 cm long, one loop per mouse) challenged (i) with PBSG (0·2 ml) or CT (2 μg in PBSG), mixed with normal (N) or immune (I) bile (0·4 ml), and (ii) with PBSG or CT (2 μg) mixed with N or I purified biliary sIgA (300 μg). Later, we found that 2 μg of CT gave consistent results also in fasted (24 hr) rat (OFA strain) upper jejunal loops. Others (C.O. Jacob, personal communication) confirmed a decreasing sensitivity to CT from upper jejunum to lower ileum in mice and rats. The loops were dissected 4 hr after challenge and weighed. Results were expressed as differences (D) between weights (mg/cm) of CT- and PBSG-challenged loops.

Erratic results were first obtained because 0·3–0·4 ml of fresh normal rat bile consistently

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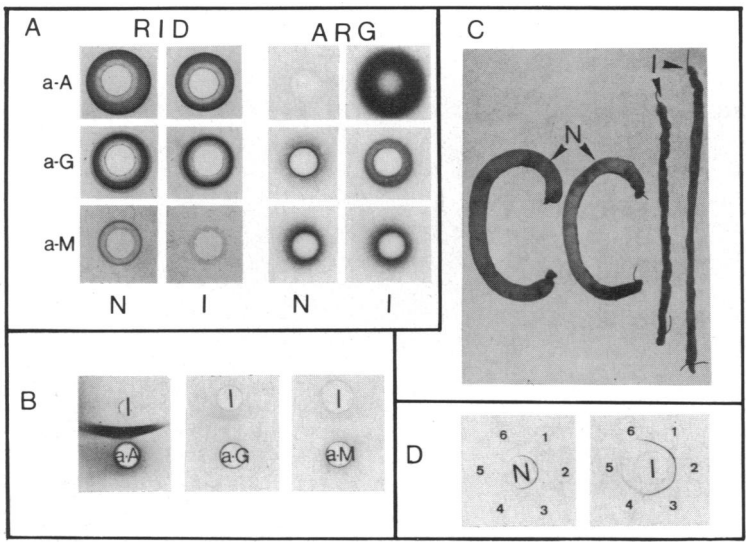

Figure 1. Anti-cholera toxin antibody activity of normal (N) and immune (I) bile sIgA. (A) Binding of ^{125}I-CT by IgA, IgG and IgM in N and I bile, revealed by autoradiography (ARG) of radial immunodiffusion plates (RID) using anti-α, -γ and -μ antisera and incubation with ^{125}I-CT. (B) Double diffusion in agarose gel of purified I bile sIgA (7·3 mg/ml) against anti-α, anti-γ and anti-μ antisera. Note the larger antigen reservoirs employed to check the absence of contaminating IgG and IgM. (C) Mouse intestinal loops challenged with 2 μg of CT mixed with 300 μg of N sIgA (left) or I sIgA (right). (D) Double diffusion in gel containing 2% polyethyleneglycol of N (left) and I (right) purified sIgA (225 μg) against doubling dilutions of CT starting at 312 μg/ml.

Table 1. Cholera-toxin neutralizing capacity of normal and immune bile, and/or of their purified sIgA fractions, assayed in mouse and rat jejunal loops

Mouse				Rat	
Bile (400 μl)*		sIgA (300 μg)		sIgA (300 μg)	
Normal†	Immune‡	Normal	Immune	Normal	Immune
143§	28	149	4	253	11
141	11	80	17	274	22
86	3	146	1	296	7
84	18	174	6	299	5
180	10	79	2		
	8				
Mean D = 126·8	14·7	125·6	6·0	280·5	11·3
SD ± 41·2	±12·4	±43·5	±6·4	±21·5	±7·6

* Bile filtered on AG1X8 resin and concentrated to its original volume.

† Normal bile was a pool of bile of four normal rats.

‡ Immune bile was from the rat with highest IgA CT-binding activity.

§ All figures are differences (D) between weights of toxin-injected loops (mg/cm) and weights of PBSG-injected loops (mg/cm). D values below 40 indicate a lack of toxic effect.

'neutralized' 2 μg of CT in the mouse loop assay, a dose which, when given in PBSG, induced fluid accumulation in 10/10 loops. The detergent effect of bile salts was thought to be responsible of this, by dissociation of the CT into its A and B units (Holmgren, 1981). In order to remove bile salts, N and I bile were filtered through an AG1X8 Biorad resin column (2·5 × 10 cm for 10 ml of bile) in PBS; the bile was then concentrated to its original volume without significant protein loss. This procedure efficiently removed the 'neutralizing' activity of N bile, but not of I bile (Table 1).

When purified N and I bile sIgA were tested in mouse and rat loops, only I sIgA neutralized CT (Table 1, Fig. 1C). Finally, upon double diffusion in 1·5% (w/v) agarose gel containing 2% (w/v) of polyethylene glycol 6000, only I bile sIgA was able to precipitate with CT (Fig. 1D).

Our results demonstrate that two intraintestinal immunizations of rats with CT without adjuvant elicit good biliary sIgA anti-CT AB capable of biologically neutralizing and precipitating CT. These AB could play a major role in the protection of the rat intestine against CT. The reactivity of these sIgA AB with the A and/or B unit of CT is currently tested and compared to that of rat anti-CT IgG AB induced by parenteral immunizations. The capacity of intraintestinal B unit, a stronger immunogen that A unit (Holmgren, 1981) to elicit bile sIgA AB is also under study. This model should further allow us to test if rats, immunized twice intraintestinally with CT (or its B subunit), are still significantly protected in their own jejunum (loop assay) when these bile sIgA AB are diverted from the gut by cannulation or bile duct ligation.

The medical relevance of these data lies much less in the human liver transport of plasma IgA in hepatic bile (~ 50-fold smaller than in rats) (Delacroix *et al.*, 1983) than in the use of rat bile sIgA as an experimental model to assess the local immunization efficiency of present and future oral cholera vaccines, including synthetic ones.

ACKNOWLEDGMENTS

This work was supported by Grant No. 3.4504.70 from the Fund for Medical Scientific Research, Brussels. D. L. Delacroix is Senior Research Assistant at the National Fund for Scientific Research, Brussels.

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