Immune responses of mice to orally administered asialo GM1-specific rabbit IgG in the presence or absence of cholera toxin

Y. UMESAKI & H. SETOYAMA *Yakult Central Institute for Microbiological Research, Kunitachi, Tokyo, Japan*

Accepted for publication 1 October 1991

SUMMARY

Cholera toxin (CT) has been shown to be a most potent mucosal immunogen and an adjuvant to orally administered unrelated antigens. We investigated the effect of the oral administration of substances with the ability to bind to intestinal epithelial cells on the immune responses against themselves in the presence or absence of CT. Mice were fed non-specific rabbit IgG (RGG) or rabbit IgG (a-GA1) specific to asialo GM1 glycolipid, a major component of the apical membrane of mouse small intestinal epithelial cells, with or without CT. Oral administration of a-GA1 evoked stronger antibody responses than that of RGG in both the serum and intestinal fluid in the presence of CT. However, when the antigens were administered singly without CT, no significant antibody response was detected. In this case, oral administration of RGG induced severe suppression of the systemic antibody response to a subsequent intraperitoneal injection of RGG. In contrast, a-GA1 could not induce oral tolerance. Together these findings suggest that substances with the ability to bind to intestinal epithelial cells are strong immunogens in the presence of CT and weak tolerogens in the absence of CT.

Immune responses to orally administered antigens are generally very weak. However, there are some substances which evoke strong antibody responses by oral administration. Examples include bacterial pili proteins, lectins,[1] cholera toxin (CT)[2,3] and the heat-labile toxin of enterotoxigenic *Escherichia coli* (LT).[4] Both CT and LT not only evoke strong immune responses to themselves but also show a mucosal adjuvant effect on an unrelated antigen which is orally administered at the same time. It is believed to be important for mucosal immunogenicity for a substance to have the ability to bind to the intestinal epithelial cells as these toxins do. However, the precise mechanism of immune responses to the orally administered compounds is still obscure.

In this study, we investigated the effect of the nature of the antigen on its immunogenicity in the presence or absence of CT. We used two antigens differing only in their ability to bind to the intestinal epithelial cell. One was asialo GM1 glycolipid-specific rabbit IgG (a-GA1) and the other was normal rabbit IgG (RCG) which was absorbed with an intestinal epithelial cell suspension to exclude components that cross-react with them. Asialo GM1 glycolipid has been shown to be a major component of the apical membrane of mouse small intestinal epithelial cells.[5] This a-GA1 did not cross-react with GM1 ganglioside, which is considered to be a receptor for CT (Fig. 1a). Histochemical examination revealed that the antibody stained the apical membrane of the villus epithelial cells and Peyer's patch-associated epithelial cells (Fig. 1b). When a-GA1 was orally administered to the mice, it was expected to bind preferentially to the villus cells, because the surface area of villus cells is much greater than that of Peyer's patch-associated epithelial cells.

a-GA1 or RGG was orally administered to the mice singly or in combination with CT. Although single administration of either antigen induced hardly any antibody response, their simultaneous administration along with CT evoked strong antibody responses in both the serum and the intestinal fluid (Fig. 2). Both serum IgG and intestinal IgA anti-rabbit IgG responses, induced in the presence of CT by a-GA1 administration, were much greater than those induced by RGG. However, there was no significant difference in antibody response between the two antigens following intraperitoneal injection (data not shown). These results suggest that antigens with the ability to bind to the intestinal epithelial cells elicit a stronger antibody

Correspondence: Dr Umesaki, Yakult Central Institute for Microbiological Research, Yaho 1796, Kunitachi, Tokyo 186, Japan.

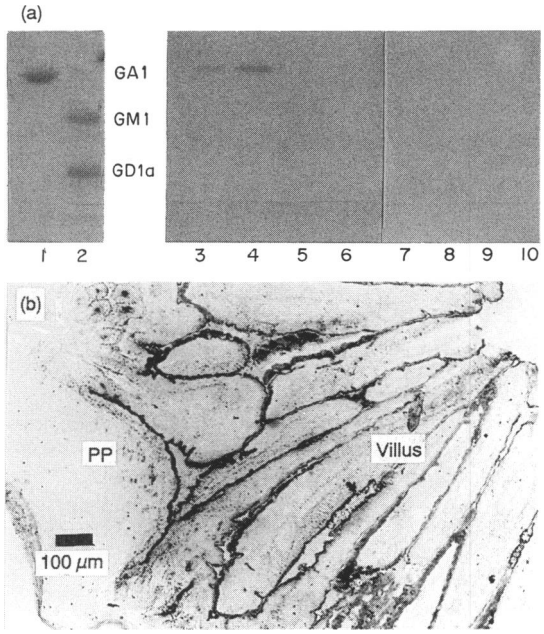

Figure 1. (a) Immunostaining of the glycolipids developed on the thin-layer plate with a-GA1 (lanes 3–6) or RGG (lanes 7–10). Asialo GM1 glycolipid (GA1) (lanes: 1, 2 μg; 3 and 7, 20 ng; 4 and 8, 100 ng) and a mixture of gangliosides (lanes: 2, 4 μg; 5 and 9, 40 ng; 6 and 10, 200 ng) were developed on a thin-layer plate in a solvent consisting of chloroform : methanol : water containing 2% $CaCl_2$, 55 : 45 : 10. The thin-layer plate was visualized by spraying 2 N H_2SO_4 containing 2% orcinol (lanes 1 and 2), or immunostained with a-GA1 (3–6) or RGG (7–10) as described elsewhere.[7] GA1 and the ganglioside mixture containing GM1 and GD1a were obtained from the small intestine and the bovine brain, respectively, and a-GA1 was prepared by asialo GM1 glycolipid-affinity and protein A column chromatography.[8] (b) Immunohistochemical staining of mouse small intestine with a-GA1. Frozen sections of the jejunum were completely dried, and fixed in cold acetone for 5 min. Then, the sections were incubated, washed and again incubated with peroxidase-conjugated anti-rabbit IgG. RGG used as a control did not stain the intestinal tissue (data not shown). PP indicates a Peyer's patch.

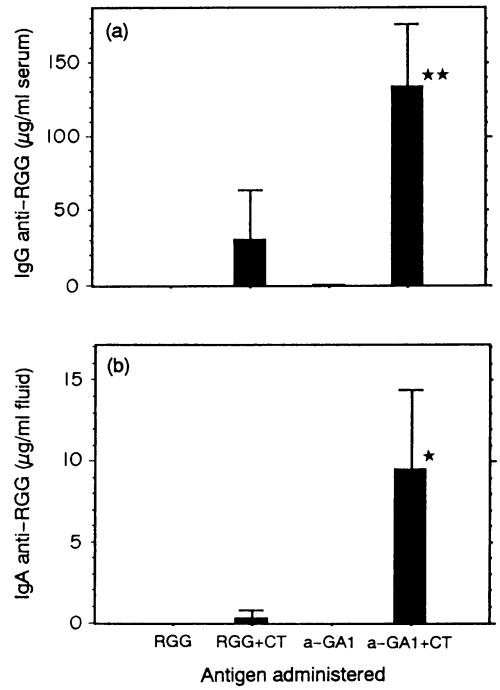

Figure 2. IgG (a) and IgA (b) anti-RGG responses to oral administration of RGG or a-GA1 singly or in combination with CT (RGG + CT or a-GA1 + CT). Mice (BALB/c, male, 8–11 weeks old) were administered RGG (40 μg) intragastrically in 0·5 ml of phosphate-buffered saline containing 40 mg $NaHCO_3$/ml in the absence or presence of CT (10 μg). This immunization was repeated 8 and 18 days after the first immunization. Antibody levels in serum and intestinal fluid obtained by washing the small intestinal lumen with a solution containing 50 mM EDTA and 1% Tween 20, were measured by an enzyme-linked immunosorbent assay (ELISA) using a microplate coated with 100 μl of antigen solution (2 μg/ml, RGG or CT) in carbonate buffer (pH 9·6). Titres of RGG- and CT-specific IgG were determined by using purified mouse anti-rabbit IgG (Jackson Immunoresearch Laboratory, West Grove, PA) and anti-CT IgG. Titres of RGG- or CT-specific IgA were determined by making an application of mouse IgA (Organon Teknika, Durham, NC) to a microplate precoated with anti-mouse IgA (Cappel, Westchester, PA). Each bar shows the mean value ± SD ($n = 5$). $^*P < 0{\cdot}05$; $^{**}P < 0{\cdot}01$, significantly different from RGG + CT by Student's and Cochran's *t*-test.

response than the usual dietary proteins. There was no difference between a-GA1 and RGG in anti-CT responses after simultaneous administration of rabbit IgG and CT. Most of the anti-rabbit IgG antibodies induced were directed to the Fc portion of IgG (data not shown). Therefore, the structural difference in the Fab portion between RGG and a-GA1 would be neglected in this immune response.

GM1 and asialo GM1 glycolipid, which are receptors for CT and a-GA1 respectively, co-localize in the small intestinal cell apical membrane, although the amount of asialo GM1 is much greater than that of GM1 in adult mice.[5,6] Therefore, CT and a-GA1 would be absorbed and processed by the same pathway in the small intestinal epithelial cells, probably villus epithelial cells, when they are administered simultaneously. This may explain why a-GA1 evokes an antibody response more effectively than RGG in the presence of CT. This explanation is supported by the observation that the antibody response to intubation of a mixture of CT and RGG in the duodenum or ileum was higher than that induced by their separate intubation in the duodenum and ileum (data not shown).

As shown in Fig. 2, single administration of RGG or a-GA1 did not induce an antibody response. Oral administration of RGG caused suppression of a systemic antibody response to a subsequent intraperitoneal injection of the antigen. On the other hand, a-GA1 did not induce clear oral tolerance (Fig. 3). We do not know, at present, whether there is a relationship between the antibody response to the orally administered antigen in the presence of CT and the oral tolerance to it in the absence of CT. Since the degree of suppression of the systemic antibody response by oral administration of RGG increased in a dose-dependent manner (data not shown), the absence of significant suppression of a systemic antibody response in the case of a-GA1 is not likely to be explained by greater absorption of the a-GA1 antigen than of the RGG antigen in the small intestine. These results suggest that the difference in anti-rabbit IgG response between the two antigens may be due to their route of uptake rather than to the amount of absorption by the intestinal cells. Together, these results suggest that the pathway of uptake of the antigen affects the handling of the antigen in the intestinal mucosa and determines its response to orally presented antigen.

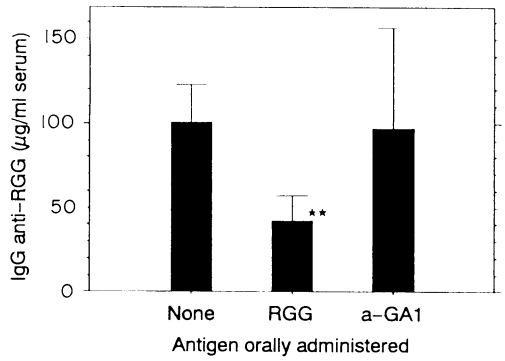

Figure 3. Effect of oral administration of RGG or a-GA1 on oral tolerance to the antigen. Forty micrograms of RGG or a-GA1 was orally administered to mice at Days 0, 1, 14 and 15. On Day 16, the mice were intraperitoneally injected with 100 μg of RGG in 100 μl of Freund's complete adjuvant. Twenty-one days later, serum antibody levels were measured as described in the legend for Fig. 2. Each bar shows the mean value $\pm$ SD ($n = 5$). $^{**}P < 0{\cdot}01$, significantly different from the control group (none) by Student's and Cochran's t-test.

ACKNOWLEDGMENTS

We thank Dr M. Nanno for his helpful discussion and comments on this manuscript. We thank the animal care staff in our institute for breeding experimental animals.

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