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Intestinal response of sheep to intraperitoneal immunization

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Summary. The aim of the present study was to develop an immunization procedure which centrally stimulated the IgA system of sheep with the release of antibody-containing cells (ACC) of the IgA class into intestinal lymph. It was found that intraperitoneal injection of ovalbumin resulted in a substantial output of ACC in intestinal lymph. ACC of the IgA class reached a peak 8–9 days after intraperitoneal injection when they comprised 1·4% of cells in lymph. ACC of the IgM and IgG1 classes comprised 3·5 and 2·9% of cells in lymph at this time. The output of ACC of the IgA, IgM and IgG1 classes in lymph at the peak of the response was respectively, $3{\cdot}7 \times 10^6$, $9{\cdot}8 \times 10^6$ and 8×10^6 cells/h. In marked contrast to rats, virtually no ACC appeared in intestinal lymph of sheep following intraduodenal infusion of ovalbumin in animals primed 2 weeks earlier by intraperitoneal injection of antigen in Freund's complete adjuvant (CFA).

INTRODUCTION

It is generally accepted that Peyer's patches are the major source of precursors of B cells of the IgA class (Craig & Cebra, 1971). There is also evidence in the rat that antigens penetrating the Peyer's patches stimulate the egress into the draining lymph of antibody-containing cells (ACC) of the IgA class, or cells with that potential. After entering the circulation, many of these cells seed the intestinal lamina propria and develop into plasma cells (Husband, Monie & Gowans, 1977). Local immunization or natural infection of the intestine has been reported to be associated with the elaboration of antibody of the IgA class into the mammary secretion (Adinolfi, Glynn, Lindsay & Milne, 1966; Saif, Bohl & Gupta, 1972; Montgomery, Rosner & Cohn, 1974), or the appearance of antibody-forming cells among the IgA lymphoid cells in human colostrum (Ahlstedt, Carlsson, Hanson & Goldblum, 1975).

Local immunization has already been applied in attempts to protect the ruminant mammary gland against bacterial mastitis and has proved partially successful (Lascelles & McDowell, 1974). The possibility of being able to maximize the local immune response in the mammary gland of ruminants by antigenic stimulation of the intestine some time prior to local mammary stimulation is now under consideration.

This paper presents some preliminary data which show that in the sheep, intraperitoneal injection of antigen stimulates intestinal lymphoid tissue resulting in a substantial output of ACC in intestinal lymph, many of which are IgA-specific.

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MATERIALS AND METHODS

Animals

Two-year-old Merino wethers were used. Sheep were housed indoors in metabolism cages and offered food and water *ad libitum*.

Antigen

Crystalline ovalbumin (Grade V) was obtained from Sigma Chemical Co.

Experimental procedure

Surgery. The intestinal lymphatic ducts of sheep were cannulated as described by Lascelles & Morris (1961). Lymph was collected aseptically into a plastic bag containing heparin solution and returned to the animal by slow intravenous infusion each day (Adams & Cripps, 1977).

Immunization. In experiments where the response to intraperitoneal injection was studied sheep were allowed 1–2 days to recover from effects of surgery before receiving an intraperitoneal injection of 5 ml (10 mg/ml) of ovalbumin emulsified in an equal volume of Freund's complete adjuvant (CFA). Output of ACC in intestinal lymph was monitored for 14 days following administration of antigen.

When studying the effect of intraduodenal infusions on ACC output, sheep received an intraperitoneal primary injection of 5 ml ovalbumin (10 mg/ml) emulsified in an equal volume of CFA and 14 days later received intraduodenally a booster dose of 500 mg of ovalbumin in 50 ml of saline.

Fluorochrome-conjugated antisera

Sheep anti-immunoglobulin sera were prepared in rabbits and conjuaged 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 & Forni (1972).

Fluorescent antibody technique. The number of antibody-containing cells (ACC) and the immunoglobulin class specificity of antibody was determined in cell spreads using the double fluorochrome labelling technique as described by Beh (1977).

Antibody assays

Anti-ovalbumin titres were measured in lymph plasma and blood serum by the passive haemagglutination of formalinized tanned sheep red blood cells treated with ovalbumin (Butler, 1963).

RESULTS

ACC in intestinal lymph following intraperitoneal immunization

The output of total cells and large basophilic cells in intestinal lymph of sheep together with the percentage of ACC at various times after intraperitoneal injection are shown in Fig. 1. There was no significant change in

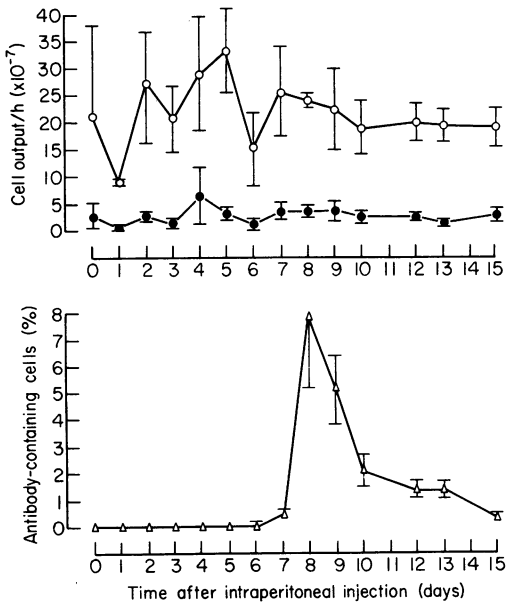

Figure 1. Output of total lymphocytes and large basophilic cells (cells/h $\times 10^{-7}$) in intestinal lymph of sheep together with results for ACC expressed as a percentage of total lymphocytes. At day 0 sheep received an intraperitoneal injection of ovalbumin in CFA. Plotted points are means ±SE for 3 sheep. o, total lymphocytes; •, large basophilic cells; ▵, ACC.

the output of total cells or of large basophilic cells in lymph following injection. ACC first appeared 6 days after injection and rose to a peak at 8 days when 7·8% of cells contained detectable antibody. The percentage of ACC in lymph then declined and very few were present by 15 days after injection.

The immunoglobulin class specificity of ACC at various times after intraperitoneal injection was determined and these results are shown in Table 1. During

Table 1. Distribution of ACC among immunoglobulin classes at various times after intraperitoneal injection of four sheep with ovalbumin in CFA. Values are means ±SE expressed as a percentage of total lymphocytes in lymph

Time after intraperitoneal injection (days)	Immunoglobulin class specificity		
	IgG1	IgA	IgM
6	0	0	<0·01
7	0·10±0·07	0·09±0·06	0·27±0·05
8	2·89±1·09	1·33±0·53	3·54±1·45
9	2·13±0·63	1·43±0·75	1·61±0·49
10	1·43±0·46	0·35±0·11	0·33±0·08
12	0·99±0·13	0·29±0·21	0·12±0·02
13	1·22±0·06	0·09±0·01	0·25±0·001
15	0·21±0·10	0·05±0·03	<0·01

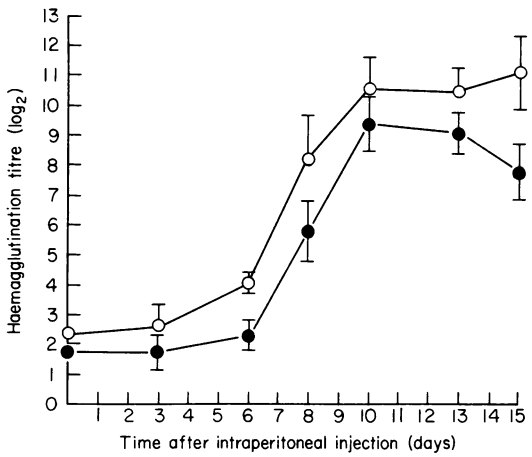

Figure 2. Changes in ovalbumin antibody titres (log_2) in blood serum and lymph plasma with time after injection. Values presented are means ±SE for four sheep. o, serum; •, lymph.

the early stages of the response, ACC of the IgM class predominated. For example, on day 8 after injection, 3·5% of total cells or around 50% of ACC were IgM-specific. On subsequent days IgG1-specific ACC predominated so that by day 15 0·21% of total cells or about 90% of ACC contained antibody of the IgG1 class. It is apparent from Table 1 that throughout the response a substantial proportion of ACC were IgA-specific. Peak levels of IgA-specific ACC occurred on day 9 when 1·43% of total cells or 28% of ACC in intestinal lymph contained antibody of the IgA class.

Table 2. ACC in intestinal lymph of four sheep at various times after intraduodenal infusion of ovalbumin in saline. Sheep received intraperitoneal injection of antigen in CFA 14 days previously. Values and means ±SE of ACC expressed as a percentage of total lymphocytes

Time after duodenal infusion (days)	ACC in lymph (%)
1	0
2	0
3	0·018±0·010
4	0·008±0·005
5	0·050±0·030
6	0·035±0·010
8	0

Antibody response following intraperitoneal injection

The antibody response in serum and lymph after intraperitoneal injection of ovalbumin in CFA is shown in Fig. 2. The onset of the antibody response occurred around 6–7 days after injection and peak titres were recorded on day 10. Antibody titres were higher in serum than in lymph and the antibody response corresponded closely with the ACC response in intestinal lymph.

Effect of intraduodenal infusion of antigen on output of ACC in intestinal lymph of intraperitoneally primed sheep

Previous studies in the rat have shown that a substantial output of ACC of the IgA class in intestinal lymph follows intraintestinal infusion of cholera toxoid in animals previously sensitized by intraperitoneal injection of antigen in oil adjuvant (Pierce & Gowans, 1975). It was therefore of interest to determine whether the ACC response in intestinal lymph could be stimulated by intraduodenal infusion of ovalbumin in intraperitoneally primed sheep. The results in Table 2 show that following intraduodenal infusion of ovalbumin a barely detectable response occurred. A maximum output of ACC in intestinal lymph of 0·05% was recorded on day 5.

DISCUSSION

The present study was undertaken to develop an immunization procedure which centrally stimulated the IgA system of sheep with the release of antibody-specific cells of the IgA class into thoracic duct lymph. It was found that intraperitoneal injection of antigen

resulted in the appearance of a large number of ACC in intestinal lymph, and many of these were IgA-specific. ACC of the IgA class reached a peak 9–10 days after intraperitoneal injection, at which time they comprised 1·3–1·5% of cells in lymph; ACC of the IgM and IgG1 class were even more abundant at this time. It was calculated that at the peak of the response, the output of ACC of the IgA, IgM and IgG1 classes were respectively $3{\cdot}7 \times 10^6$, $9{\cdot}8 \times 10^6$ and $8{\cdot}0 \times 10^6$ cells/h.

The origin of antibody-containing cells in thoracic duct lymph of sheep following intraperitoneal immunization can only be speculated upon at this time. While it is accepted that the lymphatic drainage of the peritoneal cavity is by way of the diaphragmatic lymphatics and right lymphatic duct (Yoffey & Courtice, 1970) it is suggested that the use of irritant adjuvant may have the effect of inducing a permeability of the serosa to macromolecules. In this way a direct penetration of antigen into Peyer's patches and other parts of the intestinal lymphoid apparatus may occur. Assuming the Peyer's patches in sheep are comparable with those in rats and rabbits, ACC of the IgA class in thoracic duct lymph of sheep would derive entirely from the Peyer's patches. Antigenic stimulation of the mesenteric lymph node of sheep (Beh, 1977) has been shown to give rise to a similar response to that described for the popliteal node (Beh & Lascelles, 1974). Thus any intraperitoneal antigen which is transferred into the mesenteric lymph node would be expected to give rise to the appearance of IgM and IgG1–ACC in efferent lymph a few days later. The possibility cannot be excluded, however, that antigen may be stimulating cells in the lamina propria as well as in Peyer's patches following intraperitoneal injection, and IgG1, IgM and possibly some IgA-containing cells may originate from dividing cells in the lamina propria. The CFA itself may play some part in dictating the nature of the immune response with regard to class specificity of cells emanating in the lymph. This question is presently under study.

Although intraperitoneal injection alone in sheep gave rise to a substantial output of ACC in lymph this response could not be reinvoked in a significant way by subsequent intraintestinal infusion of antigen. This contrasts markedly with the results obtained by Pierce & Gowans (1975) using cholera toxoid in rats. While no reason for the difference between the two species can be offered at this time, it is evident that it is not related to the antigen used since intraintestinal infusion of ovalbumin initiates a substantial ACC response in thoracic duct lymph of intraperitoneally primed rats (unpublished data).

Further studies are in progress to determine whether the ACC generated by intraperitoneal immunization of sheep will populate the mammary gland and other mucous sites besides the intestine, and whether this phenomenom can be exploited to enhance local immunity at these sites.

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