PEYER'S PATCHES: AN ENRICHED SOURCE OF PRECURSORS FOR IGA-PRODUCING IMMUNOCYTES IN THE RABBIT*

By SUSAN W. CRAIG‡ and JOHN J. CEBRA, Ph.D.

(From the Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218)

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The secretory glands of many mammals are known to produce large amounts of a gamma-A immunoglobulin (sIgA) which is shed into the external secretions. Immunofluorescent staining of the subepithelial mucosa of many types of secretory glands and tissues (e.g. mammary, lacrimal, salivary, bronchial, and nasal as well as the lamina propria along the whole of the gastrointestinal tract) has shown that the majority of all immunoglobulin-producing cells in these sites are producing IgA (1).

In particular, it has been reported (2, 3) that in the rabbit approximately 80% of all immunoglobulin-producing cells in the lamina propria of the small intestine stain specifically for the alpha chain of IgA. In contrast, only 8–10% of all similar cells in the spleen and lymph nodes contain this class of immunoglobulin. The observed predominance of IgA-producing cells in the lamina propria of the intestine suggested to us the possibility that the gut of a normal animal might contain a population of precursor cells which would be capable of proliferating and giving rise to IgA-producing cells.

By using a method of analysis developed by Frensdorff et al. (4, 5) in which cell transfer and subsequent quantitation of immunocytes by immunofluorescence is used to assess the proliferative and differentiative potential of rabbit lymphoid cells in irradiated allogeneic hosts, we were able to show that the Peyer's patches contain an enriched source of precursors for IgA-producing cells. These cells proliferate both in the spleen and in the intestinal lamina propria and give rise to immunoglobulin-producing cells, most of which make IgA.

Materials and Methods

Animals.—Male rabbits, weighing between 5 and 6 lb., were obtained from B and H Rabbitry (Rockville, Md.). Approximately 96% of the rabbits we have obtained from this rabbitry were homozygous for the b4 allotypic marker, an antigenic marker on the kappa

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light chain of rabbit immunoglobulins. Rabbits which were homozygous for the b5 allotypic marker were purchased from the inbred colonies at Bar Harbor, Maine (Jackson Laboratories) or received as gifts through Dr. Rose Mage, of the National Institutes of Health.

Preparation of Cell Inocula.—For each set of cell transfers, a single homozygous b5 rabbit was used as the lymphoid cell donor. These donors were anesthetized (60 mg of Nembutal [Abbott Laboratories, Baltimore, Md.] in 3.6 ml of sterile saline, given intravenously) and then exsanguinated by cardiac puncture. The blood was collected into heparin (4000 IU in 0.9% NaCl) to prevent clotting. Popliteal lymph nodes were removed and single cell suspensions were made by gently teasing the tissue apart with fine forceps in Eagle's minimal essential medium (MEM)[1] containing 2% (v/v) of Antibiotic Antimycotic (AA) solution (both from Grand Island Biological Company, Grand Island, N. Y.). Between five and seven Peyer's patches were excised from the ileum together with the sacculus rotundus, a sac-like patch which is found at the ileocecal valve.[2] The cells from these lymphoid tissues were dispersed into the same medium used for popliteal cells. The peripheral blood, lymph node cells, and Peyer's patch cells were sedimented at 700 *g* for 20 min at 4°C and then washed once in MEM containing 2% (v/v) AA solution. Portions were taken from each cell suspension, diluted with Turk's solution (30 ml glacial acetic acid/liter of distilled water with a few crystals of gentian violet added) to eliminate red blood cells, and the nucleated cells were counted with a hemocytometer. The cells were kept at 4°C during these procedures.

Cell Transfer.—The cell transfers were done in the following manner: 2 days before cell transfer, recipient b^4/b^4 rabbits received the first of the daily subcutaneous injections of penicillin and streptomycin (200,000 IU of penicillin and 100 μg of streptomycin dissolved in sterile, pyrogen-free isotonic NaCl). The following day (1 day before transfer) the recipients were anesthetized as previously described and given a lethal dose of irradiation (500 R each side). On day 0, 5×10^7 b^5/b^5 donor cells from the appropriate tissue were injected into the marginal ear vein of each recipient. When the donor allotype marker could be detected in the sera of the recipients by gel diffusion analysis, the animals were sacrificed. In a typical experiment there were three recipients for each cell source tested. Usually, all the recipients were positive by day 6 after cell transfer and were sacrificed together. The cell counts tabulated in this paper are from one particular transfer experiment. However, the generalizations concerning quantitative and qualitative repopulation of spleen and intestine with immunocytes are based on observations of stained tissue from three transfer experiments.

Preparation of Cytocentrifuge Slides.—On the day of sacrifice the host animals were anesthetized and then exsanguinated by cardiac puncture. The spleen of each animal was excised and sliced into approximately $2 \times 2 \times 4$ mm pieces. Three or four of these pieces from different parts of the spleen were quick-frozen as described below. The remaining spleen fragments were mechanically dissociated into single cells in MEM containing 1% (v/v) AA solution and 20 μg/ml of DNAase. The suspensions were allowed to incubate for 10 min at room temperature. This treatment was sufficient to prevent clumping of all the cells by the DNA released from damaged cells. After three washes in MEM containing 1% AA at 4°C, the cells were resuspended in the same medium and white cell counts were made on samples diluted with Turk's solution. Finally, the spleen cell suspensions were adjusted to a concentration of

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[1] *Abbreviations used in this paper*: AA, penicillin 10,000 units/ml, fungizone 25 μg/ml, streptomycin 10,000 μg/ml; ARC, antigen-reactive cells; MEM, Eagle's minimal essential medium; PFC, plaque-forming cells; SRBC, sheep red blood cells.

[2] In one experiment, cells from the sacculus rotundus and cells from the remaining Peyer's patches were used separately as donor cell inocula. By both quantitative and qualitative analysis, repopulation of recipient spleen and intestine by these two inocula was identical.

1×10^6 white cells/ml. Replicate cytocentrifuge slides, each containing approximately 2×10^5 cells in a circular area 0.5 cm in diameter were made in a Shandon cytocentrifuge (6). The cells were centrifuged onto the slides at 1000 rpm for 10 min. These cells were then fixed in 95% ethanol for 30 min before staining with specific fluorochrome-tagged reagents.

Preparation of Tissue for Cryostat Sections.—Pieces of spleen and ileum were taken from each of the recipients and quick-frozen in a tube of methyl butane cooled to −70°C in a dry ice acetone bath. Cryostat sections (2 μ thick) were made on an International Model CT1 cryostat microtome. The sections were fixed in 95% ethanol for 30 min before staining.

Preparation of Fluorescent Reagents.—Specific goat anti-rabbit α-chain and goat anti-rabbit γ-chain antibodies were made by immunizing goats with the appropriate rabbit immunoglobulin and absorbing the resulting antisera with normal rabbit IgG and rabbit IgA coupled to Sepharose 2B, respectively. Specific rabbit anti-b5 was prepared by immunizing b^4/b^4 rabbits with purified IgG from b^5/b^5 rabbits (4). Fluorescein (green)- and rhodamine (red)-conjugated goat anti-rabbit α-chain, goat anti-rabbit γ-chain and rabbit anti-rabbit b5 were prepared according to the method of Cebra and Goldstein (7).

Staining of Cryostat Sections and Cytocentrifuge Slides.—To double stain specimens with goat anti-rabbit α-chain and rabbit anti-rabbit b5, these two reagents were added together in proportions which were previously found to give the brightest staining and the lowest background, and a drop of the mixture was spread on top of the section. Double staining with goat anti-rabbit γ-chain and rabbit anti-rabbit b5 required sequential staining since these two reagents interact. In these cases the slides were first stained with anti-γ, washed, and then stained with anti-b5.

Microscopy.—Sections and cytocentrifuge slides were observed with a Leitz Ortholux microscope which was fitted with a dark-field condenser D = 1.20, and an Osram HBO-200 W high pressure mercury lamp. The exciting light was first passed through a heat-absorbing filter (BG38, Corning Glass Works, Corning, N. Y.). Double-stained cells (intermediate shades of yellow verging on chartreuse), cells only stained red, and cells only stained green could be simultaneously observed when the BG12 excitation filter was used in combination with a yellow glass eyepiece barrier filter (K490, Schott, Mainz, W. Germany). In order to verify that a yellow or chartreuse cell observed under this system of illumination was also stained with the rhodamine conjugate, it was subsequently observed using a green interference filter (T_{max} = 546 nm; Schott) for excitation and a red (RG1 Schott) glass eyepiece barrier filter. Filter RG1 is opaque to the yellow-green light emitted by cells stained with the fluorescein conjugate. To determine whether a fluorescing cell was stained by the fluorescein conjugate, a Wratten window filter 57A (Eastman Kodak, Rochester, N. Y.) was used in combination with the Corning BG12 filter. Filter 57A is a green window filter which, when used in double thickness, excludes most of the red-orange light emitted by cells stained with the rhodamine conjugate.

Scoring of Cells on Cytocentrifuge Slides.—Replicate cytocentrifuge slides, containing approximately 2×10^5 nucleated cells/slide in a monolayer, were double stained either with red (rhodamine) anti-b5 and green (fluorescein) anti-α chain, or green anti-γ chain and red anti-b5. Using the series of filters described above, the number of double-stained cells (observed as shades of yellow or chartreuse) was counted. Each cell counted was checked with the green excitation filter and the RG1 barrier filter to ascertain that it was indeed red as well as green. Total fluorescent cell counts were made on slides containing less than 1000 fluorescent cells. Slides containing more than 1000 fluorescent cells were sampled in the following manner. The number of microscope field diameters in the diameter of the cytocentrifuge film was determined. At × 540 magnification there were 23 such fields. 10 microscope fields were examined across the diameter of the cytocentrifuge film and the average number of double-stained cells per field was calculated. This average was multiplied by 23^2 to give

the approximate total number of double-stained cells per slide. In the same way, the total number of red b5 cells per slide was counted using the green Schott excitation filter and the RG1 barrier filter.

Photography.—Pictures of fluorescent cells were taken using a Leica camera and Anscochrome 500 film. Successful exposure times under our conditions of excitation were 3 min for green-yellow fluorescing cells and 30–45 sec for red fluorescing cells.

RESULTS

Analysis of Dispersed Cells from Recipient Spleens.—The ability of cells from different lymphoid tissues to proliferate and differentiate into immunoglobulin-producing cells was assessed by allogeneic cell transfer into lethally irradiated recipients. The distribution and number of donor cells making IgA and IgG were determined by use of immunofluorescent methods on single cell suspensions. The cell counts presented in Table I show that when popliteal lymph node or peripheral blood cells were injected, 64–80% of the b5 cells found in the recipient spleens were producing IgG, while only 6–18% were producing IgA. On the other hand, when Peyer's patches were used as the cell source, 65–79% of the b5 cells in the recipient spleens were producing IgA and only 10–32% were making IgG.

Distribution of IgA- and IgG-Producing Donor Cells in Cryostat Sections of Recipient Small Intestine.—Cryostat sections were made of recipient small intestine to determine whether or not repopulation of the gut by donor cells making α- or γ-chain could be effected by lymphoid cells from different sources. Figs. 1–4 are typical microscope fields which illustrate some of the following observations: (*a*) When 5×10^7 Peyer's patch cells were transferred into homozygous b4 recipients, there was considerable repopulation of the recipient gut by donor cells. In addition, most of the donor immunocytes in any given cross-section were producing IgA (Figs. 1–4). Donor cells producing IgG were also found, but at a much lower frequency. (*b*) When 5×10^7 popliteal lymph node cells were transferred into b4 recipients, the frequency of donor b5 cells per gut section was extremely low. In addition, the majority of donor b5 cells which were found in the sections were not producing IgA. Most of the donor immunocytes in this case were found to be producing IgG. (*c*) In the particular experiment yielding the data shown in Table I, no donor cells at all could be found in random sections taken from several regions of the small intestine when peripheral blood was used as a cell source. However, in other similar experiments it was usually possible to find a few donor cells in at least some sections. Among those few donor immunocytes observed, IgA-producing donor cells were extremely rare.

The observations recorded from stained sections of ileum from recipients of Peyer's patch and popliteal lymph node cells are interpreted to mean that Peyer's patch cells are considerably more efficient in reseeding the gut with immunoglobulin-producing cells than are an equal number of cells from the

popliteal lymph nodes. In addition, when Peyer's patches are used as the cell source, the percentage of donor cells in the gut which stain for α-chain is much greater than when a popliteal lymph node is used as a cell source.

It would seem unlikely that these findings are explicable on the basis of differential survival of popliteal lymph node and Peyer's patch cells during the handling period before transfer since the number of b5 cells in the spleens of animals which received popliteal lymph node cells was similar to the number of b5 cells in the spleens of animals which received Peyer's patch cells (Table I). That is, at least in the case of Peyer's patches and popliteal lymph node recipients, the transfers were equally successful. At this point it is not clear whether the lower number of b5 donor cells in recipients of peripheral blood reflects lability of peripheral blood cells in this system or whether it reflects the number of immunoglobulin precursor cells in 5×10^7 white cells from peripheral blood.

Distribution of IgA- and IgG-Producing Donor Cells in Sections of Recipient Spleen.—Immunofluorescent analysis by Frensdorff et al. (4, 5) of spleens which had been reseeded by donor peripheral blood cells showed that clusters of immunoglobulin-producing cells formed in the spleen, often around a small arteriole, and that most, but not all, immunocytes in a given cluster contained immunoglobulin of the same class and allotype.

In the experiments reported here, cryostat sections of spleen were made to determine how the composition of clusters in the spleen compared with the differential counts from cytocentrifuge slides. Spleen sections were double stained either with red anti-b5 and green anti-α chain or with red anti-b5 and green anti-γ chain. Figs. 5–12 illustrate some of the following observations: (*a*) The spleens of animals receiving Peyer's patch cells contained many clusters composed of donor cells with IgA in their cytoplasm. A small cluster is shown at high power to facilitate identification of the same cells in the two photographs of the same field under two different conditions of illumination (Figs. 5, 6). However, the majority of such clusters in the spleen were much larger (Figs. 7, 8) and generally contained small numbers of b5 cells which did not stain for α-chain. These are not easily discernible in low power pictures. (*b*) The spleens of animals which received popliteal lymph node cells contained clusters of b5 cells, the majority of which stained for γ-chain (Figs. 9, 10). There were a few cells in cross-sections of all such clusters which were only stained by a single conjugate. These cells might have been b5 cells which were not producing γ-chain or they might have been cells not of donor origin which were producing γ-chain. (*c*) In general, when spleen sections from lymph node cell recipients were stained with both red anti-b5 and green anti-α chain, most of the clusters were observed to contain predominantly cells stained only by the red conjugate. This observation indicates that most of the donor cells in these clusters were not

producing α-chain (Figs. 11, 12). Occasionally a b5, α-chain–producing cluster of cells was found in the spleen of a lymph node cell recipient. These were infrequent in comparison to the numbers of such clusters in the spleens of animals reseeded with Peyer's patch cells. (*d*) There were also some α-chain–producing clusters in recipients of either type of inoculum in which the majority of cells did not stain for the b5 allotype marker. These clusters must have been either host derived, i.e., b^4/b^4 or b-negative donor cells.

In summary, our observations of stained sections lead us to suggest that it is the proportion of clusters containing mainly IgA cells or IgG cells which varies with the origin of the inoculum rather than the number of cells within a given cross-section of a cluster that are making either α-chain or γ-chain. These observations are in agreement with earlier studies (4, 5) which showed that in spleen reseeded with peripheral blood cells, a given cluster contained predominantly, although not exclusively, cells staining for one class of heavy chain and one allotype of light chain.

TABLE I

Frequency of Donor (b5) IgA- and IgG-Staining Cells in Spleens of b^4/b^4 Recipients of Popliteal Lymph Node, Peripheral Blood, or Peyer's Patch Cells Sacrificed 6 Days after Transfer

Recipient rabbit	Source of cell inoculum*	Cell counts per 2 × 10^5 recipient spleen cells		% of donor cells which contain IgA	Cell counts per 2 × 10^5 recipient spleen cells		% of donor cells which contain IgG
		No. of donor (b5) IgA-producing cells‡	Total No. of donor b5 cells§		No. of donor (b5) IgG-producing cells‖	Total No. of donor b5 cells§	
480	Peyer's	14,388	18,197	79	—	—	—
	patches	12,167	16,769	73	1,645	15,870	10
481		12,695	19,467	65	6,453	19,928	32
482		7,829	10,209	77	2,433	18,303	13
483	Lymph node	89	1,443	6	—	—	—
		84	1,700	5	6,400	8,670	73
484		180	2,168	8	44,098	59,353	74
486		793	11,532	7	—	—	—
		740	9,998	8	22,300	34,914	64
488¶	Peripheral	18	106	17	154	191	80
	blood	45	248	18	—	—	—

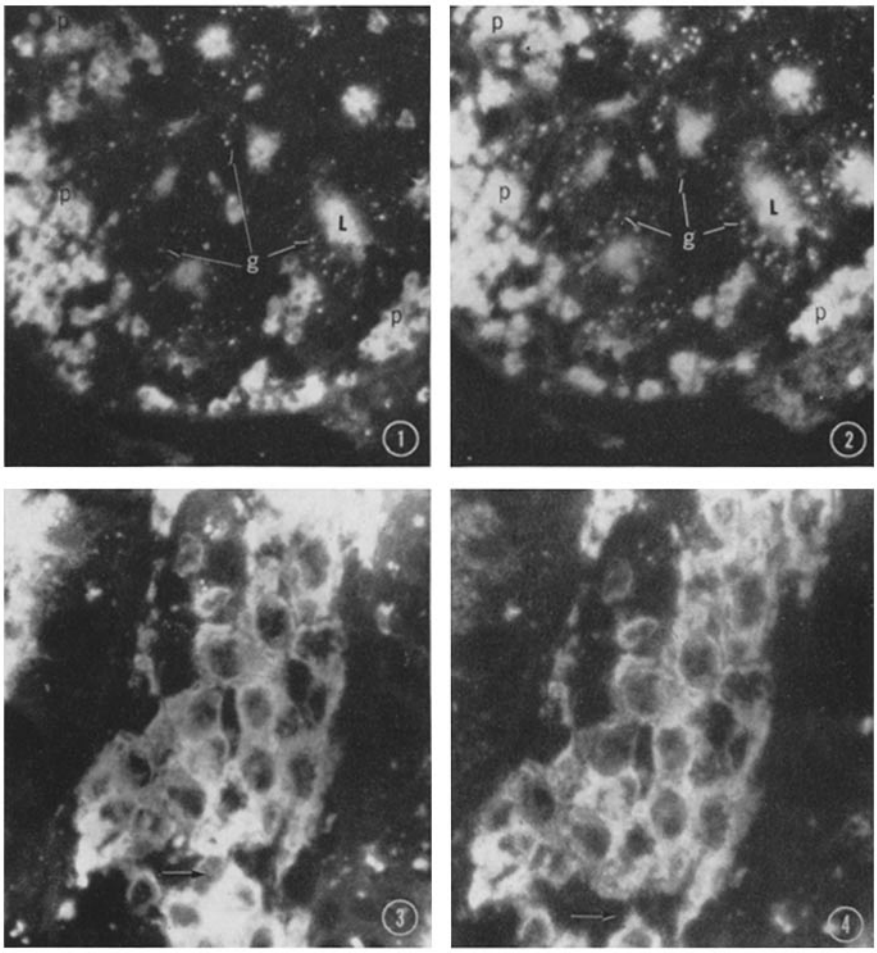
* A single b^5/b^5 rabbit was the donor of all cell inocula.

‡ Yellow cells stained by both green anti-α and red anti-b5.

§ Cells stained by red anti-b5.

‖ Yellow cells stained by both green anti-γ and red anti-b5.

¶ Two other recipients of peripheral blood from the donor used in this experiment died before day 6.



Pairs of pictures are shown in Figs. 1–12. Each pair of figures shows the same field. The left-hand picture is taken using illumination and filters which favor observation of fluorescing cells stained with fluorescein conjugates alone and of fluorescing cells stained by both a rhodamine conjugate and a fluorescein conjugate. The right-hand picture shows only those cells of donor origin stained by the rhodamine-labeled (red) anti-b5 reagent.

FIG. 1. Section of small intestine from a b^4/b^4 recipient of b^5/b^5 Peyer's patch cells stained with both green anti-α chain and red anti-b5 and observed with the BG12 and K490 filter combination. It is possible to recognize the architecture of the intestine and to note the characteristic repopulation pattern of donor cells. Specifically stained granules (17) in the epithelial cells lining the crypts of Lieberkühn glands are also visible. × 200. (L, lumen; g, granules; p, plasma cell).

FIG. 2. Same field as in Fig. 1 but observed with the Schott (T_{max} = 546 nm) excitation filter and the RG-1 barrier filter. Only red fluorescing donor cells are apparent. × 200.

FIG. 3. Section of small intestine from a b^4/b^4 recipient of b^5/b^5 Peyer's patch cells stained with both green anti-α chain and red anti-b5 and observed with the BG12 and K490 filter combination. Single-stained green cells (host) and double-stained yellow cells (donor) are both observed. × 660.

FIG. 4. The same microscope field as presented in Fig. 3 but observed with the Schott excitation filter (T_{max} = 546 nm) and the RG-1 barrier filter. Only the b5 (red) marked donor cells can be seen. Arrow indicates position of a green fluorescent cell (host) which appears under the conditions of illumination in Fig. 1, but not here. × 660.

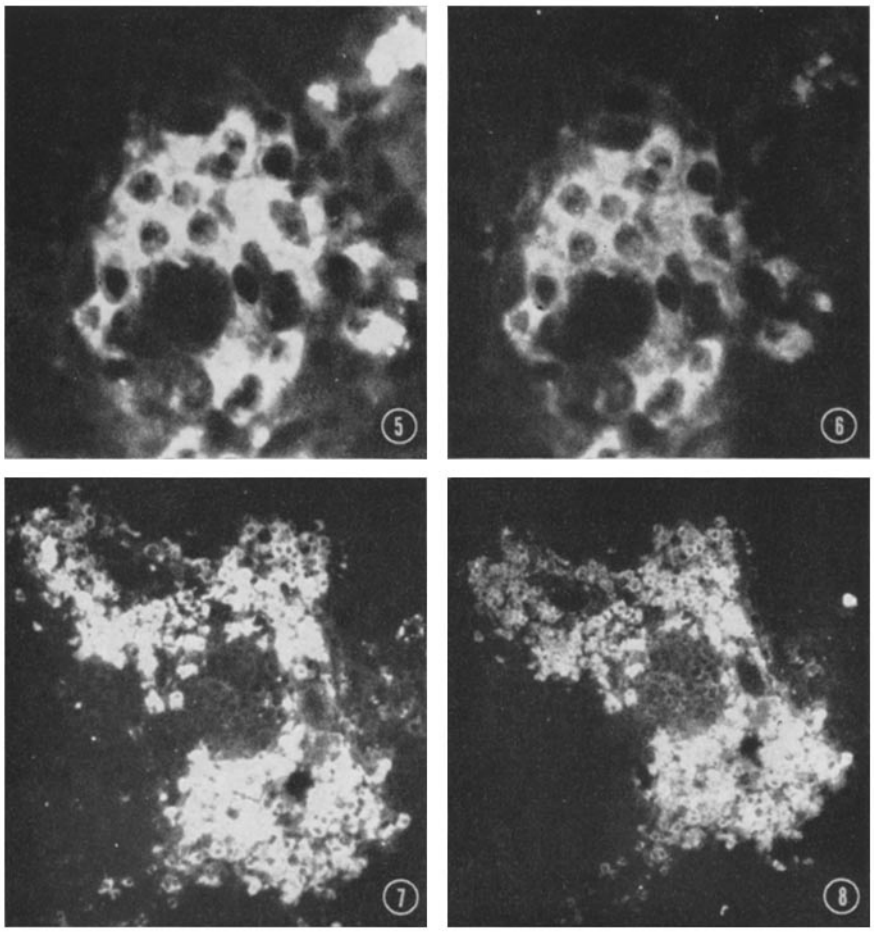


FIG. 5. Section of spleen from b^4/b^4 recipient of b^5/b^5 Peyer's patch cells stained with both red anti-b5 and green anti-α and observed with the BG-12 and K490 filter combination. All the cells appeared yellow (donor). × 600.

FIG. 6. The same field as in Fig. 5 but observed with the Schott (T_{max} = 546 nm) excitation filter and the RG-1 barrier filter. Only red fluorescing donor cells are seen. × 600.

FIG. 7. Section of spleen from a b^4/b^4 recipient of b^5/b^5 Peyer's patch cells stained with both green anti-α chain and red anti-b5 and viewed with the BG12 and K490 filter combination. × 165.

FIG. 8. The same field as in Fig. 7 but observed with the Schott (T_{max} = 546 nm) excitation filter and the RG-1 barrier filter. Only red fluorescing donor cells are seen. × 165.

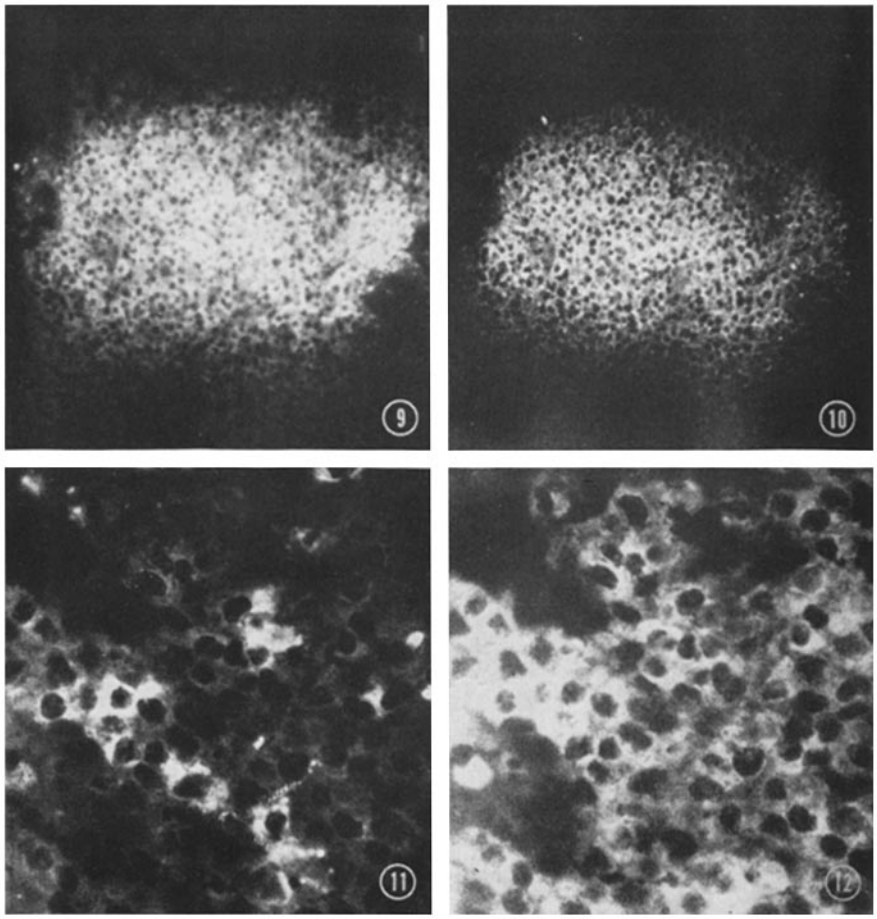


FIG. 9. Section of spleen from a b^4/b^4 recipient of b^5/b^5 popliteal lymph node cells stained with both red anti-b5 and green anti-γ chain and observed with the BG12 and K490 filter combination. A large cluster of γ-chain–containing b5 donor cells is seen. × 165.

FIG. 10. The same field as in Fig. 9 but photographed through the Schott (T_{max} = 546 nm) excitation filter and the RG-1 barrier filter. Only red fluorescing donor cells are seen. × 165.

FIG. 11. Section of spleen from a b^4/b^4 recipient of b^5/b^5 popliteal lymph node cells stained with both red anti-b5 and green anti-α chain and viewed with the BG12/K490 filter system. Both green and yellow cells are seen. × 530.

FIG. 12. The same field as in Fig. 11 but observed with the Schott (T_{max} = 546 nm) excitation filter and the RG-1 barrier filter. Only the red fluorescence is visible. Thus, although there are many donor cells (red), very few of them contain α-chain (green). × 530.

DISCUSSION

Since the Peyer's patches associated with the intestine contain a significant proportion of the total lymphoid cell population, many workers have been concerned with ascertaining whether these tissues have a special immunological role. Studies by Cooper and Turner (8), in which sheep red blood cells (SRBC) were injected directly into the Peyer's patch vein, demonstrated the induction of specific IgM plaque-forming cells (PFC) in the Peyer's patch cell population and in the draining mesenteric lymph node. Armstrong et al. (9) have reported that the number of antigen-reactive cells (ARC) in the Peyer's patches of rats immunized intravenously with purified *Salmonella adelaide* flagellin was twice the number of ARC found in the spleen. Their method of detection would record IgG-, IgM-, or IgA-producing cells.

Recently, Bazin, Levi, and Doria (10) have reported that a significant number of IgA PFC appear in the spleens and mesenteric lymph nodes of germ-free mice which have been immunized orally by allowing them to drink a 5% suspension of SRBC. The authors suggest that the PFC might have originally been derived from intestinal lymphoid tissue. Crabbé et al. (11) were able to detect specific IgA antibody-forming cells in the lamina propria, but not in the Peyer's patches, of germ-free mice which had been immunized orally with ferritin. Finally, Faulk et al. (12), in a detailed morphologic study of Peyer's patches, could not detect either IgA-containing plasma cells or specific IgA antibody-synthesizing immunoblasts in normal or hyperimmune animals.

When the above observations are considered in relation to our finding that the Peyer's patches represent a source of lymphoid cells that have the potential to seed the gut and to proliferate and differentiate into IgA-producing cells, it is not unreasonable to speculate that the function of the Peyer's patches in the immune system is to furnish IgA precursor cells or antigen reactive cells to the lamina propria of the small intestine, the mesenteric lymph nodes, and perhaps to other lymphoid tissues and secretory glands as well. The concept of there being a local secretory immune system (1) will be further strengthened if it can be demonstrated that Peyer's patches do indeed furnish IgA cells to other secretory lymphoid tissues.

Besides demonstrating the differentiative capacity of Peyer's patch cells, our results also suggest that there is a definite difference in the ability of Peyers' patch cells and popliteal lymph node cells to seed the gut of recipient rabbits. This result might be interpreted to mean that there is some intrinsic property of Peyer's patch cells which results in their having either a higher affinity than other lymph node cells for the gut tissue or a greater ability to proliferate there.

In considering the behavior of Peyer's patch and popliteal lymph node cells in our transfer system, it is relevant to point out that other workers have also reported that the final distribution of certain kinds of lymphoid cells in a trans-

fer system depends upon the source of the donor cells. For example, Gowans and Knight (13) reported that when labeled large lymphocytes obtained from rat thoracic duct lymph were injected intravenously into syngeneic recipients, the cells localized preferentially in the lymphoid tissue of the gut. They suggested that this selective localization might be due to the fact that most large lymphocytes in the thoracic duct are derived from intestinal lymphoid tissue, where they might have been "sensitized" to antigens commonly found in the gut.

More recently, Griscelli et al. (14) have shown that large lymphocytes obtained from rat mesenteric lymph nodes or thoracic duct lymph show a marked preferential accumulation in lymphoid tissue within or adjacent to the intestine while cells from peripheral nodes (popliteal, superficial and deep axillary, lumboaortic nodes, and superficial and deep cervical nodes) lodged preferentially in peripheral lymph nodes. These authors also presented evidence that the presensitization of cells with antigen influenced their homing behavior in recipient syngeneic rats.

As yet, any role that antigen or presensitization of cells by antigen might play in determining the normal distribution of immunoglobulin-producing cells, and especially of IgA cells, is experimentally undefined. For example, although recent experiments (e.g. 15) demonstrate that a local antigenic stimulus is needed to initiate the production and secretion of IgA into external secretions, it is as yet unclear whether the IgA-producing cells involved have been "called in" from other lymphoid tissues of the animal in response to the stimulus or whether they were in the subepithelial mucosa all along in the form of nonproductive IgA precursor cells which then responded to the stimulus by proliferation and differentiation. It should be possible to test these alternatives by using some further modification of the transfer system in combination with the facilitated plaque technique for detecting IgA antibody-producing cells (16).

Finally, we would like to emphasize the fact that in our artificial system, Peyer's patch IgA cells proliferate extensively in the spleen and lymph nodes as well as in the gut of recipient rabbits. However, in the normal immunologically competent rabbit, very few IgA cells are found in the spleen and lymph nodes although there are many IgA cells in the secretory glands and in the gut. There is no reason to suspect that the Peyer's patches do not contribute IgA precursor cells to the general lymphatic and blood population of circulating cells. Thus if the Peyer's patches selectively seed the intestinal and respiratory mucosae and secretory glands with IgA precursors, then either a specific "homing" must be postulated to occur under normal conditions or else a random seeding followed by selective proliferation.

The problem of specific homing *versus* "random lodging" followed by selective cell division has been dealt with fairly convincingly in embryonic systems and there, at least, the evidence favors the latter hypothesis (18). It is con-

ceivable that one of these processes also operates in determining the final distribution of classes of immunoglobulin-producing cells. At the moment, we favor the hypothesis that Peyer's patch IgA cell precursors are shed into the circulation constantly, seed various tissues on a random basis, and proliferate selectively in those tissues rich in IgA cells as a result of a specific interaction between the precursors and some component of the tissue environment. For example, either local immunogens or some local macromolecule, such as secretory component, might act on Peyer's patch precursor cells to stimulate their proliferation and to cause them to lose their migratory property.

Whatever the processes guiding the normal distribution of immunoglobulin-producing cells, future work on this problem and on other questions raised in this discussion should be facilitated by the availability of an enriched source of IgA precursor cells in the Peyer's patches.

SUMMARY

The proliferative and differentiative potential of Peyer's patch, peripheral blood, and popliteal lymph node cells was assessed by allogeneic cell transfer followed by quantitation of donor immunocytes by immunofluorescence. It was found that Peyer's patches are a highly enriched source of cells which have the potential to proliferate and differentiate into IgA-producing immunocytes and that the Peyer's patch cells are far more efficient in seeding the gut of irradiated recipient rabbits with donor cells that give rise to immunoglobulin-producing cells than cells from peripheral blood or popliteal lymph nodes.

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