THE ORIGIN AND ANTIGEN-DEPENDENT DISTRIBUTION OF IgA-CONTAINING CELLS IN THE INTESTINE

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The lamina propria of the intestine contains large numbers of IgA-producing plasma cells which arise from precursors in the gut-associated lymphoid tissue (GALT)[1] in response to antigens in the lumen of the gut (1). The precursors are large lymphocytes which travel from the GALT to the blood in the thoracic duct lymph and then migrate into the lamina propria where they secrete IgA antibody (2-4). The mechanism of this selective migration into the lamina propria is not known and, in particular, there is controversy about the role of antigen in determining the distribution of IgA-secreting cells along the intestine.

It has been reported that radiolabeled large lymphocytes from adult syngeneic donors will migrate into the intestinal lamina propria of neonatal rats (5) and into fetal intestine grafted under the kidney capsule of adult mice (6). These experiments with "antigen-free" gut are held to show that factors other than antigen determined the gut-homing of large lymphocytes. On the other hand, Ogra and Karzon (7) showed that local immunization of a segment of human colon with polio vaccine was followed by the appearance of specific IgA antibody which was entirely confined to the immunized segment. Husband and Lascelles (8) reported similar findings in sheep with two Thiry intestinal loops. When different antigens were administered into each loop, IgA antibody against each of the antigens appeared only in the correspondingly immunized loop and never in both loops. In these studies antigen appeared to be decisive in determining the localization of the blood-borne IgA precursors.

Similar contradictions were reported by Pierce and Gowans (4). In immunized rats, the density of cells in the lamina propria producing IgA antibody against cholera toxoid was always highest in that region of the intestine which had been challenged with antigen. On the other hand, the small intestine of nonimmunized rats rapidly accumulated anti-toxin-containing cells (ACC) after an intravenous injection of lymphocytes obtained from the thoracic duct of immunized donors.

In an attempt to reconcile these contradictory reports the colonization of the lamina propria with specific antibody-producing cells has been studied under more defined conditions using Thiry-Vella intestinal loops in rats and cholera toxoid as antigen. The results indicate that the accumulation of ACC in the gut is probably the result of both antigen-dependent and independent processes. The opportunity was also taken to study more closely the origin of ACC and, in particular, the role of Peyer's patches (PP) in their generation.

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[1] *Abbreviations used in this paper:* GALT, gut-associated lymphoid tissue; ACC, antitoxin-containing cells; PP, Peyer's patches; PBS, phosphate buffered saline; MLN, mesenteric lymph nodes; FCA, Freund's complete adjuvant; TDL, thoracic duct lymphocytes; [³H]Tdr, tritiated thymidine, SC, secretory component.
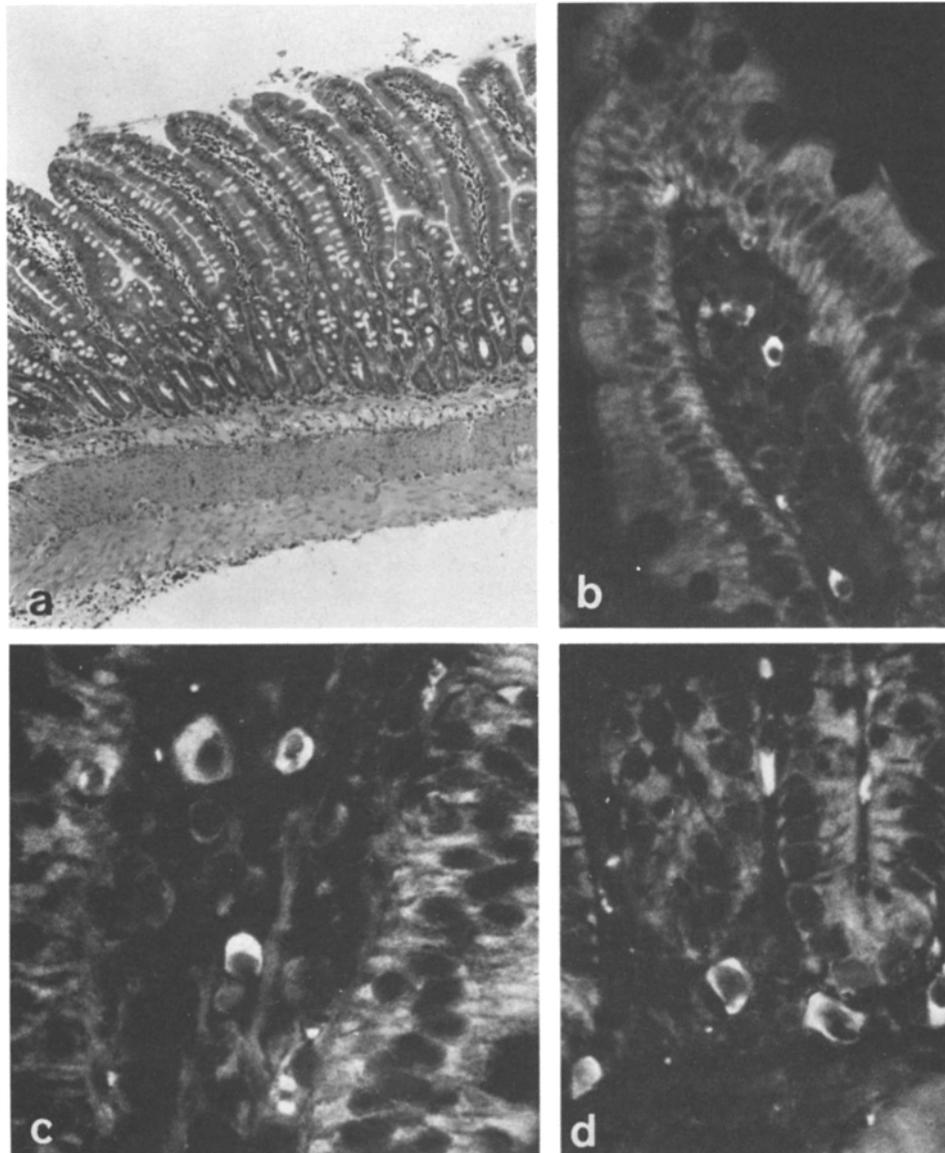
FIG. 1. Histological appearance of Thiry-Vella intestinal loops. (a) Appearance 4 wk after preparation (hematoxylin and eosin, × 160). (b–d) Fluorescent anti-toxin-containing cells in intestinal loops showing distribution in villus core (b [× 850] and c [× 1360]) and base of the mucosa (d [× 1360]). Rats were primed i.p. with 100 μg toxoid in FCA, challenged in the loop 14 days later with 1 mg toxoid, and sections taken 5 days after challenge.

Materials and Methods

Rats. Rats were males or females of the inbred PVG/c strain (hooded, Ag-B5) which were maintained in a specific pathogen-free unit. They were placed in a conventional environment 2–3 wk before use.

Thiry-Vella Loops. Single or double Thiry-Vella intestinal loops were prepared with a modification of the techniques of Markowitz et al. (9). Each loop, with its mesentery intact, was

constructed from about 10 cm of mid-small gut, the most proximal transection being at a point 10–15 cm distal to the colonic attachment of the duodenum. Both ends of each loop were exteriorized through stab incisions in the abdominal wall and the mucosal surface everted, and sutured to the skin. The continuity of the intestine was restored by end-to-end anastomosis. Loops were washed through with sterile phosphate-buffered saline (PBS) daily. Grossly and histologically (Fig. 1 a) the isolated loops had a normal appearance when examined after 4 wk. All experiments were performed between 4 and 21 days after surgery.

Thiry-Vella Loops without PP. The PP were excised from about 10–15 cm of jejunum and the intestine repaired with absorbable sutures. After 2 mo a Thiry-Vella loop was prepared from the segment without PP as described above. The rats were examined at the end of experiments to ensure the absence of PP.

Mesenteric Lymphadenectomy. The chain of mesenteric lymph nodes (MLN) was removed and 2 mo allowed for lymphatic regeneration and the restoration of normal lymph flow from the thoracic duct before loops were prepared. At the end of each experiment pontamine blue was injected subcutaneously (10) to check that the lymphadenectomy had been complete.

Immunization. Cholera toxoid was prepared from a culture filtrate of *Vibrio cholerae*, Inaba, 569B by Dr. R. O. Thomson, Wellcome Research Laboratories, Beckenham, Kent. Two preparations were employed, a crude toxoid, and an immunopurified toxoid (4). The latter preparation (but not the former) was free of toxin and thus lacked the property of binding to cell surfaces.

Unless otherwise stated, all rats were primed by an intraperitoneal injection of 100 μg of purified toxoid in 0.2 ml 0.15 M NaCl emulsified in Fruend's complete adjuvant (FCA). Loops in immunized rats were prepared 10 days after priming. Secondary intestinal challenge was given 14 days after priming. Intact rats were challenged intraintestinally by injecting 1 mg of purified toxoid into the lumen of the duodenum with a 27 gauge needle. Loops were challenged by temporarily sealing each end of the loop with plastic adhesive tape and then injecting 1 mg toxoid into the lumen with a 27 gauge needle. The plastic tape was removed after 24 h and the loops washed through with sterile PBS. These immunizations were performed either at the time of thoracic duct cannulation or by exposing the intestine through a small laparotomy.

Anti-Toxin Assay. The anti-toxin content of heat inactivated sera was measured by passive hemagglutination of tanned erythrocytes (11) sensitized with purified cholera toxin (National Institutes of Health lot 0572, supplied by Dr. J. Seal, National Institute of Allergy and Infectious Diseases, Bethesda, Md.). A control standard serum manufactured by the Swiss Serum and Vaccine Institute (Lot EC3 (A-2/67), anti-toxin content 4,470 U/ml) gave a titer of 1:2560 in this assay.

Rabbit Anti-Cholera Toxin. Antibodies to cholera toxin were prepared by hyperimmunizing rabbits with crude toxoid.

Fluorescein-Conjugated Reagents. Rabbit antibody to cholera toxoid was purified and conjugated with fluorescein isothiocyanate as previously described (4). Fluoresceinated rabbit anti-rat IgA was prepared by the methods of Williams and Gowans (12). Fluoresceinated goat anti-rabbit Ig antiserum was obtained from Nordic Laboratories (Maidenhead, England). Before use, 2.5 ml of a 1:50 dilution of this reagent was absorbed twice with 10^8 rat spleen cells.

ACC in Thoracic Duct Lymph. Thoracic duct lymph was collected at 4°C as previously described (2). Cell counts in lymph were made with a Coulter Counter, model Fn (Coulter Electronics Ltd., Dunstable, England). Smears were prepared from washed thoracic duct lymphocytes (TDL) which had been resuspended in fetal calf serum. They were fixed in 95% ethanol for 2 h at 4°C and then sequentially incubated with purified cholera toxoid (100 μg/ml in PBS), fluorescein-conjugated rabbit anti-toxin (50 μg/ml), and fluorescein-conjugated goat anti-rabbit Ig. The use of two fluorescent conjugates considerably improved the brightness of the fluorescence. Each reagent was allowed to react for 30 min at 20°C and slides were washed in PBS for 30 min after each application. The stained smears were mounted in 90% glycerol in PBS and examined with a Zeiss Universal fluorescence microscope (Carl Zeiss, Oberkochen, West Germany) under dark ground with illumination from a 12 V, 100 W tungsten-halogen bulb passed through a KP500 interference filter and a BG 38 barrier. The proportion of fluorescent cells was derived from a total count of about 1,000 lymphocytes. No fluorescent lymphocytes were seen in smears of TDL from nonimmunized rats stained as above or from immunized rats in which toxoid was omitted from the staining sequence.

ACC and IgA-Containing Cells in Gut Lamina Propria. Segments of intestine of approximately 5 mm in length were obtained from duodenum (at the point of its attachment to the colon), mid-jejunum, distal ileum (within 5 cm of the ileo-cecal valve), or from loops (at their midpoint) while rats were under ether anesthesia. Each segment was incized along the mesenteric attachment, opened out flat on filter paper moistened with ice-cold PBS, and sliced into longitudinal strips each approximately 1 mm in width. The strips were rinsed in cold PBS and then placed in the following solutions at 4°C: 96% ethanol (8 h), 100% ethanol (6 h), and xylene (12 h). The tissues were then brought to room temperature, incubated in paraffin wax under vacuum for 2 h at 60°C, and embedded at 56°C. The blocks were stored at 4°C. Sections were cut at 4 μm thickness, dried onto subbed slides for 30–60 min at 60°C, then cooled to 4°C, dewaxed, and stained. Before immunofluorescent staining sections were pretreated with Lendrum's stain (13) to block the nonspecific uptake of fluorescent reagents by eosinophils. For identifying ACC a double staining procedure was again employed in which the sections were sequentially incubated with purified toxoid (1 mg/ml in PBS), fluorescein-conjugated rabbit anti-toxin, and then fluorescein-conjugated goat anti-rabbit Ig. For detection of IgA-containing cells sections were stained with fluorescein-conjugated rabbit anti-rat IgA. Each reagent was allowed to react for 30 min at 20°C and slides were washed in PBS for 30 min after each application.

Counts of ACC and IgA-containing cells in the intestine were made with × 40 objective and × 8 eyepieces by scanning from the base of the mucosa to the tips of the villi. By viewing the various strips cut from each segment of intestine it was possible to scan 30–50 fields. The width of each field was 0.35 mm and thus a mean of 1 fluorescing cell per field was equivalent to 28.5 cells/cm of intestine. No ACC were seen in gut sections from nonimmunized animals stained as above or in sections from immunized rats in which toxoid was omitted from the staining sequence.

Radioactive Labeling of Lymphocytes. Large TDL from unimmunized donor rats were labeled in vitro with tritiated thymidine ([^{3}H]Tdr) by incubating washed cells in RPMI-1640 (Gibco Bio-Cult, Glasgow, Scotland) with 10% fetal calf serum at a concentration of 10^7 cells/ml to which was added 0.5 μCi of tritiated thymidine per ml (sp act 5 Ci/mmol; Radiochemical Centre, Amersham, England). The cells were incubated for 1 h at 37°C in an atmosphere of 5% CO_2 in air, washed, and injected intravenously into recipients.

ACC from immunized donors weighing 200–250 g were labeled in vivo by the i.v. injection of 150 μCi of tritiated thymidine every 8 h for 3 days starting immediately after the intraduodenal challenge of primed rats.

Autoradiography. To detect labeled ACC, ethanol-fixed smears and sections were stained with fluorescent reagents before preparing the autoradiographs. Tissues for autoradiography alone were fixed in formal alcohol.

Autoradiographs were prepared by dipping the slides in Ilford K2 emulsion (Ilford Ltd., Ilford, Essex, England) diluted 2:1 with water, and developed after exposure for 3–6 wk. In smears and sections which had been stained with fluorescent reagents the autoradiographic grains appeared red under the fluorescence microscope after removing the BG38 barrier filter. Autoradiographs prepared from formalin fixed sections were soaked overnight in phosphate buffer (pH 5.0) after development and then stained through the emulsion with methyl green-pyronin. The density of labeled cells in these sections was measured under dark ground illumination using the same magnification as for counting ACC, and the results expressed as labeled cells per centimeters of intestine.

Results

ACC in Thoracic Duct Lymph after Removing the MLN and after Challenging Loops Lacking PP. Large numbers of ACC appear in the thoracic duct lymph of primed rats after intraintestinal challenge with cholera toxoid (4). Since in most of the present experiments the secondary challenge of antigen was administered to a Thiry-Vella intestinal loop, it was first necessary to examine the ACC response generated in a loop. Single Thiry-Vella loops were prepared in primed rats and 4 days later the loops were challenged with antigen at the same time as the thoracic ducts were cannulated. TDL
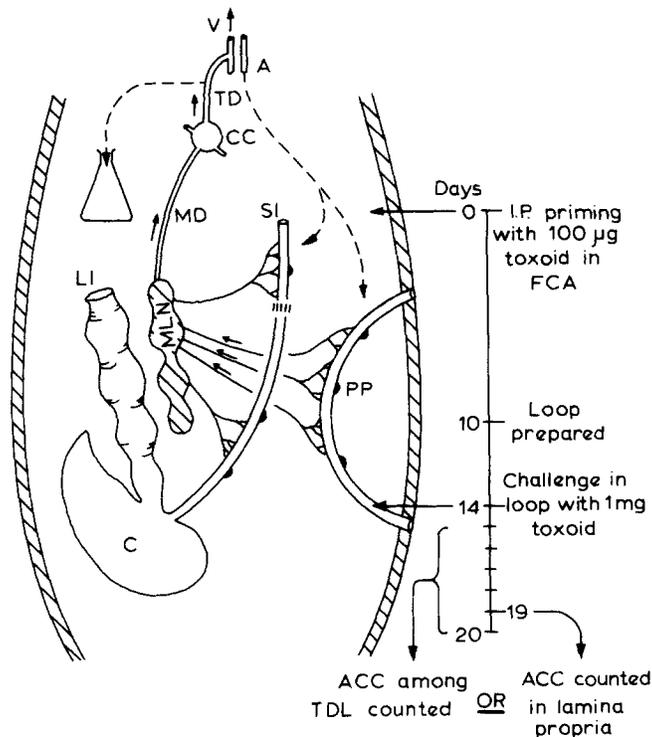
Fig. 2. Diagram showing immunization schedule of rats with Thiry-Vella loops, and pathway of migration of lymphocytes from challenged loop via thoracic duct (TD) to blood and thence to the lamina propria of the small intestine. Lymphocytes were either collected through a fistula or allowed to pass normally into the blood; counts of ACC were performed on either smears of thoracic duct lymph or on sections of gut. PP, Peyer's patch; MLN, mesenteric lymph nodes; MD, mesenteric duct; CC, cysterna chyli; V, vein; A, artery; SI, small intestine; LI, large intestine; C, caecum.

were collected for 6 days and the daily output of ACC determined. The design of the experiments is illustrated in Fig. 2. For comparison, primed intact rats were given a secondary challenge intraduodenally at the time of cannulation and similar collections made. The contribution of the PP and MLN to the response was examined in a similar way by challenging loops from which the PP had been removed surgically and by challenging loops in rats from which the MLN had been removed. Fig. 3 shows that in intact animals large numbers of ACC began to appear in lymph 2 days after intraduodenal challenge and reached a peak of around 250,000 ACC/h on day 4, after which the numbers declined rapidly, confirming the results of Pierce and Gowans (4). The kinetics of the response was similar after challenge into the lumen of a Thiry-Vella loop but the magnitude was reduced. In rats lacking MLN the response from a challenged loop was almost identical to that from normal loops but there was virtually no response from loops without PP.

Influence of Antigen on Distribution of ACC in Gut Lamina Propria. The influence of antigen on the location of ACC in primed rats after intraintestinal challenge was studied using double Thiry-Vella loops so that the localization of antigen could be controlled. The challenging dose of toxoid was given to either the proximal or the distal loop and the density of ACC in the lamina propria of each loop was estimated
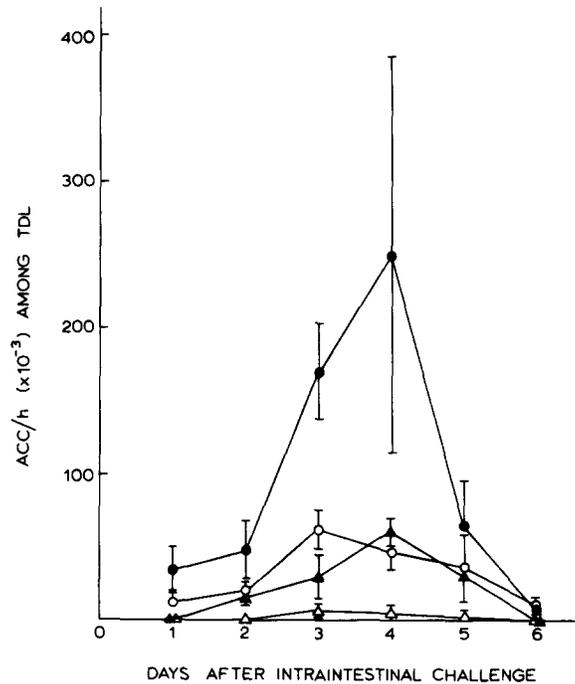
Fig. 3.   Output of ACC in thoracic duct lymph after intraintestinal challenge of primed rats with 1 mg toxoid. 14 days after priming, challenge was given into the duodenum of five normal intact rats (●); and into single Thiry-Vella loops (prepared 10 days after priming) of eight normal rats (○), of four mesenteric lymphadenectomized rats (▲), or of five rats in which the loops lacked PP (△). Points are means, and vertical bars, standard errors.

5 days later. The appearance of the fluorescent ACC in gut loops is illustrated in Fig. 1 (b–d). Table I shows that the greatest number of ACC was consistently found in the loop which had been challenged. As a control for nonspecific homing of ACC to challenged loops, replicate sections of tissues from rats challenged in the distal loop were stained with anti-IgA reagent and IgA-containing cells (of which the ACC are a subpopulation [4]) were counted. Approximately equal numbers of IgA-containing cells were found in both the challenged and nonchallenged loops (Table I), although the numbers in the loops were fewer than in the remaining intact intestine.

Distribution of IgA-Containing Cells in the Small Intestine.   It can be seen from Table I that the density of IgA-containing cells in the duodenum was about double that in the ileum. Table II confirms this observation in the intestine of normal rats in which there is clearly a progressive decrease in the density of IgA-containing cells from duodenum to ileum.

Homing of Radiolabeled Large TDL to Loops.   To determine whether the presence of fewer IgA-containing cells in isolated loops than in the intact intestine would be reflected by a similar difference after the i.v. injection of large lymphocytes from syngeneic donors, large cells in thoracic duct lymph of normal syngeneic rats were labeled in vitro with [³H]Tdr and injected i.v. into four normal rats with single Thiry-Vella loops. The numbers of labeled cells in the laminae propriae of loop, duodenum, and ileum of each rat 24 h after injection are shown in Table III. A consistently smaller number of labeled cells was found in isolated loops.

TABLE I

Distribution of ACC and IgA-Containing Cells in the Intestine of Primed Rats with Double Thiry-Vella Loops after Challenge with Cholera Toxoid into One Loop

| | Loop challenged | | |
| | Proximal (4)* | Distal (6)* | |
	ACC/cm	ACC/cm	IgA-Containing cells/cm
Duodenum	13 (10–16)‡	15 (7–22)	2094 (1,638–2,569)
Proximal loop	69 (62–89)	15 (10–21)	822 (693–921)
Distal loop	16 (12–20)	73 (52–121)	730 (662–870)
Ileum	16 (8–26)	18 (12–25)	1122 (988–1265)

* Number of rats.
‡ Mean, with range in parentheses.
  Loops prepared 10 days after i.p. priming with 0.1 mg cholera toxoid in FCA. One loop challenged by injection of 1 mg toxoid into the lumen 4 days after surgery. ACC and IgA-containing cells scored in gut sections 5 days after challenge.

Kinetics of Accumulation of ACC.   Although the results in Table I indicate an influence of antigen on the accumulation of ACC in the lamina propria, the presence of ACC in the nonchallenged gut and the previous finding (4) of ACC in the intestine of nonimmunized rats 5–6 h after a transfusion of TDL from immunized donors, weakens the concept that the accumulation of ACC in the lamina propria is exclusively mediated by antigen. However, counts of ACC in histological sections at a single time may have given an incomplete picture so observations on the accumulation of ACC in the gut were made at various times after the injection of immune TDL into the blood.

TDL were collected from the thoracic ducts of immunized donors from day 3 to day 4 after intraduodenal challenge and injected i.v. into normal, nonimmunized recipients. The recipients were killed at various times after transfer and the density of ACC in the jejunum determined. Table IV shows that there was a progressive decrease in density between 6 and 48 h after cell transfer. This result together with the data in Table 1 suggest that antigen does not dictate migration into the gut but that it determines the ultimate distribution of ACC in the lamina propria. To test this possibility it was necessary to compare the accumulation of ACC in challenged and nonchallenged gut under conditions where the normal continual supply of ACC to the circulation over the period of the response was interrupted. It should be recalled that the thoracic duct is the major source of ACC in the intestine (4).

A group of 21 primed rats with double Thiry-Vella intestinal loops were cannulated and challenged with cholera toxoid in the distal loop. TDL from each rat were collected from day 3 to day 4 after challenge, washed, and then reinfused i.v. back into the rats in a single dose. The thoracic duct cannulae continued to drain throughout the experiment. The rats were killed at various times up to 48 h after TDL injection and the density of ACC in the challenged loop compared with that in the nonchallenged loop. It can be seen from Fig. 4 that early after injection (3–6 h) there were approximately equal numbers of ACC in both loops, their density at 6 h being only slightly less than in challenged loops of uncannulated rats 5 days after challenge (Table I). By 18 h the numbers in the nonchallenged loops had declined to

TABLE II

Distribution of IgA-Containing Cells in the Intestine of Normal Rats

	IgA-containing cells/cm*
Duodenum	1,953 (1,588–2,429)
Jejunum	1,235 (1,005–1,652)
Ileum	963 (712–1,404)

* Mean of 7 rats, range in parentheses.

zero. However in the challenged loop the numbers continued to increase and reached a peak of about 300 ACC/cm at 36 h, after which they declined.

Local Proliferation of ACC after Homing. Since the number of ACC in immunized loops continued to increase after all their precursors had left the circulation (i.e. after ACC had ceased to appear in nonimmunized loops) it appeared that ACC might be proliferating in the lamina propria of the intestine. To investigate this possibility four primed rats with single loops were cannulated and challenged in the loop. TDL were collected from day 3 to day 4 after challenge, washed, and injected i.v. back into each rat. Each was then given 150 μCi [^{3}H]Tdr i.v. 18 h after the injection of cells (by which time all ACC or their precursors were judged to have left the circulation, from data in Fig. 4) and this dose was repeated at 26 and 32 h. The rats were killed at 40 h and sections of loop tissue processed for fluorescence and autoradiography. Table V shows that about 10% of all ACC in each rat were labeled. This is prima facie evidence that the ACC were dividing in the lamina propria.

Fate of ACC in Nonimmunized Intestine. Since ACC had disappeared from the nonchallenged loops by 18 h after transfer (Fig. 4) they either died in the tissues or left by way of the lymphatics. The second alternative was investigated by first collecting TDL from primed rats which had been challenged intraintestinally with toxoid. These donor TDL were then injected into eight normal, nonimmunized recipients in which thoracic duct fistulae had already been prepared. ACC of donor origin which migrated from the intestine of the recipients would then be detected in smears of recipient TDL. The mean dose of TDL given to the eight recipients was 2.5 $\times$ 10^8 cells (range 1.2–4.6 $\times$ 10^8) and the mean number of ACC in each dose was 3.6 $\times$ 10^6 (range 1.6–8.2 $\times$ 10^6). A mean of 3.2 $\times$ 10^5 ACC (range 1.7 – 5.8 $\times$ 10^5) were recovered among TDL of recipients and first appeared in lymph between 12 and 24 h after transfer. Apparently, blood-borne cells which had entered the lamina propria left it again by way of the lymph and were detected as ACC in the thoracic duct.

To establish whether the blood-borne precursors of the recovered ACC were ACC or cells at an earlier stage of differentiation the experiment was repeated in two rats using donor TDL which had been labeled in vivo with [^{3}H]Tdr (Materials and Methods). Although 100% of ACC in each dose of cells was subsequently shown by autoradiography to have been heavily labeled (20–30 grains per cell after 2 wk exposure) none of the ACC recovered among recipient TDL was labeled after 6 wk exposure, showing that the recirculating ACC had differentiated from unlabeled cells in the donor TDL which were not themselves ACC; that is, they did not contain detectable amounts of anti-toxin at the time of transfer. On the other hand, the ACC which were transferred and which localized in the lamina propria did not migrate back into the lymph; they presumably died in the gut.

To determine whether the presence of antigen would prevent the recirculation of

TABLE III

Distribution of [³H] Tdr-Labeled Normal TDL after Injection into Normal
Rats with a Single Thiry-Vella Loop

Rat	Labeled cells in- jected ($\times$ 10^{-6})	Labeled cells/cm		
		Loop	Duodenum	Ileum
1	4.2	17	36	37
2	7.9	21	40	37
3	28.8	166	191	186
4	18.9	66	106	91
5	8.2	42	51	57

Distribution of labeled cells (>5 grains/cell) scored in autoradiographs of gut taken 24 h after i.v. injection of TDL among which the large lymphocytes had been labeled in vitro with [³H]Tdr. Loops were prepared 8–9 days before injection of cells.

these ACC precursors, two nonimmunized rats were each injected with 2.2 $\times$ 10^8 TDL (containing 2.1 $\times$ 10^6 ACC) and crude cholera toxoid was added to their drinking water to provide an oral dose equivalent to 5 mg purified toxoid per day. This had no effect on the recirculation of ACC into thoracic duct lymph, 5.1 $\times$ 10^5 and 6.5 $\times$ 10^5 ACC, respectively, being recovered among recipient TDL.

Accumulation of ACC in Loops without PP.   Although the ACC response in lymph was negligible after challenging loops without PP in primed rats (Fig. 3) it was important to know whether the presence of PP influenced the accumulation of ACC in the adjacent lamina propria. Two primed rats with single Thiry-Vella loops from which PP had previously been removed were accordingly challenged in the loop and then given TDL from primed and intraduodenally challenged donors 4 days after loop challenge. Sections of loop and intact intestine were taken 48 h after cell transfer and ACC counted. Table VI shows that ACC did accumulate in the challenged loop despite the absence of PP although the density was less than that in normal challenged loops at 48 h after TDL injection (Fig. 4). This difference may simply reflect the importance of PP as the main portal of entry for antigen to the lamina propria.

Discussion

The aim of this paper has been to define the factors which determine the distribution of IgA-containing (and therefore presumably IgA-secreting) cells in the lamina propria of the small intestine. The IgA-containing cells studied were those generated in response to intestinal challenge with cholera toxoid in rats which had been primed intraperitoneally (4). Cholera toxoid is an antigen to which the rats had not been previously exposed and all the ACC were thus generated as a direct result of experimental immunization. The majority of ACC produced in this way were of IgA specificity (4).

The use of Thiry-Vella loops made it possible to challenge a defined segment of intestine by presenting antigen intraluminally. After challenging such loops ACC appeared in the draining thoracic duct lymph and the peak of the cellular response was about 25% of that seen after immunization of the whole intact intestine. Since each loop represented about one quarter of the length of the small intestine it appears that the response from the gut is proportional to the length exposed to antigen.

TABLE IV

*ACC in Jejunum of Nonimmunized Rats at Various Times after Transfer of
TDL from Immunized Donors*

Time after TDL injection	Rats	ACC/cm in Jejunum*
h		
6	2	27 (25–29)
24	3	12 (8–15)
48	2	8 (7–8)

* Mean, with range in parentheses.
TDL collected from immunized donors from day 3 to day 4 after intraduodenal challenge and injected i.v. into nonimmunized recipients. Mean dose of TDL was 1.7×10^8 cells (range 0.9–2.5×10^8); mean number of ACC in each dose was 1.8×10^6 (range 1.0–3.1×10^6).

Excision of the PP from a Thiry-Vella loop before immunization abolished the subsequent ACC response in the lymph indicating that the ACC had originated exclusively from the patches, confirming the earlier work of Craig and Cebra (1) that the PP tissue is an enriched source of the precursors of IgA-secreting cells. Removal of the mesenteric nodes before immunization caused no significant reduction in the ACC response and suggested that engagement with antigen occurred entirely within PP.

Previous studies had shown that the ACC which accumulated in the lamina propria of primed rats after intraintestinal boosting were derived from precursors in the draining thoracic duct lymph (4). The point now at issue is the extent to which antigen in the gut dictates the accumulation of ACC in the lamina propria. To study this point, primed rats with two loops were boosted in one loop only and TDL, collected at the height of the ACC response, were reinjected as a single large dose of cells. The thoracic duct fistula remained open to cut off any further supply of precursors from the draining lymph. 6 h after injection, similar numbers of ACC were present in both loops (Fig. 4), but whereas the numbers declined thereafter in the unchallenged loop they increased dramatically in the challenged loop. Thus antigen had a profound effect on the number and distribution of ACC in the lamina propria although their precursors could clearly enter the lamina propria independently of antigen.

In an experiment identical in design to that just described, tritiated thymidine was administered to the recipient after it could be assumed that all the injected precursors of ACC had left the circulation. The result showed that about 10% of the ACC in the lamina propria became labeled, suggesting that part at least of the dramatic increase in the number of ACC in the challenged loop was due to the proliferation of ACC or their precursors in the gut itself.

A further question concerns the nature of the lymph-borne precursors of intestinal ACC. IgA plasma cells are known to be derived from nonrecirculating precursors among the large lymphocytes in thoracic duct lymph (2, 14, 15), many of which already contain specific antibody (4); that is, intestinal ACC are derived from lymph-borne ACC. However, the present experiments showed that there is also a population of undifferentiated precursors in lymph which do recirculate and which can develop into ACC. In this connection, Williams and Gowans (12) described a population of
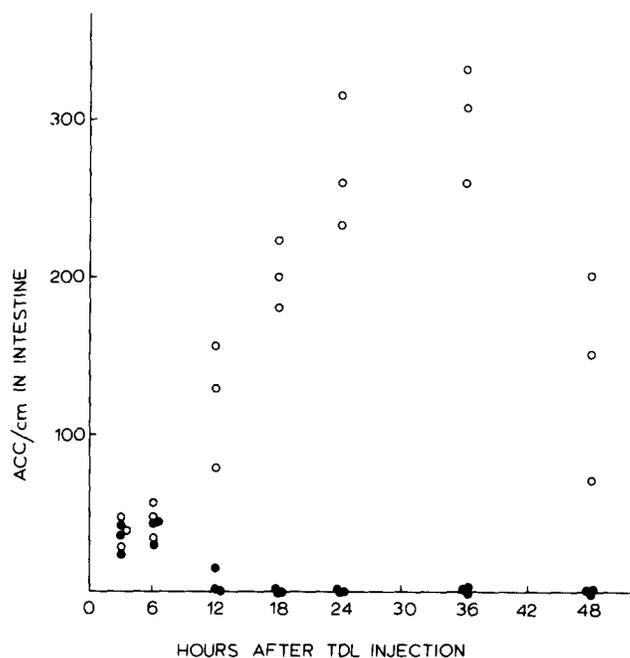
Fig. 4.　Density of ACC in proximal loop (●) and distal loop (○) of primed rats at various times after an injection of TDL. The thoracic ducts were cannulated and 1 mg toxoid injected immediately into the lumen of the distal loop. TDL were collected from day 3 to 4 after loop challenge, washed and injected i.v. back into each rat. Three rats were killed at each time interval and ACC counts performed on proximal and distal loops. In all rats the thoracic duct was drained throughout the experiment.

small lymphocytes comprising at least 20% of cells in rat thoracic duct lymph which carried IgA on their surface but which lacked internal IgA, and these were thought to be precursors of IgA-containing cells. The recruitment and differentiation of these recirculating cells may have contributed to the antigen-induced accumulation of ACC in the challenged loops (Fig. 4). On the other hand it is clear from the labeling studies that lymph-borne ACC do not reappear in the lymph after they have migrated into the lamina propria, whether or not the specific antigen is presented to the gut. Thus the disappearance of ACC from the nonchallenged loops (Fig. 4) cannot be accounted for by recirculation; presumably the cells die in situ. In our own previous experiments (4) the disappearance of ACC from the lamina propria of nonimmunized animals after a transfusion of immune TDL was not recorded since localization was studied only at 6 h after cell-transfer.

The present experiments suggest an explanation of the apparent contradiction that while nonrecirculating precursors of IgA plasma cells (large lymphocytes) migrate randomly into the small intestine, specific intraluminal challenge of a segment of the gut with antigen results in a local IgA response of considerably increased magnitude. Thus, nonantigen-specific emigration of large lymphocytes accounts for the homing of large lymphocytes into antigen-free gut in neonatal rats (5) and into grafts of fetal intestine (16); it also accounts for the small ACC response which was observed in unchallenged Thiry-Vella loops and, presumably, for an early secretory antibody response at intestinal sites remote from local stimulation. The effect of antigen in

TABLE V

Incorporation of [³H]Tdr by ACC in Thiry-Vella Loops

Rat	Total ACC counted	No. labeled ACC (>5 grains/cell)	Labeled ACC
			%
1	199	18	9.1
2	211	20	9.5
3	172	18	10.5
4	215	22	10.3

Primed rats with single Thiry-Vella loops were cannulated and challenged in the loop. TDL were collected from day 3 to day 4 after challenge, washed, and injected i.v. back into each rat. Rats were then given doses of 150 μCi [³H]Tdr i.v. at 18, 26, and 32 h after TDL injection. They were killed 8 h after the last injection of isotope and sections of loops processed for fluorescence and autoradiography.

increasing the local density of IgA cells develops more slowly and is due partly to the recruitment and differentiation of recirculating IgA precursors (small lymphocytes) at the site of deposited antigen. Cell division in the lamina propria also contributes to the antigen-induced local response. It is not known whether division is restricted to the new population which is recruited or whether plasma cells in the gut also divide in response to restimulation by antigen. It is clear that cells begin to secrete antibody while still capable of division (17) but it is generally assumed that mature plasma cells do not divide (18). Indeed, Mattioli and Tomasi (19) have made estimates of the lifespan of IgA plasma cells in the gut based on this assumption. In interpreting the present experiments it should be borne in mind that prolonged drainage of lymph from the thoracic duct may itself have provided some stimulus for local proliferation in the lamina propria.

The large increment in the number of ACC in the gut at the site of locally applied antigen may involve both antigen-dependent recruitment and cell division in the lamina propria but it is not known whether antigen provides any additional signal for the migratory step itself. The element in the antigen-free gut which dictates the tissue specificity of homing has not been identified. Several authors have attempted to implicate surface-IgA and secretory component (SC) in such a receptor system, but it has been demonstrated in this laboratory that ¹²⁵I-labeled rabbit SC does not bind to the surface of living large TDL in vitro despite its avid binding to IgA-containing cells in fixed smears of TDL(H-J Grosse, unpublished data) and further, the pretreatment of recipient mice with antibodies directed against either SC or IgA failed to alter the homing to the gut of injected mesenteric lymph node cells (20).

It should be emphasized that a distinction between antigen- and tissue-specific homing to TDL to the gut was possible in the present study because a specific antigen was employed to which rats are not normally exposed. Large lymphocytes from mesenteric lymph nodes also show a predilection for the gut (3, 20, 21). It would be interesting to determine if large lymphocytes from nodes remote from the gut would home to the intestine if they were generated by an antigen which was later introduced into the gut. The study of a specific IgA antibody response in the present experiments also avoided any complications arising from the homing of T cells as well as B cells to the lamina propria after the transfusion of labeled lymphoid cells. Radiolabeled T

TABLE VI

Accumulation of ACC in Thiry-Vella Loops without PP

Rat	ACC/cm		
	Loop (−PP)	Duodenum	Ileum
1	40	11	10
2	44	11	11

PP were removed from a segment of intestine and the rats primed i.p. 6 wk later. Single Thiry-Vella loops were prepared from the segment lacking PP 10 days after priming and rats were challenged in the loop 4 days later. TDL from immunized donors (2.3 × 10⁸ cells, containing 3.4 × 10⁶ ACC, per recipient) were injected i.v. 4 days after challenge. ACC were counted in sections taken 48 h after cell transfer.

blasts (22–24) may not share the same migratory properties as B blasts of GALT origin.

In a study of the distribution of all IgA-containing cells, irrespective of antibody specificity, it was found that they were present in smaller numbers in isolated Thiry-Vella loops than in the intact intestine. Similarly, smaller numbers of radiolabeled large lymphocytes homed into the loops. These observations no doubt reflect the removal of the normal antigenic load from the loops by their isolation from the flow of digesta. Conversely, the increased number of IgA-containing cells which were observed in the proximal small intestine is probably due to the greater stimulation of this region by antigens in ingested material. This distribution of IgA-containing cells is in agreement with similar data in the calf (25) and human (14) and appears to reflect the antigenic load of dietary antigens rather than that of the commensal gut flora which is heaviest in the distal small intestine.

Summary

The aims of this paper were to establish the origin of cells producing IgA antibody to cholera toxoid in the lamina propria of the small intestine and to define the role of antigen in their distribution. The use of Thiry-Vella loops made it possible to restrict antigenic challenge to a defined segment of the intestine in rats which had been primed i.p. with toxoid in Freund's complete adjuvant. The anti-toxin-containing cells (ACC) which appeared in the draining thoracic duct lymph after challenge of a loop were almost all of IgA specificity and their numbers were proportional to the length of intestine exposed to antigen. The abolition of this cellular response which occurred when Peyer's patches (PP) were removed from a loop before challenge and the failure of mesenteric lymphadenectomy significantly to affect the response indicated that ACC originated exclusively from PP. Cell transfer studies showed that although nonrecirculating large lymphocytes gave rise to ACC in the lamina propria, some of the recirculating small lymphocytes also developed subsequently into ACC.

Counts of ACC in the lamina propria of challenged loops were consistently greater than in nonchallenged loops although some ACC were always present in the latter. However, a time-course study on the appearance of ACC in the lamina propria of cannulated rats given a single dose of thoracic duct lymphocytes from immunized donors demonstrated that ACC continued to accumulate and persist in challenged loops but only appeared transiently in nonchallenged loops. These transients did not

migrate from the lamina propria back into the lymph and they presumably died in situ. The increase in the number of ACC in loops which had been challenged with antigen was probably due both to cell division in the lamina propria and to the development of new ACC from recirculating lymphocytes which had been recruited into the loop. Thus, the cells which give rise to intestinal ACC can migrate into the lamina propria independently of antigen, but antigen has a profound effect on the location, magnitude, and persistence of the response.

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