Many of the IgA producing plasma cells in murine gut are derived from self-replenishing precursors in the peritoneal cavity

Frans G. M. Kroese, Eugene C. Butcher, Alan M. Stagg, Paul A. Lalor, Sharon Adams and Leonore A. Harzenberg

Departments of Pathology and Genetics, Stanford University Medical School, Stanford, Ca 94305, USA

Key words: Ly-1 B cells, lamina propria, adoptive transfer

Abstract

Long term B lineage chimeras are used here to study the origin of plasma cells in the mouse. Chimeric mice are constructed by reconstituting lethally irradiated mice with peritoneal cells (PerC) and bone marrow cells from congenic pairs of mice differing in IgG1 allotype. All conventional B cells in these mice express the allotype of the bone marrow donor and nearly all Ly-1 B lineage cells express the allotype of the PerC donor. FACS analysis and immunohistology of these mice shows that virtually all (IgG1) B cells in peripheral lymphoid organs are derived from the bone marrow donor. However, despite this overwhelming number of bone marrow-derived B cells in these animals, immunohistological staining of lymphoid organs and gut shows that nearly half of the IgM, IgG, and IgA plasma cells derive from the PerC donor. These data demonstrate that the peritoneal cavity contains a major reservoir of self-replenishing cells that play a significant role in the mucosal immune response. The possibility that these are B cells that belong to the Ly-1 B lineage is discussed.

Introduction

The secretion of IgA by numerous plasma cells located in the intestinal lamina propria is a prominent feature of the humoral immune response of the intestine (and other mucosal tissues) (for review see 1). This immune response provides a crucial defence against pathogenic micro-organisms in the gut. Its importance to the overall immune system is reflected by the fact that in the mouse there are at least ten times more IgA producing plasma cells than IgM producing plasma cells (2).

IgA plasma cells in the intestine are generally thought to be derived from precursor cells located in gut associated lymphoid organs of the small intestine (Peyer's patches) (3-8). According to this theory, committed B cells (IgM+IgD-IgA-) leave the Peyer's patches after antigenic stimulation, migrate to the mesenteric lymph nodes and via the thoracic duct into the blood circulation to the spleen where they expand and differentiate further. Finally, many of these cells are thought to lodge in the lamina propria of the gut where they mature into IgA secreting plasma cells.

This view appears to be correct for roughly half of the gut IgA plasma cells; however, data from studies presented here demonstrate that the other half of these cells are derived from self-replenishing (or very long-lived) cells that are readily accessible in the peritoneal cavity and may belong to the Ly-1 B lineage. The Ly-1 B lineage represents a distinct lineage of self-replenishing B cells that differ from the conventional bone marrow-derived B cells in phenotype, localization, function, and reconstitution properties (9, 10). These cells are rare in peripheral lymphoid organs but can be found in high frequencies in the peritoneal and pleural cavity (11, 12, and Kroese et al., manuscript in preparation). Functionally, these cells are responsible for producing much of the serum IgM, many of the 'natural' IgM antibodies and a variety of autoantibodies found in normal and autoimmune mice (12, 13). In addition, they have been shown to produce IgH antibodies to micro-organisms coat antigens such as dextran (14), phosphoryl choline (PC) (Stall et al., manuscript in preparation), and phosphatidyl choline (15).

In studies presented here, we examine the origin of murine IgA plasma cells in the small intestine of stable bone-marrow/peritoneal-cell chimeras constructed with cells from Ig heavy chain allotype congenic donors. In these mice, cells derived from either donor population can be distinguished on the basis of the

---

* Partly presented at the 11th Convocation on Immunology, 12-16 June 1988, Buffalo, NY, USA

* Present address: Department of Histology and Cell Biology, University of Groningen, Oostersingel 89/1, 9713 EZ Groningen, The Netherlands

* Present address: Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Victoria 3052, Australia

* Correspondence to: Frans G. M. Kroese, Department of Histology and Cell Biology, University of Groningen, Oostersingel 89/1, 9713 EZ Groningen, The Netherlands.

Received 24 November 1988, accepted 12 December 1988
76 Origin of gut IgA plasma cells

Ig-C allotype they express. Essentially all of the 'conventional' B cells in these chimeras are derived from bone marrow and essentially all of the Ly-1 B lineage cells derive from Ig+ cells in peritoneum (16, 17). Furthermore, we show that up to half of the gut IgA plasma cells (and IgM plasma cells in the spleen) in such animals are derived from transferred peritoneal cells that persist and give rise to IgA plasma cells for the life of the animal.

Materials and Methods

Animals

BALB/c (lgh), their lgh congenic strains BAK25 and C.B17, CBA/Ca (lgh), its lgh congenic strain CBA/bb and B6C20 mice (lgh) were bred under conventional conditions in the animal facility of the Stanford Department of Genetics. Breeding pairs of B6Ly5.2 (lgh) mice were a kind gift from Dr Edward Boyse, Memorial Sloan-Kettering Cancer Center, New York, USA and were bred in the animal facility of the Stanford Department of Pathology.

Antibodies

The following mouse monoclonal antibodies were used: anti-Igh-5a (IgD of 'a' allotype, AMS 9.1), anti-Igh-5b (IgD of 'b' allotype, AF6-122.2), and anti-Igh-6b (IgM of 'b' allotype, AF6-78.25) as described (18); anti-Igh-6a (IgM of 'a' allotype, D5-1), kindly donated by Dr Donna Siekmann (19); anti-Igh-2a (IgA of 'a' allotype, HY16) (20); anti-Igh-1a (IgG2a of 'a' allotype, 20-8.3) (21, 22) and anti-Igh-4a (IgG1 of 'a' allotype, 20-10.9) (21, 22). Monoclonal rat antibodies employed were: anti-IgM (331.12) (23); anti-Ly-1 (53.7.B) (24); anti-Mac-1 (M1/70) (25) and anti-IgA (71.14) produced by Dr Eric Pillemer at the Department of Pathology, Stanford University (20). Monoclonal antibodies were isolated from either culture supernatants or ascitic fluid, purified by ion exchange chromatography on DEAE Sephacel or QAE Sephadex (26). Conjugation of antibodies to biotin, fluorescein, allophtocyocyanin and of avidin to Texas Red have been described previously (26, 27).

Preparation of cells

Peritoneal washout cells (PerC) were harvested from mice by injecting chilled RPMI medium (Applied Scientific, Santa Ana, CA, USA) supplemented with 3% newborn calf serum as described (16). Suspensions from peripheral lymphoid tissues were prepared in the same medium using the frosted ends of microscope slides. Bone marrow cell suspensions were made by flushing femurs with chilled medium. Lamina propria cells were isolated from the small intestine based upon a method described by Tseng (28). Briefly, fragments of small intestines (without Peyer's patches) were incubated for 5 min in Ca and Mg free Hank's Balanced Salt Solution (HBSS) containing 1 mM dithiothreitol (Sigma, St Louis, MO, USA) and for 5 min in Ca and Mg free HBSS containing 5 mM EDTA. Segments were subsequently incubated for 2 h (room temperature) in HBSS supplemented with 20% newborn calf serum and containing 10 U/ml Collagenase Type VII (Sigma) to release lamina propria cells. Lamina propria cells were then purified using a 100/50% Percoll (Pharmacia, Uppsala) gradient and the (lymphoid) cells from the interface between 100% and 50% Percoll were collected.

Construction of chimeras

Irradiated chimeras were constructed by transfer of 2.5-5 x 10^6 BALB/c PerC (lgh) and 1-2.5 x 10^6 bone marrow cells from allotype congenic IgM (BAB/CB or C.B17) mice (dissolving in Ig heavy chain allotype) to lethally irradiated recipients as described (16, 17). Recipients were always of the same strain as the bone marrow donors. Neonatal chimeras were produced by treating neonatal IgM mice (CBA/bb) from birth with monomeric anti-Igh-6b antibody (78.25) (total of 2 mg antibody over a 4 weeks period) and injected with 5 x 10^6 PerC from Igh-C congenic donors (CBA/Ca, lgh) as described (29).

Cell suspension staining and FACS analysis

Three colour immunofluorescence staining of cell suspensions was carried out in microtitre plates as described previously (27). Briefly, cells (500,000 cells/sample) were incubated for 15 min on ice with monoclonal antibodies conjugated to FITC, biotin, or allophtocyocyanin. After washing, a 15 min incubation followed with avidin coupled to Texas Red, to detect biotinylated antibodies. Multiparameter flow cytometry was carried out on an extensively modified fluorescence activated cell sorter (FACS II, Becton and Dickinson, Mountain View, CA, USA) interfaced with a VAX11/780 computer (Digital Equipment, Maynard, MA, USA) (27). Data were collected from 20,000-30,000 individual viable (i.e. propidium iodide negative) cells.

Immunohistological staining procedure of frozen tissue sections and cytocentrifuge preparations

Sections from frozen tissue were stained by an immunoperoxidase method as described elsewhere (30). Briefly, acetic fixed sections were incubated with biotinylated monoclonal antibodies followed by peroxidase conjugated avidin (Vector Laboratories, Inc.). Peroxidase activity was demonstrated using 3,3-diamino benzidine tetrahydrochloride (Sigma) at a concentration of 0.5 mg/ml in 0.05 M Tris/HC1 buffer (pH 7.6) containing 0.01% H2O2. Sections were counterstained with hematoxylin.

Double immunofluorescence staining was carried out on methanol/acetic (50%/50% v/v) fixed frozen sections from the gut and on cytocentrifuge preparations from single cell suspensions prepared from spleen and intestinal lamina propria. Cytosols were air dried for at least 1 h and fixed with acetone. Sections/cytosols were incubated for 20 min with FITC conjugated monoclonal anti mouse IgA or anti mouse IgM and with biotinylated anti-Igh-2a or anti-Igh-6a respectively. After rinsing in PBS, sections/cytosols were incubated for 15 min with avidin-Texas Red. Finally they were mounted in an embedding compound containing an anti-fading agent (Citfluor Ltd., The City University, London, UK). The proportion of PerC-derived IgM or IgA containing cells was estimated by scoring at least 250 IgM or IgA plasma cells/plaques (green fluorescence) for their reactivity with IgG or IgA-2a (red fluorescence), respectively.

Fluorescence immunoassay (FIA)

Serum total IgA and IgG-2a were assayed by fluorescence immunoassay: a modified enzyme-linked immunoassay (31) using a fluorogenic substrate. Briefly 96-well plates (E.I.A. II Plus, Flow Laboratories) were incubated with 50 µl of a 10 µg/ml solution of HY-16 (anti-Igh-2a) or 71-14 (anti IgA) monoclonal antibodies for 1 h. Plates then had successive 1 h incubations with: 50 µl serum
samples at the appropriate dilutions; 50 µl biotin-conjugated 71-14 (5 µg/ml), and 50 µl streptavidin-conjugated alkaline phosphatase (8 µg/ml) (Southern Biotechnology Associates, Birmingham, AL, USA). Plates were extensively washed with PBS containing 1% BSA between each incubation. Enzyme activity was assayed using the fluorogenic substrate 4-methylumbelliferylphosphate (MUP) (Sigma). Following the last wash plates were incubated for 30 min with 100 µl of 4-MUP (0.5 mg/ml in Ethanolamine buffer, pH = 9.5). Fluorescence was read with a Titerpak Fluoroscan (Flow Laboratories). Serum levels were calculated from standard curves using purified MOPC-460 (IgA4).

Results

Transferred peritoneal cells give rise to substantial numbers of plasma cells in chimeric recipients

B lineage chimeras were constructed, as in previous studies (16, 17), by reconstituting lethally irradiated mice with a mixture of syngeneic bone marrow cells and peritoneal cells (PerC) from IgH-C allotype congenic donors. FACS analyses shown in Figure 1 confirm that, as reported earlier (10, 17), nearly all of the (IgE+) B cells in spleen, lymph nodes, and Peyer's patches in these animals are conventional B cells derived from the bone marrow donor. In contrast, very few PerC-derived B cells are found in these organs.

Immunoperoxidase staining of frozen histologic sections 3 or 7 months after transfer confirm and extend these findings (see Figs. 2 and 3). The B cell areas of the lymphoid organs in the chimeric animals are almost exclusively composed of bone marrow derived (Igh) B cells. A few PerC-derived (Igh) B cells are detectable. These are mainly located in primary follicles and in the corona (mantle zone) of secondary follicles. Some PerC-derived B cells could be present in germinal centres however, the presence of these cells is difficult to ascertain since paraffin staining with class specific anti IgH of PerC donor allotype occurs in these regions. This staining, which is most likely due to immune

Fig. 1. Two colour FACS analysis of lymphoid cells taken from various lymphoid organs and the peritoneal cavity from an irradiation chimera after staining for Igh-5a and Igh-6a (PerC donor IgD and IgM, respectively). Only very low numbers of PerC-derived (Ly1 B lineage) cells are found in peripheral lymphoid organs.
Fig. 2. Immunoperoxidase staining of Peyer's patches from an irradiation chimera, 7 months after transfer of IgM− ParC and IgM− bone marrow cells. Serial sections were stained for (a) IgM (both allotypes), (b) IgM− IgD+ (IgM of bone marrow donor), (c) IgM− IgA (IgM of ParC donor), (d) IgM− IgA (IgD of ParC donor), and (e) IgM− IgA (IgA of ParC donor) or (f) with peroxidase conjugated avidin alone. Virtually all B cells are from bone marrow donor origin; only rare scattered IgM+, IgD+, or IgA+ cells are found in the corona of the lymphoid follicle (F). Note the dendritic staining (arrows) with anti-IgA in the germinal centre (GC) due to immune complexes trapped by follicular dendritic cells. Magnification × 897.
Fig. 3. Immunoperoxidase staining from the spleen (a–c) and lymph node (d) from B lineage chimeras (7 and 3 months after irradiation and reconstitution, respectively). Serial sections from the spleen were stained for (a) IgM-60 (IgM of bone marrow donor) and (b) IgM-6a (IgM of PerC donor) and show that the vast majority of IgM⁺ cells in the lymphoid follicles (F) are derived from the bone marrow donor. Only very few PerC-derived IgM⁺ are found in the follicles (small arrows). Intense dendritic staining of immune complexes bound to follicular dendritic cells is found in the follicles of anti IgM-6a stained spleen sections (big arrows in b). High numbers of PerC-derived plasma cells are observed after staining with anti IgH-6a in the splenic red pulp (c) or with anti IgG-1a (PerC donor IgG2a) in the lymph node medulla (d). Magnification approximately ×256.
complexes localized on the surface of follicular dendritic cells (32), can obscure the presence of germinal centre B cells expressing low levels of surface Ig.

Relatively large numbers of PerC-derived B cells, in contrast, are detectable in the peri-tonal cavity of recipient mice (see Fig. 4). As shown previously and confirmed here, these PerC-derived cells belong to the Ly-1 B lineage and demonstrate the characteristic ly-1 B phenotype (IghM<sup>−</sup>, IghD<sup>−</sup>, Mac-1<sup>−</sup>, and Ly-1<sup>−</sup>) or Ly-1<sup>+</sup>) (9, 16, 17). The total number of PerC-derived B cells in the animal, however, is still far below the number of conventional (BM-derived) B cells.

Surprisingly, despite this overwhelming preponderance of BM-derived B cells, large numbers of plasma cells expressing the Igh-C allotypes of the PerC donor are detectable in peripheral lymphoid organs and gut in the chimeric mice. The location of these PerC-derived plasma cells corresponds to the normal distribution for plasma cells producing the various Ig isotypes, that is, most of the IgM-producing plasma cells are found in the spleen (in the red pulp, see Fig. 3) and lymph nodes and IgG-producing plasma cells are mainly found in lymph nodes. Only IgG1 (data not shown) and IgG2a (Fig. 3) were tested. IgA plasma cells, as expected, are found in large numbers in the lamina propria of the gut (see Fig. 5) and in smaller numbers in spleen (data not shown). Other typical sites for plasma cells (e.g. mammary tissue, bone marrow) were not examined.

Up to half of the IgM and IgA plasma cells in spleen and gut are derived from transferred peri-tonal cells. The proportions of PerC-derived IgA plasma cells in the gut and IgM plasma cells in the spleen were measured by double immunofluorescence staining of cytocentrifuge preparations of cell suspensions from the spleen and gut and tissue sections from the gut. In essence, roughly half of the IgM containing cells in the spleen and approximately 45% of the IgM containing cells in the lamina propria proved to be derived from the PerC donor in chimeric mice 3 or 11 months after reconstitution (see Fig. 5 and Table 1).

Very few sigA<sup>+</sup> cells are detectable in the peri-tonal cavity.

FACS analysis of PerC from normal mice shows that only a small proportion of the peri-tonal cells stain with anti IgA. To estimate the proportion of sigA<sup>+</sup> cells, we stained PerC from BALB/c (Igh<sup>+</sup>)

**Fig. 4.** Multparameter (three colour) FACS analysis of peri-tonal cells from a B lineage chimera, 3 months after reconstitution. PerC were stained by anti Ly-1 coupled to allophycocyanin in combination with fluoresceinated anti IgH-6a and biotinylated anti IgH-5a (left FACS plots) of fluoresceinated anti IgH-6b and biotinylated anti IgH-6b (right FACS plots). Biotinylated antibodies were revealed with Texas Red conjugated to avidin. These FACS plots show that Ly-1 B lineage cells express the PerC-donor Ig allotype, whereas conventional B cells express the bone marrow donor Ig allotype.
Fig. 5. Double immunofluorescence staining of the gut from a B lineage chimera. Two colour immunofluorescence staining of frozen sections from the small intestine from an irradiation chimera mouse 11 months after transfer of cells with (a) fluoresceinated anti mouse IgA and with (b) biotinylated anti Igh-2a followed by avidin-Texas Red. The majority of IgA containing cells in the lamina propria also react with anti Igh-2a and thus are derived from the PerC donor. Magnification approximately x 300.
Origin of gut IgA plasma cells

Table 1. PerC-derived cells give rise to a major proportion of the IgA plasma cells in the gut lamina propria and the IgM plasma cells in the spleen

<table>
<thead>
<tr>
<th>Donor cell source*</th>
<th>Host B cell depletion</th>
<th>Time after transfer</th>
<th>% PerC derived plasma cells**</th>
</tr>
</thead>
<tbody>
<tr>
<td>PerC Bone marrow</td>
<td>BALB/c</td>
<td>X irr.</td>
<td>3 mo</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>X irr.</td>
<td>11 mo</td>
</tr>
<tr>
<td></td>
<td>BALB/c</td>
<td>X irr.</td>
<td>3 mo</td>
</tr>
<tr>
<td></td>
<td>CBA/Ca</td>
<td>anti μ</td>
<td>7 mo</td>
</tr>
</tbody>
</table>

* Lethally irradiated Ig− mice were reconstituted with 2.5 million syngeneic bone marrow cells mixed with 5 million PerC from allogeneic congenic (Igh−) mice. Igh− mice treated from birth with anti-Igh−6a antibodies were treated i.p. by 4 days of age with 5 million peritoneal cells harvested from allogeneic congenic (Igh−) adult mice (2-3 months old). The IgA and IgM values are shown for individual animals respectively.

** Tissue sections from guts or isolated lamina propria cells (in the third group of mice) were stained by double immunofluorescence for total IgA and IgM-2A (PerC-derived IgA). Cytospin preparations from spleen cell suspensions were stained by double immunofluorescence for total IgM and IgM-6a (PerC donor derived IgM). At least 250 IgA or IgM plasmacells/biasts were scored for their reactivity with anti-Igh−6a or IgM-6a, respectively.

n.d. = not done.

mice with two anti IgA reagents, one that detects sIgA on all mouse cell and one that detects an allogeneic determinant on sIgA on BALB/c cells. Cells that express sIgA will react with both reagents in a correlated manner. Autofluorescent and non-specifically stained cells, in contrast, tend to show differential 'staining' with one reagent or the other. Thus, by staining with the two reagents, taking data for 1-2 x 10⁶ cells per sample, and gaging on the cells demonstrating correlated fluorescence levels, we were able to substantially decrease background and arrive at a tentative estimate of the very low frequency of peritoneal sIgA+ cells. With some reservations, we therefore estimate the sIgA+ to be no more than 1% of total peritoneal cells.

Because of the presence of relatively large numbers of non-specifically stained cells and the low frequency of the PerC IgA+ cells, we have not as yet been able to obtain satisfactory three and four colour FACS analyses that would unambiguously define the phenotype of these latter cells. Preliminary evidence from these studies, however, suggests that a large proportion do not express detectable levels of Ly-1.

PerC-derived IgA plasma cells are found in the lamina propria at 2 weeks after reconstitution.

Studies with chimeric animals sacrificed at various time intervals after irradiation and transfer demonstrate that PerC-derived IgA plasma cells are detectable at high frequencies (>50%) in the intestinal lamina propria as early as 2 weeks after transfer, when Peyer's patches are virtually absent. These cells are not detectable at 1 week after transfer, although sizable numbers of PerC-derived IgM plasma cells are detectable in spleen at this time. Virtually no PerC-derived B cells are detectable in the peritoneum at 1 week after transfer; however, PerC-derived Ly-1 B are clearly detectable 2 weeks after transfer in the peritoneum (data not shown). Thus, the arrival of the PerC-derived IgA plasma cells in the gut 2 weeks after transfer effectively correlates with the appearance of PerC-derived (Ly-1) B cells in the peritoneal cavity of the recipient mouse.

PerC-derived IgA and IgM plasma cells in neonatal B cell chimeras

It is unlikely that the homing of PerC-derived cells into the gut is due to non-specific (possibly irradiation-induced) mechanisms. Studies with another type of B lineage chimera, constructed (in non-irradiated recipients) without disrupting the lymphoid organs or the gut, confirm the clear presence of PerC-derived IgM plasma cells in spleen and IgA plasma cells in the gut (see Table 1). In these chimeras, IgM-C congenic PerC are transferred into neonatal allogeneic homoyzocytes treated from birth with anti-IgM allotype antibodies. This treatment permanently deploys the recipient Ly-1 B cells but does not affect the long term development of recipient conventional B cells.

Donor Ly-1 B cells (PerC-derived) reconstitute a self-replenishing population that occupies the normal Ly-1 B sites and apparently functions normally throughout life in these (non-irradiated) chimeras (29). Furthermore, as indicated above, precursors present in donor PerC persist and give rise to IgA plasma cells that can be found in the gut in these chimeras many months after transfer. Thus, radiation-induced cell death and tissue destruction is not required either for the establishment of the chimeras or for the homing of PerC-derived IgA plasma cells to the gut.

Taken together, these findings demonstrate that the peritoneal cavity in the mouse contains a reservoir of precursors for gut IgA plasma cells and suggest that this reservoir serves as a source of a reasonable proportion of the gut IgA plasma cells in normal animals.

Ig produced by PerC-derived cells is present in serum in B lineage chimeras

The presence of large number of IgM, IgA, and IgD plasma cells in chimeric animals suggests that these cells make a major contribution to the production of these immunoglobulins. This prediction is substantiated by data from measurements of serum Ig levels, which show that up to half of the serum IgM (9, 29) and
Table 2. Half the serum IgA in irradiation chimeras is produced by Perc-derived cells.

<table>
<thead>
<tr>
<th>Months after transfer</th>
<th>Total Perc b/a</th>
<th>Percential donor IgA, IgA (lgk-2a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/ml</td>
<td>mg/ml</td>
</tr>
<tr>
<td>3</td>
<td>1.5 ± 0.05</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>1.7 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>12</td>
<td>5.0 ± 0.7</td>
<td>2.9 ± 0.7</td>
</tr>
</tbody>
</table>

* 650 R irradiated BALB/c (lgk) mice received 5 million BALB/c (lgk) peritoneal cells and 2.5 million BALB/c (lgk) bone marrow cells. Animals were bled at the indicated times and assayed for total serum IgA and serum Igk-2a levels by IA. All values are an average of three animals and expressed as mg/ml of serum.

significant amounts of IgG (9) and IgA (Table 2) in the chimeras are produced by Perc-derived cells.

Discussion

Evidence from studies presented here, in which we use Perc bone marrow chimeras to investigate the origin of plasma cells in lymphoid organs and the lamina propria of the gut, demonstrate that a major proportion of the Ig-producing (plasma) cells in these animals are derived from self-replicating cells that can be readily harvested from the peritoneal cavity.

The lineage origin of these plasma cells is not completely established; however, several lines of evidence indicate that they may represent a terminally differentiated, functional component of the Ly-1 B lineage. First, all evidence from Perc bone marrow transfers (including those described here) demonstrate that the transferred Perc selectively reconstitute Ly-1 B lineage cells (both CDS - + and CDS - /sister cells) and do not reconstitute detectable numbers of conventional B cells (16, 17). Thus, if Ly-1 B cells do not give rise to the Perc-derived plasma cells, these must be being supplied from a very small reservoir of self-replicating conventional B cells.

Secondly, the precursors for the Perc-derived plasma cells appear to self-replicating, like Ly-1 B cells (16, 17). The vast majority of IgA plasma cells in the gut, for example, are known to turn over rapidly with a half-life of approximately 5 days (33).

The Perc-derived intestinal lgA plasma cells (and Perc-derived serum IgA), however, are still found at high levels 11 months after transfer. Similarly, Perc-derived lgG and IgM plasma cells are also detectable at other lymphoid sites many months after transfer. Thus, either these plasma cells are all extremely long lived or they are being supplied by self-replicating precursors.

Thirdly, FACs sorting studies indicate that many Perc-derived lgG and lgG secreting cells in chimeric animals are derived from Ly-1 B cells. In chimeras made with FACs-sorted Ly-1 B cells, roughly half of serum IgM and substantial amounts of the serum lgG in chimeric mice reconstituted several months previously carries the allotype of the Ly-1 B donor (9). Thus, a fair proportion of IgA plasma cells can also be expected to derive from Ly-1 B lineage cells. Consistent with this argument, a Ly-1 B cell line, Ch12, has been shown to switch readily in vitro, from IgM expression to lgA expression (34).

Finally, the kinds of antibodies that Ly-1 B cells are known to produce also recommend these cells as precursors for lgA producing cells in the gut. That is, Ly-1 B cells and/or Ly-1 B cell tumours have been shown to produce antibodies to microbial cell surface antigens, for example, 1-1.2 dextran, phosphoryl choline, phosphatidyl choline, and an uncharacterized surface determinant on E. coli (14, 15, and Staal et al. manuscript in preparation).

Thus, the gut region represents a major source of exposure to antigens of the type recognized by Ly-1 B cells and therefore constitutes a site at which antibody-secreting progeny of Ly-1 B cells might be expected to localize.

Drawing definitive conclusions with respect to the lineage origin of the IgA plasma cells, however, requires transfer studies with sorted cells. We do not, for example, know the origin of the lgG B cells that we detected in the peritoneum or whether these cells are self-replicating memory B cells that contribute to the observed reconstitution of lgA plasma cells. Similar B there could be a key peritoneal B cell population whose phenotype and frequency makes it hard to distinguish under the conditions that we use.

In any event, the demonstration that nearly half of the IgA plasma cells in the intestinal mucosa derive from the self-replicating cells in the peritoneum contradicts the prevailing paradigm regarding the origin of the IgA producing cells in the gut. These cells are commonly thought to be derived from lgM-lgD+ precursor B cells in Peyer's patches (9). Cells with this phenotype are exclusively located in the corona (mantine zone) of the Peyer's patch lymphoid follicles (29); however, data presented here show that despite the large number of Perc-derived IgA plasma cell seen in the gut, very few cells derived from the Perc donor are found in the lymphocyte corona of the Peyer's patches.

Thus, although the Peyer's patches are likely to be an important source of intestinal IgA producing cells, they do not appear to be the sole source of these cells. Previous findings support this conclusion. First, surgical removal of Peyer's patches in rats does not greatly reduce the number of plasma cells in the gut (35, 36). Secondly, concentrations of IgA in the thoracic duct plucks Peyer's patches derived cells but does not result in a corresponding depletion of intestinal IgA plasma cells (37). These findings were difficult to explain with the earlier model; however, they are clearly consistent with the idea that two independent B cell lineages contribute to the IgA plasma cell population in the gut.

Acknowledgements

The technical help of Auk S. Wubbena and F. T. Gadus is greatly appreciated. F. G. M. K. was a recipient of a NATO Science felicitation from the Netherlands Organization for Scientific Research (N.W.O.) Supported by N.H. grants AI-19957 and DK-38707 (E.C.B.) and HD-01327 and CA-42509 (A.I.H. A. M. S. is a Special Fellow of the Leukemia Society of America.

Abbreviations

BMM bone marrow
FACS fluorescence-activated cell sorter
lgG immunoglobulin heavy chain locus
Perc peritoneal washout cells

References

Origin of gut IgA plasma cells

of immunoglobulin-secreting cells in the murine small intestine to the background immunoglobulin production. Immunology 62:551


