MESENTERIC LYMPH NODE B LYMPHOBLASTS WHICH HOME TO THE SMALL INTESTINE ARE PRECOMMITTED TO IgA SYNTHESIS*

By MICHAEL McWILLIAMS,† JULIA M. PHILLIPS-QUAGLIATA,§ AND MICHAEL E. LAMM

(From the Department of Pathology, New York University School of Medicine, New York 10016)

Immunoglobulin A (IgA)-secreting plasma cells beneath secretory epithelia probably represent the terminal differentiation stage of lymphocytes originating in Peyer's patches. Here, it is postulated (1), lymphocytes acquire a commitment to IgA synthesis. They proceed to the mesenteric lymph nodes (MN), where they divide and differentiate into plasmablasts, then emigrate via the thoracic duct and blood to the lamina propria, principally of the small intestine, where they lodge and undergo terminal differentiation to IgA-producing plasma cells. Several types of evidence implicate mesenteric nodes as the proximate source of IgA-secreting plasma cells in mucous membranes. Among them are these observations (2–4): (a) A subpopulation of the DNA-synthesizing large lymphocytes of rat and mouse mesenteric nodes homes to the lamina propria of the small intestine shortly after intravenous transfer into syngeneic recipients; mesenteric node blasts are unique in this capacity, which is not manifested by the bulk of resting cells nor by blasts from other lymphoid organs including Peyer's patches. (b) IgA-bearing B cells are relatively abundant in mesenteric nodes. (c) Most of the homing cells bear surface Ig, though the particular class of Ig has not hitherto been unequivocally identified. (d) Many of the homing cells differentiate into IgA-secreting plasma cells within 24 h of transfer.

Despite this suggestive evidence, there is as yet no formal proof that the precursors of the IgA-producing plasma cells in the lamina propria are already committed to IgA synthesis before leaving the mesenteric nodes. We now report direct evidence that homing B blasts bear surface IgA while still resident in MN. Furthermore, the homing cells themselves produce the IgA which they bear, i.e., it is not passively adsorbed.

Materials and Methods

Mice. Balb/c mice, 6–12-wk old and of either sex, were obtained from The Jackson Laboratory, Bar Harbor, Maine or Carworth Farms, Jamaica, N. Y.

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Abbreviations used in this paper: [125I]UDR, [125I]iododeoxyuridine; MN, mesenteric lymph nodes; PN, peripheral lymph nodes.

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Preparation of Cells for Transfer. These procedures are fully described in (5). Monodisperse suspensions of cells were prepared from peripheral lymph nodes (PN), including inguinal, axillary, brachial, superficial, and deep cervical nodes, and MN in RPMI 1640 medium with HEPES buffer (Grand Island Biological Co., Grand Island, N.Y.) at 0°C. They were subsequently incubated for 1 1/2 h at 37°C in medium containing 10% fetal calf serum (Gibco) and 2 μCi/ml of [125I]iododeoxyuridine ([125I]iododeoxyuridine (125IUDR; Amersham/Searle Corp., Arlington Heights, Ill.) in order to label cells synthesizing DNA. In those experiments in which immunofluorescence was combined with autoradiography of tissue from recipients of labeled cells, 2 μCi/ml of [3H]methylthymidine (2 Ci/mmol; New England Nuclear, Boston, Mass.) was used.

After labeling, the cells were washed three times and filtered twice through nylon wool in order to prepare suspensions of single viable cells free of unbound label. Filtration involves such rapid passage through the nylon filters that no selective B-cell depletion results. In all experiments described in reference 4, and in some of those described below, centrifugations were done at 4°C, and ice-cold medium was used for suspending the cells between centrifugations. Alternatively, in order to promote shedding of passively adsorbed Ig (6-8), prewarmed medium was used to suspend the cells, which were incubated for 5 min at 37°C between centrifugations at room temperature.

Approximately 10^7 cells per recipient were transferred intravenously at this stage, or were first exposed to normal rabbit serum, polyvalent rabbit antiserum to mouse Ig reactive with α- and γ-chains, or antiserum specific for mouse α-, γ-, or μ-heavy chains in the presence of guinea pig complement for 45 min at 37°C in medium 199 (Gibco). Viability was checked by trypan blue dye exclusion. Cells were then washed and filtered as above in ice-cold RPMI 1640 medium to remove dead cells in preparation for transfer.

Recovery of Label. 16-24 h after intravenous transfer of cells to 3-5 mice per treatment group, organs were removed and radioactivity was measured in a Nuclear Chicago gamma counter as previously described (4). Counts recovered are expressed as a percentage of the radioactivity injected, plus or minus one standard deviation.

Antisera. The preparation and specificity of these reagents are fully described in reference 5. Rabbit antiserum to mouse Ig was raised against purified normal mouse IgG. Antisera to mouse α-, γ-, and μ-heavy chains were raised against heavy chains isolated from partially reduced and alkylated myeloma proteins. These antisera, and normal rabbit serum, were absorbed with mouse thymic leukemia cells (L1210) before use. All antisera, with the exception of anti-α-chain, proved cytotoxic for MN cells in the standard assay (5, 9). The lack of detectable activity of anti-α chain antiserum presumably reflects the paucity of α-positive cells in the cell suspension. Nevertheless, it was cytotoxic in the sense that it prevented homing of MN lymphocytes (vide infra). Specificity of the anti-α-chain antiserum was checked by absorbing with the IgA myeloma protein TEPC-15 coupled to APB-cellulose (10). Absorption removed all anti-α-chain activity detectable by Ouchterlony analysis.

Aggregation of Immunoglobulins. 10 mg/ml immunoglobulin solutions in 0.15 M NaCl were heated at 83°C for 30 min.

Immunofluorescence and Autoradiography. IgG fractions of the rabbit antisera were prepared, fluoresceinated, and used to stain the surfaces (5) of viable MN lymphocytes rinsed either in the cold or at 37°C. When the indirect technique was used for combined immunofluorescence and autoradiography, 4-μm tissue sections of MN or the coiled proximal 10 cm of small intestine were prepared in a cryostat and fixed for 10 min in acetone, treated with unconjugated whole rabbit antiserum diluted 1:20 in saline, rinsed, stained with fluoresceinated goat anti-rabbit IgG (Hyland Laboratories, Costa Mesa, Calif.) diluted to a concentration of 0.5 mg/ml in saline, rinsed, fixed again in acetone for 5 min, and dipped in NTB-3 Nuclear Track Emulsion (Eastman Kodak Co., Rochester, New York). Slides were incubated for 1-2 days before developing. Fluorescence microscopy was done with a Leitz Orthoplan microscope and the Ploem system of epiillumination; transillumination with visible light was used to reveal silver grains in the same field.

Results

Homing of MN Lymphocytes. Under the conditions of labeling employed, about 2% of the lymph node cells incorporate label. All are large lymphocytes. Approximately 10% of these cells contain α-chain. The percentage of injected 125I recovered from various organs of recipients of 125IUDR-labeled PN or MN cells is shown in Table I. About 10-15% of the injected label is recovered from the
recipients; the remainder is excreted (4). Much more of the injected label is recovered in the wall of the small intestine of MN cell recipients than of PN cell recipients. In 24 MN cell and 5 PN cell transfer experiments, the percentage of label recovered from the small intestine ranged from 4.1 to 10.4 (average 6.7) and 1.7 to 3.1 (average 2.3), respectively. This preference for a particular site by MN lymphocytes, over and above that of PN cells for the same site, is homing by our definition. Homing of MN cells to MN also occurs. This is best seen in the P/M ratio, the ratio of percentage of the injected label per gram of PN to the same figure for MN in the same recipient. Ratios significantly less than one indicate preference of the injected cells for MN of the recipients. Differences in other organs have not consistently been found.

Effect on Homing of Pretreatment with Antiserum to Ig or Individual Classes of Ig. To determine whether any immunoglobulin markers could be demonstrated on the surface of homing cells, MN cells labeled with 125IUDR at 37°C, but otherwise washed and maintained in the cold, were incubated with normal rabbit serum, antiserum reacting with all classes of mouse Ig, or class-specific antisera plus complement. Results of two experiments are shown in Table II. Anti-Ig antiserum decreased migration to the gut under these conditions, but had no significant effect on homing to MN. Anti-α-chain antiserum mimicked the effects of anti-Ig, but antisera to μ- and γ-chains were without effect. Despite the fact that anti-α-chain antiserum gave no evidence of cytotoxicity in the dye exclusion test, active complement was necessary for the antiserum to prevent homing. Separate experiments showed that antiserum in conjunction with heat-inactivated complement had no effect.

Effect of Rinsing at 37°C on Cell Surface Ig. Cell surface Ig may be either native or passively adsorbed. Recent reports (6-8) have indicated that Ig which is passively adsorbed to the surface of human lymphocytes can be removed by rinsing at 37°C, and we therefore investigated MN cells to see whether this is also true of mouse lymphocytes. Viable cells were washed three times at 0°C or 37°C and stained in suspension with fluoresceinated antibody at 0°C or room temperature. Results of these experiments are shown in Table III. The frequency of Ig-positive murine cells does decline after rinsing at 37°C, and this decline is accounted for by a diminution in the frequencies of IgG- and IgA-positive cells. The frequency of IgM-positive cells remains unchanged.
TABLE II

Effect of Various Antisera, Plus Complement, on Homing of Mesenteric Node Blast Cells
Rinsed at 0°C Before and After Labeling

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Cytotoxic Index</th>
<th>Percent of injected label recovered</th>
<th>P/M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Small intestine</td>
<td>PN</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%/g</td>
<td>%/g</td>
</tr>
<tr>
<td>Exp. no. 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRS</td>
<td>-</td>
<td>9.5 ± 1.0</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td>Anti-Ig</td>
<td>20</td>
<td>7.2 ± 0.7</td>
<td>4.9 ± 1.2</td>
</tr>
<tr>
<td>Anti-μ</td>
<td>19</td>
<td>7.9 ± 0.9</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>Anti-γ</td>
<td>3</td>
<td>7.4 ± 0.9</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Anti-α</td>
<td>0</td>
<td>5.8 ± 0.8</td>
<td>3.1 ± 0.7</td>
</tr>
<tr>
<td>Exp. no. 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRS</td>
<td>-</td>
<td>10.4 ± 0.6</td>
<td>2.2 ± 1.4</td>
</tr>
<tr>
<td>Anti-Ig</td>
<td>24</td>
<td>7.6 ± 1.9</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Anti-μ</td>
<td>9</td>
<td>10.0 ± 0.7</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>Anti-γ</td>
<td>10</td>
<td>9.2 ± 1.2</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Anti-α</td>
<td>0</td>
<td>6.8 ± 0.5</td>
<td>2.1 ± 0.4</td>
</tr>
</tbody>
</table>

TABLE III

Effect of Rinsing Mesenteric Node Cells at 37°C Versus 0°C on Surface Ig

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Ig</th>
<th>μ</th>
<th>γ</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0°</td>
<td>37</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>14</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>20</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>26</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>20</td>
<td>12</td>
<td>11</td>
</tr>
</tbody>
</table>

* 200-500 cells were examined in each instance.
‡ Rinsing temperature, °C.
§ Not done.

Other experiments showed two rinses to be sufficient to remove passively adsorbed Ig under these conditions; no further reduction in the frequencies of Ig-, IgG-, and IgA-positive cells was observed after three or four rinses. Frequencies of IgG- and IgA-positive cells at least as great as those observed in the cold-rinsed populations could be restored by incubating warm-rinsed cells in the cold with heat-aggregated IgG or IgA; treatment with heat-aggregated IgM had no effect.

We believe these results indicate that rinsing at 37°C is an effective means of removing passively adsorbed Ig from the surface of mouse cells, but that cells bearing IgG or IgA integral to their membranes do exist, albeit at lower frequencies than we had previously reported for cells washed in the cold (5).

Effect of Anti-α-Chain Antiserum on Homing of Cells Rinsed at 37°C. Results of the transfer of cells rinsed in the cold or at 37°C and subsequently treated with antiserum to Ig or α-chain plus complement are shown in
Table IV. Both antisera prevented homing to the small intestine, regardless of the temperature at which the cells had been rinsed. Our conclusion is that IgA on the surface of homing cells is not passively adsorbed, but is integral to their membranes and produced by the cells which bear it.

Maturation of Homing Cells in the Recipient. Sections of the MN and duodenum were studied by combined immunofluorescence and autoradiography to determine what proportion of the homing lymphoblasts had differentiated into Ig-secreting cells 16-20 h after transfer of 3-6 x 10^7 cells. Table V shows the results.

More than half of the cells that migrated to the lamina propria of the duodenum after treatment with normal serum or anti-μ-chain antiserum and complement had readily detectable Ig in their cytoplasm, and the great majority of these contained IgA. Most did not appear to be mature plasma cells, but were larger cells with a large central nucleus, and should probably be considered plasmablasts. A typical cell is shown in Fig. 1. Treatment with anti-α-chain antiserum and complement reduced the frequency of Ig-positive donor cells.

In contrast to the frequency of IgA-positive cells found in the gut, few or none of the cells that homed to the MN were Ig positive. This agrees with the results of Guy-Grand et al. (3), and is, of course, in accord with the observation that anti-Ig has no effect on homing here. The nature of the cells homing to MN is more fully explored in reference 4.
TABLE V
Combined Immunofluorescence and Autoradiographic Studies of Recipients of [3H]Thymidine-Labeled Mesenteric Node Cells

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Pretreatment of transferred cells</th>
<th>MN</th>
<th>Small intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ig A-positive</td>
<td>Ig A-positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>per number donor cells counted in recipient*</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>NRS</td>
<td>0/10</td>
<td>35/63 (56%)</td>
</tr>
<tr>
<td></td>
<td>Anti-α</td>
<td>0/10</td>
<td>7/38 (18%)</td>
</tr>
<tr>
<td>2</td>
<td>Anti-μ</td>
<td>1/17</td>
<td>44/60 (73%)</td>
</tr>
<tr>
<td></td>
<td>Anti-α</td>
<td>3/20</td>
<td>5/33 (15%)</td>
</tr>
</tbody>
</table>

* Donor cells distinguished by autoradiography; Ig and IgA by indirect immunofluorescence.

Discussion

Previous work implicating MN in the IgA cell cycle, i.e., as a source of precursors of intestinal IgA plasma cells, has provided strong inferential evidence but no direct demonstration of precommitment to IgA synthesis. In the present work, we have shown that these precursor cells are in fact already committed to IgA while still in the mesenteric nodes.

The argument for precommitment to IgA synthesis is based on two grounds. The first is that pretreatment in vitro with antiserum to either Ig or α-heavy chains, but not to γ- or μ-chains, in conjunction with complement, is equally effective in preventing homing of mesenteric node lymphoblasts to the gut as assessed from recovery of cell-associated radioactivity. Presumably the potential homing cells, although too few in number to be detected in the standard cytotoxicity assay, are killed in this reaction since lytically active complement is necessary for the effect. Alternatively, the cells are opsonized and removed in vivo. Inasmuch as such treatment is equally efficacious when passively adsorbed, surface Ig is eluted beforehand by rinsing cells at 37°C, the IgA being attacked by antibody is apparently integral to the membrane of the cells that bear it. This conclusion is substantiated by the inability of anti-γ-chain antiserum to prevent homing of cells previously washed either in the cold or at 37°C, even though IgG has at least as marked a tendency to be passively adsorbed by cells with Fc receptors.

The second argument stems from observations of Guy-Grand et al. (3), confirmed in the present work, namely, that a significant proportion of the homing large lymphocytes matures into IgA-secreting plasma cells within hours of cell transfer. This result, by itself, however, does not exclude the possibility that a local environmental influence causes a B lymphocyte not previously synthesizing IgA to switch to IgA synthesis and differentiate to a plasma cell. This
hypothesis is rendered less plausible, and the idea of precommitment to IgA synthesis is supported, by the present additional finding that, after treatment with anti-\(\alpha\) chain antiserum, few of the transferred cells that lodge in the gut of the recipients differentiate into IgA-secreting plasma cells. Any additional stimulus to proliferation or differentiation which the local environs of the gut

**Fig. 1.** An IgA-secreting donor cell in the lamina propria. (a) Fluorescence photomicrograph of a section treated with anti-IgA antiseraum. (b) Transmitted light photomicrograph of the same area after staining with hematoxylin and eosin. \(x \approx 540\).
may exert on other cells over a longer period remains unexplored; some cells could multiply and differentiate in situ and thus account in part for the high concentrations of IgA-secreting plasma cells found there.

Under ordinary circumstances cells homing to mucous membranes probably enter blood via the thoracic duct, thus accounting for the frequency of large lymphocytes in thoracic duct lymph which migrate to the gut (11). One of the most intriguing questions left unresolved concerns the actual mechanism of homing. One possibility is that homing involves interaction between cell surface IgA and secretory component in the mucous membrane. However, since free secretory component is apparently confined to the lining epithelial cells, and not released into interstitial fluid, it is difficult to envision where such an interaction could occur. In addition, pretreatment of recipients with large intravenous doses of IgA, or antiserum to secretory component, both calculated to interfere with in vivo combination between cell surface IgA and secretory component, had no effect on homing (4). Furthermore, Brandtzaeg (12) has reported that human peripheral blood lymphocytes do not bind secretory component; however, thoracic duct lymphocytes have not been studied in this regard. A second possibility is that the cells are homing to antigen. However, homing occurs to antigen-free grafts of fetal gut (3, 13). Nevertheless, the presence of antigen does have some influence; Pierce and Gowans (14) have shown that primed IgA-containing cells home best at or distal to the site of intralumenal injection of antigen into the intestine. Of course, even if homing were wholly directed by antigen, this would not explain why the antigen receptor should be of the IgA class.

A second major question not yet completely resolved involves initiation of the IgA cell cycle. Craig and Cebra (15, 16) and Rudzik et al. (17, 18) have shown that rabbit Peyer's patches are especially rich in precursors of IgA-secreting plasma cells which become evident in the spleen and gut of irradiated allogeneic, or the gut of autologous recipients 7 days after transfer. Presumably some tissue receptor interacts with the homing cell surface. Again, IgA is not necessarily involved since Peyer's patch cells, some of which bear surface IgA, do not home in short-term experiments (3, 4). Conceivably, however, Peyer's patch cells do not bear dimeric IgA which may be requisite for homing (3). Jones et al. (19), using a fluorescence-activated cell sorter, demonstrated that the precursor cells in Peyer's patches bear surface \( \kappa \), but not \( \mu \)-chains. Subsequently, the \( \mu \)-minus, \( \kappa \)-positive population, which contains the precursors of the IgA-producing plasma cells, was shown to contain \( \alpha \)-positive cells detectable with antisera to IgA allotypes (20, 21). However, the precursor cells have not directly been shown in transfer experiments to bear surface \( \alpha \)-chains.

After cell transfer, a week is required for IgA-producing plasma cells to appear in the irradiated rabbit. This may explain why Peyer's patch cells, unlike MN cells, do not home to intestinal mucosa within 24 h of transfer to a syngeneic mouse. Taken together, the various studies support the idea that the IgA cell cycle does indeed begin in the organized lymphoid tissue of the intestinal tract, such as Peyer's patches, but the precursor cells must undergo a maturational step in the mesenteric lymph nodes. From the mesenteric nodes they can quickly pass to the thoracic duct, bloodstream, and reach the lamina propria of the gut.
Summary

The fate of mesenteric lymph node lymphoblasts labeled with either [125I]iododeoxyuridine or [3H]thymidine can be studied after intravenous transfer into syngeneic mice both by measurement of radioactivity in various organs and by combined immunofluorescence and autoradiography of recipient tissues. Many of the lymphoblasts home to the lamina propria of the small intestine within hours of transfer; of these, many visibly secrete IgA. To determine whether the cells that will ultimately secrete IgA are already committed to IgA synthesis before their arrival in the gut, mesenteric lymph node cell populations were treated with various class-specific antisera to mouse immunoglobulins before transfer. Treatment with antiserum to IgA, plus complement, reduced the fraction of injected label recovered from the recipients' intestines, and also reduced the proportion of donor (labeled) cells containing IgA. We conclude that mesenteric lymph nodes are probably the principal source of IgA-secreting plasma cells in the lamina propria of the gut, and that the cells become committed to IgA synthesis and develop cell surface IgA before emigrating. This IgA is apparently synthesized by the cells that bear it since it is not removed by extensive rinsing at 37°C, a maneuver that elutes passively adsorbed immunoglobulin.

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References


