Immunology 1979 **37** 279

Antibody-containing cell response in lymph of sheep after intra-intestinal infusion of ovalbumin with and without DEAE–dextran

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Received 5 October 1978; accepted for publication 6 November 1978

Summary. The output of antibody-containing cells (ACC) was monitored in efferent ileal lymph after continuous infusion of ovalbumin into the ileum of sheep with and without the adjuvant DEAE-dextran. When ovalbumin was infused at the slow rate of 5 ml/h, maximum outputs of $2{\cdot}9 \times 10^5$ and $2{\cdot}4 \times 10^5$ ACC/h were observed on days 9 and 16 respectively. When infused at the faster rate of 15 ml/h, peak levels of $6{\cdot}9 \times 10^5$ and $11{\cdot}7 \times 10^5$ ACC/h were recorded on days 10 and 16 respectively. The maximum response was substantially enhanced when ovalbumin was infused simultaneously with DEAE-dextran when a mean output of $51{\cdot}7 \times 10^5$ ACC/h occurred on day 10. With all treatments the distribution of ACC amongst various immunoglobulin classes was similar. During the first few days of the response IgM-specific ACC predominated and later IgG1-specific ACC were most abundant. Throughout the response a substantial proportion (10–81%) of ACC in efferent ileal lymph were IgA-specific.

INTRODUCTION

Presentation of antigen to the immune apparatus via the lumen of the intestine results in locally produced antibody in gut secretions. This was first demonstrated by Batty & Marrack (1955) and subsequently Crabbé, Nash, Bazin, Eyssen & Heremans (1969) showed that prolonged oral intake of ferritin led to an increase in IgA-specific secretory plasma cells in the lamina propria of the intestine of mice and to an increase in levels of circulating IgA antibody. Other studies have shown that prolonged oral administration of bacterial 'O' antigen resulted in an increase in antibody, mainly of IgA class specificity in gut secretions of pigs and calves (Allen & Porter, 1975; Porter, Kenworthy, Noakes and Allen, 1974).

While the presence of IgA antibody in gut secretions can be taken as evidence that intra-luminally administered antigen is acting on precursor cells in the lamina propria, the effect of intra-intestinal antigen on other parts of the lymphoid system is less clear. There are reports that intra-intestinally administered antigen is apparently absorbed and stimulates organised lymphoid tissue, especially the mesenteric lymph nodes (Cooper, Halliday & Thonard, 1967; Rothberg, Kraft & Farr, 1967; Robertson & Cooper, 1972). These studies showed that antigen given by either the intestinal or parenteral routes produced systemic antibodies of similar immunoglobulin classes. It follows from these results that systemic immunity can be stimulated orally.

The potential of the oral route to stimulate systemic as well as local (IgA mediated) immunity may be explored by studying the immunoglobulin classes of antibody-containing cells (ACC) produced in response to intra-intestinally infused antigen. The ACC

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0019-2805/79/0500-0279$02.00

response would also reveal information about the normal function of various components of the gut associated lymphoid system during response to antigen presented from the intestinal lumen.

The experiments reported in this paper were carried out to determine the nature of the response to ovalbumin infused continuously into the lumen of the ileum of sheep by determining the number and immunoglobulin class of ACC in lymph efferent to the ileal lymph node. When it was found that the response to intra-luminally administered antigen was meagre compared with responses previously obtained from lymph nodes (Beh & Lascelles, 1974; Beh, 1977), attempts were made to enhance the response by simultaneous intra-intestinal infusion of antigen with the polycation adjuvant DEAE-dextran.

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 solutions

Ovalbumin (Grade V) was obtained from Sigma Chemical Co. For infusion a 1 mg/ml sterile solution was prepared in phosphate buffered saline pH 7·4.

DEAE-dextran was obtained from Pharmacia Pty. Ltd. To test its adjuvant effect when infused intra-intestinally, DEAE-dextran was added to the ovalbumin solution at the rate of 5 g/100 ml.

Experimental procedure

Surgery. The efferent duct of the ileal lymph node was cannulated as described by Beh (1977). During surgery to cannulate the lymphatic duct, a polyvinyl chloride cannula (I.D. 1·40 mm; O.D. 1·90 mm; Dural Plastics & Engineering) was inserted into the ileum immediately proximal to the area draining to the cannulated node.

Antigen infusion. The day after surgery, the continuous infusion of the solutions into the ileum commenced. A peristaltic pump (Perpex; LKB Produkter) was used to achieve a continuous infusion rate for ovalbumin solution of either 5 ml/h (three sheep) or 15 ml/h (five sheep). When testing the effect of DEAE-dextran on the immune response, DEAE-dextran ovalbumin solution was infused into the ileum at the rate of 5 ml/h (five sheep).

Lymph collection. Lymph was collected into plastic bottles tied to the side of the sheep. A fresh sample of lymph was collected daily and from this sample cell counts were carried out with a haemocytometer and cells in lymph were differentiated on Leishman stained smears.

Fluorescent antibody technique

Fluorescent anti-sheep immunoglobulin sera were prepared as described by Beh & Lascelles (1974). An anti-ovalbumin serum was prepared in rabbits and labelled with tetramethylrhodamine isothiocyanate. To determine the immunoglobulin class of ACC the double fluorochrome labelling technique was carried out as described by Beh (1977). Methanol-fixed cytocentrifuge smears were incubated with ovalbumin (0·5 mg/ml), washed and then incubated with a mixture consisting of equal parts of fluorescein isothiocyanate labelled anti-immunoglobulin reagent and tetramethylrhodamine isothiocyanate labelled rabbit anti-ovalbumin serum. After washing and mounting in 1% glycerine in phosphate buffered saline pH 7·4, slides were examined under narrow band excitation using a Zeiss Photomicroscope III equipped with an epi-fluorescence condenser III RS.

Antibody assays

Antibody to ovalbumin was measured in lymph plasma using the haemagglutination technique (Stavitsky, 1954). Formalinized, tanned sheep red blood cells were sensitized with ovalbumin as described by Butler (1963).

RESULTS

Duration of lymph flow

Of the three sheep in the slow infusion rate group lymph flow stopped in one after 18 days and continued beyond 20 days in the other two. Five sheep were included initially in the fast infusion rate group. Lymph flow ceased in one after 6 days, one after 9 days, one after 13 days and one after 17 days. The remaining sheep in this group flowed beyond 20 days. Of the five sheep infused with DEAE-dextran ovalbumin solution lymph flow stopped in one after 6 days, one after 11 days, one after 13 days and another after

17 days. The remaining sheep in this group flowed beyond 20 days.

Lymphocyte outputs

The mean output of total lymphocytes (small and medium lymphocytes plus large basophilic cells) for the three treatment groups is shown in Fig. 1. For the slow infusion rate and DEAE-dextran ovalbumin groups, the mean output of total lymphocytes varied between $5–10 \times 10^7$ cells/h whereas mean output for sheep in the fast infusion rate group was higher varying between $10–20 \times 10^7$ cells/h. Outputs of total lymphocytes for all groups were relatively constant over the 20 day observation period.

Blast cell outputs for sheep in the three groups are shown in Fig. 2. Outputs for all groups were low during the first few days and thereafter rose to levels between 5 and 15×10^6 cells/h. Levels then remained relatively constant for the remainder of the treatment period.

Antibody-containing cell outputs

The output of ACC in efferent ileal lymph for sheep in the three treatment groups is shown in Fig. 3. The results show that the slow infusion of ovalbumin solution into the ileum produced a barely detectable ACC response in efferent ileal lymph. ACC were first detected on day 6 and on day 9 $2{\cdot}9 \times 10^5$ ACC/h were being discharged into efferent lymph. At this time ACC represented, on average, only 0·24% of total cells in efferent lymph. Later in the response on day 16 a mean output of $2{\cdot}4 \times 10^5$ ACC/h was recorded.

Figure 3 also shows the ACC response when ovalbumin solution was infused into the ileum at the faster rate of 15 ml/h. Faster infusion of the ovalbumin solution resulted in a larger response than was obtained with the slow infusion. ACC first appeared in lymph on day 7 and a peak output of $6{\cdot}9 \times 10^5$ ACC/h was recorded on day 10. This meant that at this time 0·44% of total cells in efferent ileal lymph contained detectable antibody. A further peak was apparent on day 16 when a mean of $11{\cdot}7 \times 10^5$ ACC/h were leaving the ileal lymph node via the efferent lymph. At this time 0·53% of cells in efferent lymph contained antibody.

The ACC output following infusion with DEAE-dextran ovalbumin solution is also shown in Fig. 3. ACC were first detected 6 days after the start of infusion and peak levels were reached at 10 days when the mean output of ACC was $51{\cdot}7 \times 10^5$ ACC/h. This constituted 4·3% of total cells in efferent ileal lymph. Output then fell so that by 14 days after the start of infusion, ACC output was comparable to that obtained with the fast infusion rate of ovalbumin solution without adjuvant.

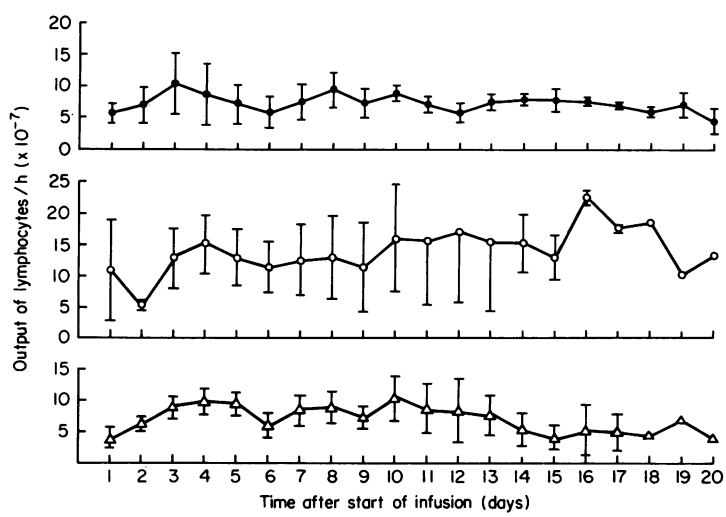

Figure 1. The output of total lymphocytes in efferent ileal lymph at various times after the start of infusion of ovalbumin or DEAE-dextran ovalbumin solutions. Plotted points are the mean output of total lymphocytes $\times 10^{-7} \pm$ standard error. •, infusion of ovalbumin solution at 5 ml/h; ○, infusion of ovalbumin solution at 15 ml/h; △, infusion of DEAE-dextran ovalbumin solution at 5 ml/h.

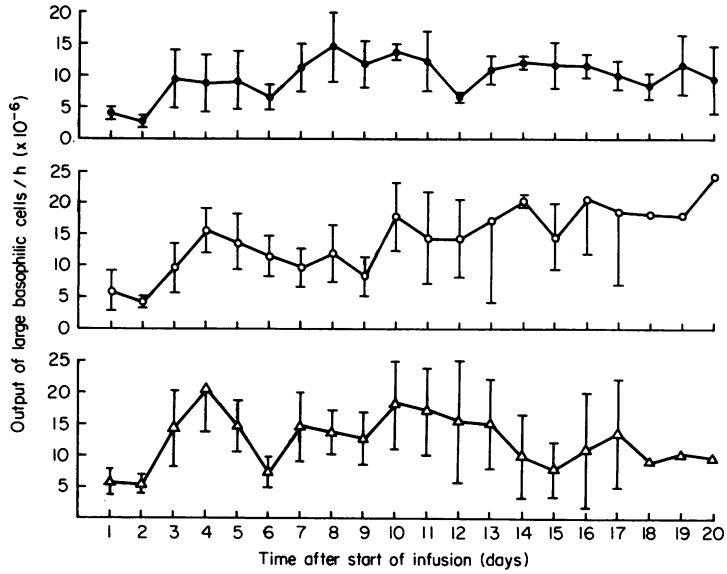

Figure 2. Output of large basophilic cells in efferent ileal lymph at various times after start of infusion of ovalbumin or DEAE-dextran ovalbumin solutions. Plotted points are mean output of large basophilic cells $\times 10^{-6} \pm$ standard error. •, infusion of ovalbumin solution at 5 ml/h; ○, infusion of ovalbumin solution at 15 ml/h; △, infusion of DEAE-dextran ovalbumin solution at 5 ml/h.

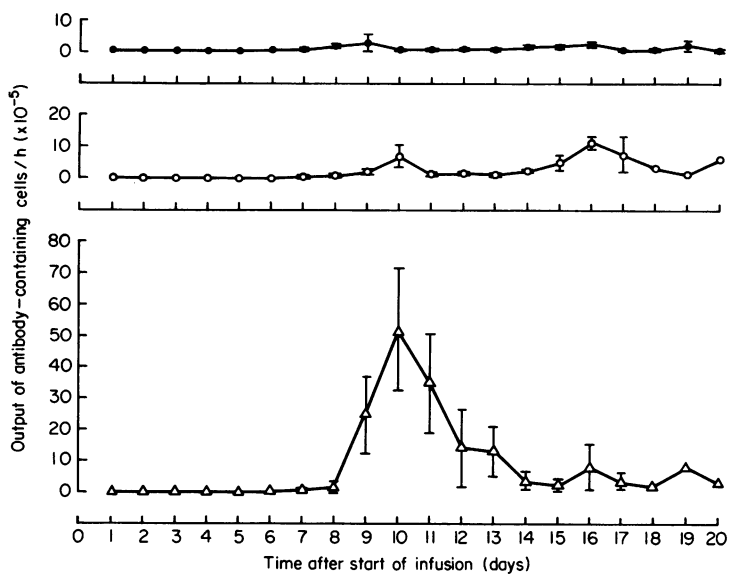

Figure 3. Output of antibody-containing cells in efferent ileal lymph at various times after start of infusion with ovalbumin or DEAE-dextran ovalbumin solutions. Plotted points are the mean output of antibody-containing cells/h $\times 10^{-5} \pm$ standard error. •, infusion of ovalbumin solution at 5 ml/h; ○, infusion of ovalbumin solution at 15 ml/h; △, infusion of DEAE-dextran ovalbumin solution at 5 ml/h.

Immunoglobulin class of ACC

Slow infusion of ovalbumin solution. The distribution of ACC amongst immunoglobulin classes when ovalbumin solution was infused into the ileum at the slow rate is shown in Table 1. For the first few days of the response IgM-specific ACC predominated and on subsequent days IgG1 ACC predominated. Only a small number of IgG2-specific ACC were detected. Throughout the response IgA-specific ACC comprised a substantial fraction (13–33%) of total ACC. Peak output of IgA-specific ACC occurred on day 8 when $0{\cdot}48 \times 10^5$ IgA-specific cells/h were leaving the node and these constituted 33% of total ACC output.

Fast infusion of ovalbumin solution. Distribution of ACC amongst various immunoglobulin classes when ovalbumin solution was infused at the fast rate is shown in Table 2. IgM-specific ACC were most abundant early in the response, none being detected in the

Table 1. Distribution of ACC amongst immunoglobulin classes at various times after start of infusion with ovalbumin solution at the rate of 5 ml/h. Values shown are means output/h $\times 10^{-5}$ ± standard error

Days after start of infusion	Output of ACC/h $\times 10^{-5}$			
	IgG1	IgG2	IgA	IgM
6	0	0	0	0
7	0·02 ± 0·02 (22)*	0	0·02 ± 0·02 (22)	0·05 ± 0·05 (56)
8	0·26 ± 0·25 (18)	0	0·48 ± 0·47 (33)	0·70 ± 0·70 (49)
9	1·86 ± 1·82 (64)	0	0·40 ± 0·37 (13)	0·66 ± 0·65 (23)
10	0·22 ± 0·11 (41)	0	0·13 ± 0·04 (24)	0·20 ± 0·08 (35)
11	0·54 ± 0·30 (71)	0·01 ± 0·01 (1)	0·14 ± 0·09 (18)	0·08 ± 0·04 (10)
12	0·69 ± 0·40 (74)	0·01 ± 0·01 (1)	0·20 ± 0·08 (21)	0·04 ± 0·02 (4)
14	1·01 ± 0·36 (70)	0	0·36 ± 0·02 (25)	0·07 ± 0·04 (5)
16	1·69 ± 0·66 (72)	0·01 ± 0·01 (< 1)	0·61 ± 0·11 (26)	0·05 ± 0·01 (2)
18	0·39 ± 0·16 (75)	0·002 ± 0·002 (< 1)	0·11 ± 0·07 (21)	0·02 ± 0·01 (4)
20	0·62 ± 0·20 (69)	0·01 ± 0·01 (1)	0·24 ± 0·11 (27)	0·03 ± 0·03 (3)

* Values shown in parentheses are the means output/h of ACC of each immunoglobulin class expressed as a percentage of total ACC output.

Table 2. Distribution of ACC amongst immunoglobulin classes at various times after start of infusion with ovalbumin solution at the rate of 15 ml/h. Values shown are mean output/h $\times 10^{-5}$ ± standard error.

Days after start of infusion	Output of ACC/h $\times 10^{-5}$			
	IgG1	IgG2	IgA	IgM
6	0	0	0	0
7	0	0·01 ± 0·01 (63)	0	0·003 ± 0·003 (37)
8	0·10 ± 0·05 (20)*	0	0·27 ± 0·18 (54)	0·13 ± 0·11 (26)
9	0·83 ± 0·80 (65)	0	0·31 ± 0·10 (24)	0·14 ± 0·13 (11)
10	2·79 ± 1·31 (40)	0·06 ± 0·04 (1)	1·52 ± 0·49 (22)	2·65 ± 2·21 (38)
11	0·65 ± 0·54 (47)	0·01 ± 0·01 (1)	0·53 ± 0·36 (39)	0·18 ± 0·11 (13)
12	1·14 ± 0·90 (59)	0·01 ± 0·01 (1)	0·69 ± 0·02 (36)	0·09 ± 0·04 (5)
14	2·09 ± 1·07 (72)	0·04 ± 0·04 (1)	0·44 ± 0·32 (15)	0·35 ± 0·35 (12)
16	9·18 ± 0·02 (79)	0	2·25 ± 1·76 (19)	0·15 ± 0·04 (1)
18	1·89 (50)	0	1·91 (50)	0
20	1·24 (19)	0	5·13 (81)	0

* Values shown in parentheses are the mean output/h of ACC each immunoglobulin class expressed as a percentage of total ACC output.

latter stages of infusion. A significant number of IgG1-specific ACC were present early in response, but the level was maximal on day 16 when a mean output of $9{\cdot}18 \times 10^5$ IgG1-specific ACC/h was recorded. This was equivalent to 79% of total ACC output. Only low outputs of IgG2-specific ACC were observed during the response. A substantial population of IgA-specific ACC was present throughout the response with peak outputs of $1{\cdot}52 \times 10^5$ and $2{\cdot}25 \times 10^5$ ACC/h on days 10 and 16 respectively. In the single sheep remaining on day 20 output of IgA-specific ACC was $5{\cdot}13 \times 10^5$/h which comprised 81% of the total ACC output on that day.

Infusion of DEAE-dextran ovalbumin solution. Table 3 shows the distribution of ACC amongst immunoglobulin classes following infusion of DEAE-dextran ovalbumin solution into the ileum. Again IgM-specific ACC were most abundant in the early stages of the response. The peak output of these cells of $19{\cdot}7 \times 10^5$/h occurred on day 10, although the greatest proportion occurred on day 8 when 46% of ACC in efferent lymph were IgM-specific. Peak output of IgG1-specific ACC ($20{\cdot}9 \times 10^5$/h) occurred on day 10 and these cells predominated on day 12 when 52% of ACC discharged into lymph were IgG1-specific. A small population of IgG2-specific cells was also pre-

Table 3. Distribution of ACC amongst immunoglobulin classes at various times after start of infusion with DEAE-dextran ovalbumin solution. Values shown are mean output/h $\times 10^{-5}$ ± standard error

Days after start of infusion	Output of ACC/h $\times 10^{-5}$			
	IgG1	IgG2	IgA	IgM
6	0	0	0	0
7	0·06 ± 0·04 (50)*	0	0·05 ± 0·05 (42)	0·01 ± 0·01 (8)
8	0·77 ± 0·50 (44)	0	0·18 ± 0·09 (10)	0·82 ± 0·74 (46)
9	9·48 ± 6·15 (38)	0·42 ± 0·28 (2)	5·37 ± 4·09 (21)	9·73 ± 5·39 (39)
10	20·92 ± 9·29 (40)	2·13 ± 1·58 (4)	9·10 ± 4·26 (18)	19·67 ± 8·19 (38)
11	14·24 ± 9·46 (41)	0·83 ± 0·74 (2)	10·54 ± 3·78 (31)	8·99 ± 3·84 (26)
12	7·50 ± 6·49 (52)	0·67 ± 0·67 (5)	2·64 ± 2·09 (19)	3·49 ± 3·36 (24)
14	1·75 ± 1·68 (47)	0·09 ± 0·09 (2)	1·65 ± 0·63 (45)	0·21 ± 0·04 (6)
16	3·14 ± 3·08 (38)	0	4·73 ± 4·22 (58)	0·29 ± 0·28 (4)
18	0·26 (14)	0	1·53 (81)	0·10 (5)
20	0·73 (22)	0	2·46 (75)	0·11 (3)

* Values shown in parentheses are the mean output/h of ACC of each immunoglobulin class expressed as a percentage of total ACC output.

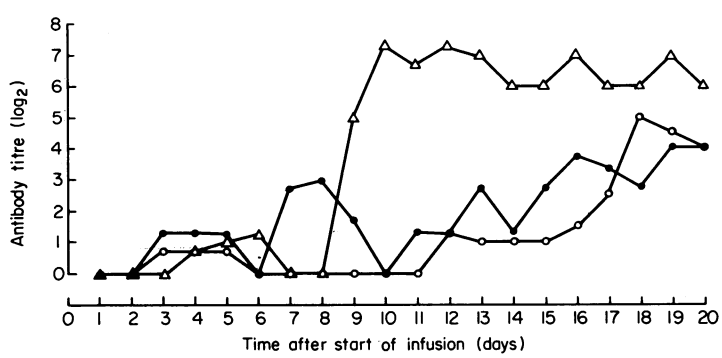

Figure 4. Change in the antibody titre in efferent ileal lymph with time after start of infusion of ovalbumin or DEAE-dextran ovalbumin solution. Plotted points are mean $\log_2$ antibody titre determined by haemagglutination assay. •, infusion of ovalbumin solution at 5 ml/h; ○, infusion of ovalbumin solution at 15 ml/h; △, infusion of DEAE-dextran ovalbumin solution at 5 ml/h.

sent in efferent lymph and on day 12 these comprised 5% of total ACC output. Output of IgA-specific ACC was maximum on day 10 when $9{\cdot}1 \times 10^5$ ACC/h were IgA-specific. This comprised 18% of the mean ACC population in efferent lymph and in the later stages of the response IgA-specific ACC predominated, so that in the single sheep flowing on day 20 75% of ACC in efferent ileal lymph were IgA-specific.

Antibody response to infusions

Figure 4 shows the antibody levels in efferent lymph treatment groups at various times after the start of infusion. Low titres were recorded for sheep in the groups receiving ovalbumin solution without adjuvant at the slow or fast infusion rates. For these groups highest titres occurred during the later stages of the period of observation.

In sheep infused with the DEAE-dextran ovalbumin solution antibody was detected in lymph by day 9 and rose sharply to peak levels by day 10. Antibody response in these sheep corresponded closely with the ACC response in efferent ileal lymph.

DISCUSSION

The purpose of the experiments described in this paper was to study the response of the ileum and its associated lymph node to intra-luminally administered antigen by following the output of ACC in lymph. The results showed that infusion of antigen into the ileum resulted in an initial output of mainly IgM-specific ACC. Later IgG1-specific ACC were most abundant in the efferent lymph. In these respects the response to intra-luminally administered antigen was similar to the response of an antigenically stimulated lymph node (Beh & Lascelles, 1974). Throughout the response to antigen given intra-luminally, however a significant proportion of cells leaving the node were IgA-specific. Therefore the response to intra-intestinal antigen differs in this important way from the response obtained when antigen is introduced directly into the node. The results presented in Figs 1 and 2 also show that, unlike the response from an antigenically stimulated lymph node, the ACC response to intra-luminally infused antigen was not associated with any detectable change in the output in efferent lymph of either total lymphocytes or blast cells. It is possible that any cell response from this region would be masked by the high cell traffic and blast cell production normally associated with the intestine.

In addition the results clearly show that the response to intra-luminally administered antigen can be enhanced if antigen is infused simultaneously with the adjuvant DEAE-dextran. DEAE-dextran was shown to have adjuvant properties in pigs when injected intra-muscularly with foot and mouth disease virus vaccine (Wittmann, Dietzschold & Bauer, (1975). The present results show that infusion of antigen with DEAE-dextran into the gut lumen resulted in a significant increase in the output of ACC in efferent ileal lymph. For example, with the fast rate of infusion of ovalbumin solution ACC output at the peak of the response on day 10 was $6{\cdot}9 \times 10^5$ ACC/h, whereas, even when DEAE-dextran ovalbumin solution was infused at the slow rate, output on day 10 was about eight-fold greater. During the latter stages of the response ACC outputs were comparable. DEAE-dextran has been shown to enhance pinocytosis *in vitro* and this is probably the basis for its adjuvant effect (Werner, Maral, Floc'h & Jouanne, 1977). When used to enhance the immune response to locally administered antigen the mechanism of action of the adjuvant can only be speculated upon. It could be acting on the specialized cells lining the Peyer's patches (Bockman & Cooper, 1973) stimulating their pinocytotic activity and hence increasing uptake of antigen from the gut lumen, or DEAE-dextran could be absorbed, enter the draining lymph node, and exert its adjuvant action there.

Although infusion with adjuvant substantially increased the magnitude of the response, the distribution of ACC amongst various immunoglobulin classes was similar to the distribution when antigen was infused without adjuvant. The IgM and IgG1 component of the response could be accounted for if antigen found its way to the mesenteric lymph node. Previous work has shown that antigen injected into the mesenteric lymph node stimulates the production ACC of IgG1 and IgM classes (Beh, 1977) and Warshaw, Walker, Cornell & Isselbacher (1971) have demonstrated that macromolecules can enter the mesenteric lymphatic circulation from the gut lumen. Apparently mesenteric lymph nodes contain only IgM and IgG1 precursor cells so stimulation of mesenteric lymph nodes by absorbed antigen could not account for the substantial IgA component of the immune response, unless it is postulated that processing of antigen by epithelial cells lining the intestine rendered antigen capable of driving cells to IgA synthesis.

It has been shown that the lymphoid cells in the lamina propria or the Peyer's patches are the precursors of IgA containing cells normally found in sheep intestinal lymph (Beh, 1977). The contribution of the Peyer's patches to the observed IgA response in the present study cannot be determined from the results presented in this paper. It is known, however, that Peyer's patches in germ-free animals are capable of responding to antigens in the gut lumen with germinal centre formation (Carter & Collins, 1975) and more recently Robertson & Cebra (1976) have shown in rabbits that the magnitude of the IgA antibody response in secretion of isolated intestinal loops is correlated with the presence of a Peyer's patch.

In conclusion, the roles assigned in this discussion to various points of the lymphoid tissue associated with the intestine in response to intra-luminal antigen is in accordance with the known facts on the immunoglobulin class specificity of their precursor cell populations. These roles, however, require confirmation by further experiments which are in progress. These studies are being facilitated by the finding that it is possible to enhance both IgA and IgM/ IgG ACC responses to intra-luminal antigen with DEAE-dextran.

ACKNOWLEDGMENTS

I wish to thank D. Himsley for assistance with the surgery and the skilled technical assistance of M. Booth and Mrs J. Darlington is also gratefully acknowledged.

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