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IgA-Containing cells in the ruminant intestine following intraperitoneal and local immunization

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Summary. Experiments are described which demonstrate that a single intraperitoneal (i.p.) injection of antigen in Freund's complete adjuvant results in the appearance of IgA-specific antibody-containing cells (ACC) in the intestinal lamina propria of sheep and that these cells reach the intestine via the intestinal lymph and blood circulation. Following intraintestinal administration of antigen to sheep immunized i.p. 2 weeks previously, an enhanced ACC response occurs in the intestine but cannulation and drainage of the intestinal duct does not interfere with this enhancement. Evidence is presented which suggests that the enhanced ACC response may be accounted for by antigen-induced local proliferation of ACC in the lamina propria of the intestine.

INTRODUCTION

In an investigation of regimes of immunization which would establish a high density of IgA-specific antibody-containing cells (ACC) in the small intestine of rats, Pierce & Gowans (1975) found that the best response was achieved by an intraperitoneal (i.p.) dose of antigen in Freund's complete adjuvant (FCA) followed by an oral boost 2 weeks later. This regime resulted in the appearance of large numbers of ACC in the thoracic duct lymph following oral boosting and these cells subsequently populated the lamina propria of the intestine. The i.p. dose of antigen on its own did not give rise to a significant response in lymph or in the gut, but seemed to prime the gut for a secondary response following local administration of antigen.

In marked contrast to rats, Beh, Husband & Lascelles (1979) have reported that an IgA-specific ACC response was observed in intestinal lymph of sheep following a single i.p. injection of antigen in FCA. However, virtually no ACC appeared in lymph following intraduodenal boosting of animals primed 2 weeks previously by i.p. immunization.

Those experiments indicated that in sheep a single i.p. injection of antigen in FCA centrally stimulates the IgA system, resulting in the appearance of IgA-specific ACC in intestinal lymph. The experiments described in this paper extend those findings by demonstrating that the cells which appear in intestinal lymph following i.p. immunization populate the lamina propria of the intestine of sheep and in addition the role of local antigen in enhancing the ACC response in intestinal tissue has been investigated.

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MATERIALS AND METHODS

Animals

Adult Merino wethers were used. The sheep were housed in indoor pens and offered food and water *ad libitum*.

Antigens

Crystalline ovalbumin (grade III) was obtained from Sigma Chemical Co., St Louis, U.S.A.

Surgical procedures

The intestinal lymphatic duct was cannulated as described by Lascelles & Morris (1961) and lymph allowed to drain throughout the period of observation.

Double Thiry-Vella loops were prepared from two segments of proximal small intestine each 30–40 cm in length which were resected with mesentery intact. Both ends of each loop were exteriorized through stab incisions in the abdominal wall and the mucosa sutured to the skin. The continuity of the intestine was restored by end-to-end anastomosis. Loops were washed through with sterile phosphate buffered (pH 7·6) saline (PBS) twice daily. The gross and histological appearance of the isolated loops was normal when examined at the end of the experiment.

Immunization

Intraperitoneal (i.p.) immunizations were performed by injection of 5 ml of a 10 mg/ml solution of ovalbumin emulsified in an equal volume of FCA. For intraintestinal immunizations 50 ml of a 10 mg/ml solution of ovalbumin in saline was injected into the lumen of the duodenum or infused directly into the proximal end of a Thiry-Vella loop.

Fluorochrome-conjugated antisera

Sheep anti-immunoglobulin sera were prepared in rabbits and conjugated to fluorescein isothiocyanate (FITC) as described by Beh & Lascelles (1974).

Rabbit anti-ovalbumin serum was prepared as described by Beh (1977) and conjugated to tetramethylrhodamine isothiocyanate as described by Amante, Ancona and Forni (1972).

Enumeration of antibody-containing cells (ACC)

Lymph smears. The number of ACC and their immunoglobulin class specificities was detected in cell smears using the double fluorochrome labelling technique described by Beh (1977) and Pierce & Gowans (1975).

Gut sections. Pieces of intestine were processed for fluorescent antibody staining using the method of Sainte-Marie (1962). The double fluorochrome labelling technique was used to detect ACC and determine their immunoglobulin class specificity. The mean number of ACC per cm of intestine was calculated by counting the number of ACC per field in 30–50 fields using a 40× objective and 8× eyepiece (field diameter = 350 μm) scanning from the base of the mucosa to the tips of the villi. A mean of 1 fluorescing cell per field was equivalent to 28·6 cells/cm of intestine.

RESULTS

ACC in gut lamina propria after i.p. immunization

Seven sheep were immunized i.p. with ovalbumin and then killed at 14 days (four sheep) or 21 days (three sheep) and the density of ACC in the intestinal lamina propria and their immunoglobulin class specificities determined (Table 1). Large numbers of ACC were observed in the intestine of all sheep with a slightly higher response at 14 days than at 21 days. ACC of the IgA class predominated in all tissues except the duodenum of sheep killed at 21 days in which IgG-ACC predominated.

ACC in gut lamina propria after intraduodenal infusion of antigen in i.p. immunized sheep

The number and immunoglobulin class specificity of ACC in intestinal lamina propria 6 days after intraduodenal infusion of ovalbumin in six sheep primed intraperitoneally 14 days previously is presented in Table 2.

The results show that the density of ACC in the lamina propria of the intestine after the infusion was greater than in sheep given only i.p. immunizations (Table 1). Using Student's t test, it was computed that the differences were statistically significant ($P < 0{\cdot}001$ for duodenum; $P < 0{\cdot}01$ for jejunum and $P < 0{\cdot}02$ for ileum). The data in Table 2 also shows that in the duodenum of infused sheep, most of the ACC were IgG-specific, although a substantial proportion were IgA-specific, whereas in the jejunum and ileum IgA-specific cells predominated.

Table 1. Density and immunoglobulin class specificity of ACC in the intestine of sheep at 14 and 21 days after i.p. immunization, presented as means ± SE values. Class specificities are percentages of ACC which stained with the respective antisera

	14 days (4)*			21 days (3)*		
	ACC/cm	% IgG	% IgA	ACC/cm	% IgG	% IgA
Duodenum	63 ± 5	43·7 ± 3·6	56·1 ± 5·0	53 ± 3	77·3 ± 8·9	34·8 ± 15·4
Jejunum	40 ± 10	42·1 ± 2·7	66·5 ± 5·2	20 ± 5	46·8 ± 9·3	57·5 ± 4·6
Ileum	21 ± 4	21·9 ± 4·5	69·3 ± 3·2	13 ± 7	—	—

* Number of sheep in parentheses.

Table 2. Density and immunoglobulin class specificity of ACC in the intestine of sheep given an intraduodenal immunization 14 days after i.p. immunization and killed 6 days later. Values are means ± SE of observations from six sheep. Class specificities are percentages of ACC which stained with the respective antisera

	ACC/cm	% IgG	% IgA
Duodenum	145·0 ± 19·0	60·3 ± 3·8	46·0 ± 1·3
Jejunum	87·0 ± 14·0	40·0 ± 2·5	55·0 ± 3·5
Ileum	52·0 ± 10·0	39·9 ± 2·5	54·7 ± 3·5

ACC in gut lamina propria after i.p. immunization and chronic lymphatic drainage

To determine whether the ACC present in the gut after i.p. immunization originated from intestinal lymph, the intestinal lymph duct was cannulated in four sheep, i.p. immunization administered after the sheep had recovered from surgery and lymph allowed to drain throughout the experiment. Lymph smears were prepared each day for 14 days after which the sheep were killed and sections prepared from the intestine.

The results in Table 3 demonstrate that chronic intestinal duct drainage almost completely prevented the appearance of ACC in the intestine of i.p. immunized sheep and the few cells which were present were predominantly of the IgG class. An ACC response was obtained in the intestinal lymph of these sheep which was similar to that previously observed in the lymph of i.p. immunized sheep (Beh *et al.* 1978).

Table 3. Density and immunoglobulin class specificity of ACC in the intestine 14 days after i.p. immunization of cannulated sheep. Lymph was allowed to drain throughout the experiment. Values are means ± SE of observations from four sheep. Class specificities are percentages of ACC which stained with the respective antisera

	ACC/cm	%IgG	%IgA
Duodenum	5 ± 3	77·4 ± 0·5	17·8 ± 0·5
Jejunum	2 ± 1	—	—
Ileum	0	—	—

ACC in gut lamina propria after intraduodenal infusion of antigen in i.p. immunized, cannulated sheep

The enhancement of the numbers of ACC in the gut following local secondary immunization (Table 2) raised the question of the origin of these additional cells and to investigate the possibility that they may have arisen from precursors in intestinal lymph, four sheep which had been immunized i.p. 14 days previously were cannulated and at the time of surgery antigen was infused into the duodenum. After 6 days, during which time the intestinal lymph was allowed to drain continuously, the sheep were killed and ACC enumerated in sections of the gut. The results in Table 4 show that the ACC response after chronic lymph drainage was still much greater than in sheep receiving only i.p. immunization (Table 1) and these differences were statistically significant according to Student's *t* test ($P < 0{\cdot}01$ for each level of the gut). Indeed the data in Table 4 was similar to that observed in uncannulated sheep (Table 2), there being no statistically significant differences in ACC densities at any level of the gut in these two groups of sheep.

ACC response in Thiry-Vella loops

To determine whether the enhanced response obtained after local application of antigen was restricted to the

site of antigen exposure, the ACC response was measured in immunized and non-immunized Thiry-Vella loops. Loops were prepared in four sheep which had been immunized i.p. 12 days previously and antigen was infused into one loop 2 days later. The sheep were killed after a further 6 days and ACC enumerated in sections from both Thiry-Vella loops and from the intact jejunum.

Table 4. Density and immunoglobulin class specificity of ACC in the intestine of four sheep which were cannulated and given an intraduodenal immunization 14 days after i.p. immunization, and killed 6 days later. Values are means ± SE. Class specificities are percentages of ACC which stained with the respective antisera

	ACC/cm	%IgG	%IgA
Duodenum	112 ± 26	62·9 ± 10·5	41·5 ± 8·7
Jejunum	76 ± 22	39·8 ± 7·7	62·6 ± 6·6
Ileum	68 ± 11	34·0 ± 9·0	77·7 ± 4·7

The results in Table 5 demonstrate than an enhanced ACC response was only observed in the loop receiving a local infusion of antigen and the majority of these ACC were IgA-specific. The response in the non-immunized loop and in the jejunum was equivalent to that observed in normal sheep 21 days after i.p. immunization (Table 1).

Table 5. Density and immunoglobulin class specificity of ACC in jejunum and Thiry-Vella loops of four sheep. Sheep were immunized i.p. on day 0, loops prepared on day 12, antigen infused into one loop on day 14 and the response measured on day 20. Values are means ± SE. Class specificities are the percentage of ACC which stained with the respective antisera

	ACC/cm	%IgG	%IgA
Immunized loop	1831 ± 419	38·0 ± 3·9	53·5 ± 3·6
Non-immunized loop	45 ± 9	55·4 ± 6·9	46·1 ± 0·5
Jejunum	30 ± 4	52·7 ± 5·9	43·6 ± 6·6

DISCUSSION

The data in Tables 1 and 3 demonstrate that an IgA-specific ACC response occurs in the gut of sheep following a single i.p. injection of antigen and that these cells reach the gut via the intestinal lymphatic duct, and presumably therefore, are generated in the gut associated lymphoid tissue, probably in Peyer's patches (Beh, 1977). This extends the findings of Beh *et al.* (1979) that the i.p. route of immunization has potential as a means of inducing IgA immunity in the intestine of sheep. It also confirms that in sheep, as in rats (Gowans & Knight, 1964), there is a migration pathway for the precursors of IgA plasma cells from organized lymphoid tissue associated with the gut to the lamina propria of the intestine via the intestinal and thoracic ducts and blood circulation.

The ACC response in the intestine in i.p. immunized sheep given a secondary local (intraduodenal) immunization (Table 2) was much greater than that observed after i.p. immunization alone (Table 1). The enhancement of the ACC response induced by local administration of antigen could result from antigen-induced proliferation of ACC already in the lamina propria, a selective trapping of ACC still in the circulation following the i.p. immunization, or an influx to the gut, via the intestinal lymph, of a population of ACC precursors newly generated in gut-associated lymphoid tissue in response to local antigen.

The latter explanation can be excluded on the basis of the data in Table 4 which shows that in i.p. immunized sheep the ACC response was enhanced by intraduodenal infusion of antigen despite chronic drainage of intestinal lymph, the response being greater than in sheep receiving only i.p. immunization (Table 1). Indeed the response shown in Table 4 was equivalent to that recorded in similarly immunized uncannulated sheep (Table 2). As the enhancing effect of local antigen is not dependent on an intact supply of intestinal lymph then the additional cells are almost certainly not generated in gut-associated lymphoid tissue. It is also unlikely that antigen-dependent trapping of circulating ACC could account for the enhanced response considering the paucity of ACC still emanating from intestinal lymph by 14 days after i.p. immunization (Beh *et al.*, 1979). Thus the absence of an ACC response in intestinal lymph following intraduodenal administration of antigen to i.p. immunized sheep (Beh *et al*, 1979) and the failure of chronic lymph drainage to interfere with the enhancement of the ACC response by local antigen (Table 4) indicate that the enhanced response following local immunization may be accounted for by antigen-induced local proliferation of ACC or their precursors *in situ* in the intestinal lamina propria. This conclusion was supported by the experiments carried out in sheep with Thiry-Vella loops (Table 5) where the enhancing

effect was restricted to the locally immunized loop. This finding was in conformity with the results of earlier findings in experiments using loop preparations in sheep (Husband & Lascelles, 1974) and in rats (Husband & Gowans, 1978).

The results presented in this paper show that intestinal IgA responses in ruminants may be maximized by oral immunization of animals which have been previously immunized intraperitoneally. This procedure induces local proliferation of IgA-containing cells or their precursors, which reach the gut via the intestinal lymph following the systemic exposure to antigen. It has already been demonstrated that young lambs can be protected from *Salmonella typhimurium* challenge using this immunization protocol (Husband, 1978).

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