Immunological Investigations
A Journal of Molecular and Cellular Immunology

ISSN: 0882-0139 (Print) 1532-4311 (Online) Journal homepage: http://www.tandfonline.com/loi/iimm20

A Major Peritoneal Reservoir of Precursors for Intestinal IgA Plasma Cells

Frans G.M. Kroese, Eugene C. Butcher, Alan M. Stall & Leonore A. Herzenberg

To cite this article: Frans G.M. Kroese, Eugene C. Butcher, Alan M. Stall & Leonore A. Herzenberg (1989) A Major Peritoneal Reservoir of Precursors for Intestinal IgA Plasma Cells, Immunological Investigations, 18:1-4, 47-58, DOI: 10.3109/08820138909112226

To link to this article: https://doi.org/10.3109/08820138909112226

Published online: 07 Jul 2009.

IMMUNOLOGICAL INVESTIGATIONS, 18(1-4), 47-58 (1989)

A MAJOR PERITONEAL RESERVOIR OF PRECURSORS FOR INTESTINAL IgA PLASMA CELLS

Frans G.M. Kroese*, Eugene C. Butcher*, Alan M. Stall# and Leonore A. Herzenberg#

*Departments of Pathology and #Genetics, Stanford University Medical School, Stanford, CA 94305 and *VA Medical Center, Palo Alto.

ABSTRACT

Studies presented examine the origin of IgA plasma cells in B lineage chimeric mice constructed by reconstituting lethally irradiated mice with a mixture of syngeneic bone marrow cells and peritoneal cells from Ig heavy chain allotype congenic donors. In these mice, essentially all B cells in spleen and Peyer's patches are derived from the bone marrow donor; however Ly-1 B lineage cells which have been mainly detected in the peritoneum are derived from the peritoneal cell donor. Surprisingly, roughly half of the IgA plasma cells in the lamina propria of the gut are also derived from the peritoneal cell donor, suggesting an important role for peritoneally-derived B cells in the mucosal immune response.

INTRODUCTION

The immune responses of the intestinal mucosa play an essential role in the first line of defense against pathogenic microorganisms. The secretion of IgA by the numerous plasma cells located in the lamina propria forms a major contribution to these immune responses [for review see ref. 1]. IgA, produced by the plasma cells, becomes associated with secretory component, is transported through the epithelial lining and secreted into the gut lumen to react with antigens present there.

The vast majority of the IgA producing cells in the gut are commonly thought to be derived from precursor B cells in the Peyer's patches in the small intestine [2-4]. Short term reconstitution studies suggest that antigen-stimulated B cells ($IgM^+IgD^+IgA^-$) leave the Peyer's patches via the lymph and peripheral blood and lodge in the mucosal tissues, where they further differentiate to mature IgA secreting plasma cells [5]. IgM^+IgD^+ cells are located in the corona (mantle zone) of the Peyer's patch lymphoid follicles [6].

Nevertheless, there are some indications that the Peyer's patches are not the sole source of intestinal IgA plasma cells [7-9]. For example, surgical removal of Peyer's patches in rats does not greatly reduce the number of plasma cells in the gut [7,8]. Furthermore, continued cannulation of the thoracic duct to deplete Peyer's patch derived cells doesn't markedly deplete intestinal plasma cells [9]. Finally, as we show here, roughly half the plasma cells in the lamina propria are derived from a bone marrow independent B cell population that is well represented in the peritoneum. This precursor population appears to be related to the recently described Ly-1 B lineage.

The Ly-1 B lineage contains phenotypically distinct, self-renewing B cells that are rare in spleen, lymph nodes and Peyer's patches but predominate in the peritoneal cavity [10,11]. These cells have been shown to produce much of the IgM and many of the autoantibodies found in serum in normal and autoimmune mice [12,13]. In addition, they appear to make certain key antibody responses to bacterial coat antigens, e.g., anti DEX and anti PC [A.M.S., unpublished observation; 14].

In studies presented, we evaluate the origin of the lamina propria cells by producing stable B lineage irradiation chimeras in which the vast majority of the B cells are derived from Ig^- cells in the donor bone marrow while the Ly-1 B cells (and related B cells) are derived from mature, self-replenishing (Ly-1) B cells in the donor peritoneal cavity [11,15]. By reconstituting the irradiated recipients with a mixture of syngeneic bone marrow and peritoneal cells (PerC) from an Ig allotype congenic donor, the bone marrow derived "conventional" B cells are clearly distinguishable from the peritoneally-derived B cells on the basis of the IgM, IgD, IgG and IgA heavy chain allotypes they produce.

Using these chimeras, we show that roughly half of IgA containing cells in the lamina propria are derived from self-replenishing donor PerC, whereas the Peyer's patch IgM^+IgD^+ corona B cells are almost exclusively derived from bone marrow precursors. These findings suggest an important role for PerC-derived B cells in the mucosal immune response.

MATERIALS AND METHODS

Animals. Balb/c (Igh^a) and its Igh^b congenic strain BAB/25 were bred under conventional conditions in the animal facility of the Stanford Department of Genetics.

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) as described [16]; anti Igh-6a (IgM of "a" allotype, DS-1), donated by Dr. Donna Sieckmann [17] and anti Igh-2a (IgA of "a" allotype, HY16) [6]. Monoclonal rat antibodies employed were: anti IgM (331.12) [18]; anti Ly-1 (53-7.8) [19]; anti Mac-1 (M1/70) [20] and anti IgA, (71.14) produced by Dr. Eric Pillemer at the Department of Pathology, Stanford University [6]. Monoclonal antibodies were isolated from either culture supernatants or ascitic fluid, purified by ion exchange chromatography on DEAE Sephacel or QAE Sephadex and conjugated to biotin, fluorescein or allophycocyanin as described [21,22].

Preparation of cells. Peritoneal washout cells (PerC) were harvested from mice by injecting chilled RPMI medium (Applied Scientific, Santa Ana, CA) supplemented with 3% newborn calf serum as described {11]. Suspensions from lymphoid tissues were prepared in the same medium using the frosted ends of microscope slides.

Construction of chimeras. Irradiation chimeras were constructed by transfer of 2.5×10^6 PerC and 1×10^6 bone marrow cells from allotype congenic mice (differing in Ig heavy chain allotype) to lethally irradiated recipients as described [11,15]. Mice were analysed 2-7 months after irradiation and transfer of cells.

Cell staining and FACS analysis. Three color immunofluorescence staining of cell suspensions was carried out in 96-well plates as described previously [21]. Briefly, cells (500,000 cells/sample) were incubated for 20 minutes on ice with monoclonal antibodies conjugated to FITC, biotin or allophycocyanin. After washing, a 15 minutes incubation followed with avidin coupled to Texas Red, tc detect biotinylated antibodies. Multiparameter flow cytometry was carried out on an extensively modified fluorescence activated cell sorter (FACS II; Beckton and Dickinson, Mountain View, CA) interfaced with a VAX11/780 computer (Digital Equipment, Maynard, MA) [21]. Data were collected from 20,000 - 30,000 individual, viable (i.e. propidium iodide negative) cells.

Immunohistological staining procedure of frozen tissue sections. Sections from frozen tissues were stained by an immunoperoxidase method as described elsewhere [23]. Briefly, acetone fixed sections were incubated with biotinylated monoclonal antibodies followed by peroxidase conjugated avidin (Vector Laboratories, Inc., Burlinghame, CA). Peroxidase activity was demonstrated using 3,3' - diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) at a concentration of 0.5 mg/ml in 0.05 M Tris/HCl buffer (pH 7.6) containing 0.01% H_2O_2. Sections were counterstained with hematoxylin.

Double immunofluorescence staining for IgA and Igh-2a (IgA of "a" allotype) was carried out on acetone fixed frozen sections from the gut. Sections were incubated for 20 minutes with FITC conjugated monoclonal anti-mouse IgA (71.14) and biotinylated anti Igh-2a (HY16). After rinsing in PBS, sections were incubated for 15 minutes with avidin-Texas Red. Finally sections were mounted in an embedding compound containing an anti-fading agent (Citifluor Ltd, The City University, London, U.K.).

RESULTS

B cells of the Ly-1 lineage cells are predominantly found in the peritoneum. Multiparameter FACS (three color) analysis of peritoneal washout cells (PerC) from normal, untreated mice initially distinguished two types of B cells [10-15]: conventional B cells, which

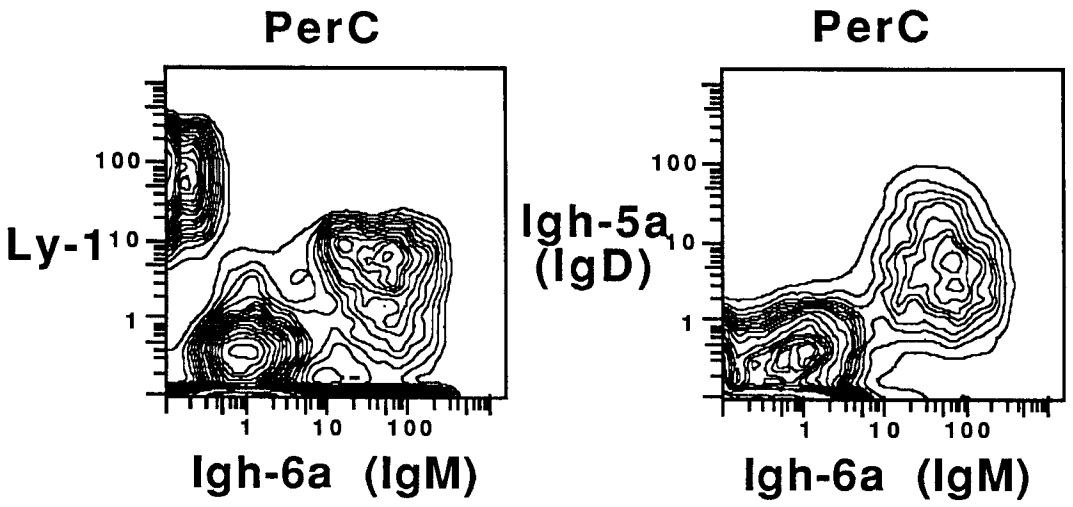

Figure 1. Three color FACS analysis of peritoneal cells from PerC/BM irradiation chimeras. Peritoneal cells from a chimeric mouse (3 months after reconstitution) were stained with fluoresceinated anti Igh-6a (IgM of peritoneal cell donor), biotinylated anti Igh-5a (IgD of peritoneal cell donor) and allophycocyanin conjugated to Ly-1. Biotin was detected by Avidin-Texas Red. All cells with the Ig allotype of the peritoneal cell donor are Ly-1 B lineage cells.

have the $IgM^{low}IgD^{high}Ly\text{-}1^{-}Mac\text{-}1^{-}$ phenotype; and Ly-1 B cells, which are phenotypically $IgM^{high}IgD^{low}Ly\text{-}1^{low}Mac\text{-}1^{low}$. Recently, the Ly-1 B lineage has been subdivided into a majority subpopulation, which contains the typical Ly-1 B cells, and a minority subpopulation, which contains so-called "sister population" cells that have essentially the same function, phenotype, distribution and reconstitution characteristics as typical Ly-1 B cells but lack surface Ly-1(CD5). Ly-1 B cells (both typical and sister) are are abundant in the peritoneal cavity and rare in spleen. They are virtually absent in lymph nodes and Peyer's patches [10].

Peritoneal cells selectively reconstitute Ly-1 B lineage populations in irradiation chimeras. Irradiation chimeras were constructed by repopulation of lethally irradiated Igh^{b} (BAB/25) mice with syngeneic bone marrow cells and peritoneal cells derived from Igh^{a} (Balb/c) congenic donors. FACS analysis of PerC from these chimeric

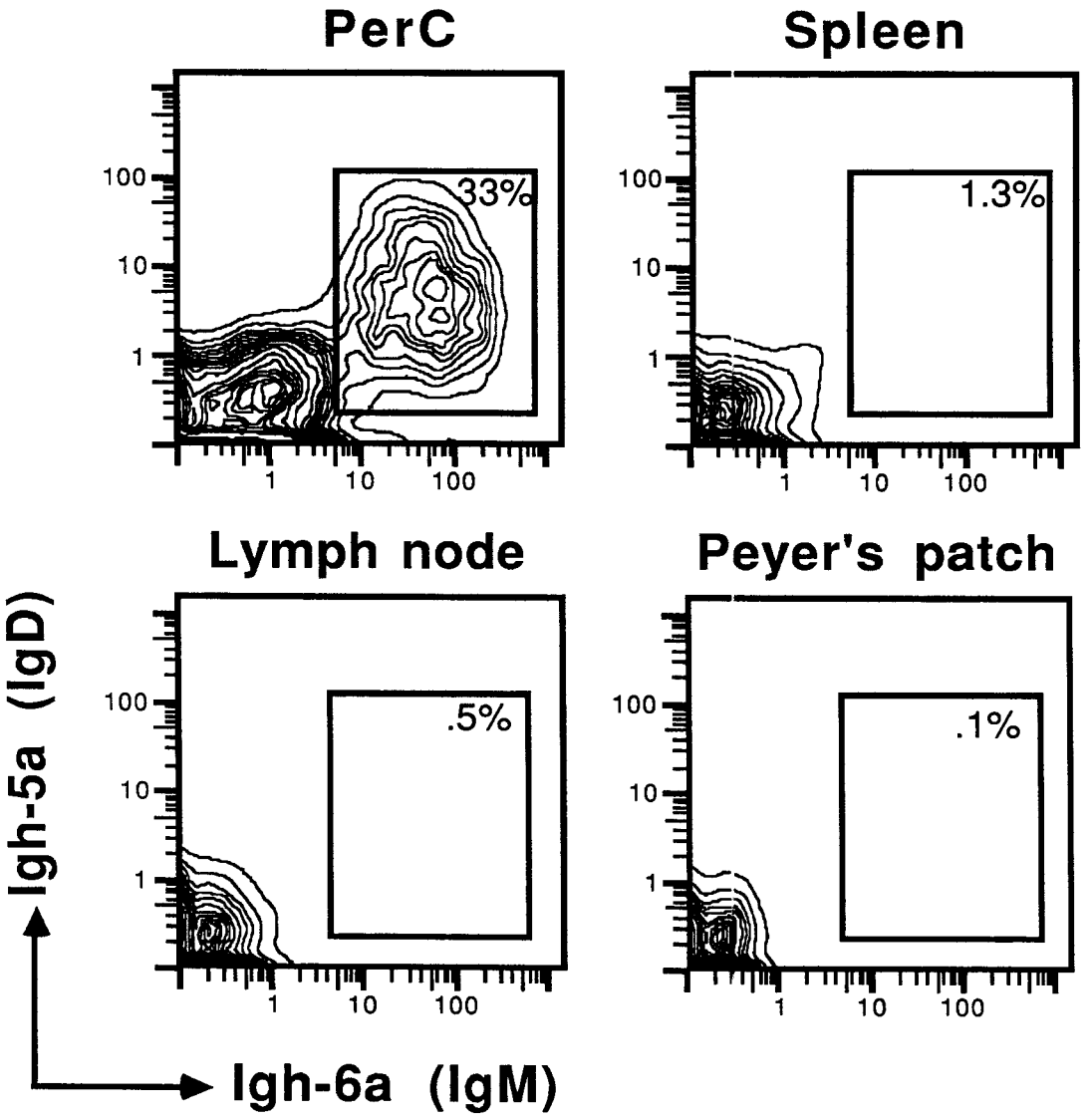

Figure 2. FACS analysis of cells taken from different organs from an irradiation chimera. (The same mouse is shown in figure 1). Cells were stained with fluoresceinated anti Igh-6a (IgM of peritoneal cell donor allotype) and with anti Igh-5a (IgD of peritoneal cell donor allotype), followed by avidin-Texas Red. Only in recipient PerC, significant numbers of B cells from the peritoneal cell donor were found.

mice confirmed previous observations that virtually all Ly-1 B lineage cells, both typical Ly-1 B cells and the $CD5^-$ "sister" population, express the IgM allotype (Igh-6a) of the PerC donor and thus are derived from the transferred PerC (see fig. 1). On the other hand, the conventional B cells in recipient PerC virtually all express the IgM allotype (Igh-6b) of the bone marrow donor and thus are derived from precursor cells in the bone marrow. Only a few PerC-derived B cells were detected in spleen (approx. 1%) and even fewer (approx. 0.5%) were detected in lymph nodes and Peyer's patches (see fig. 2).

The anatomical localization of PerC-derived cells in spleen and Peyer's patches. To study the localization of PerC derived cells *in situ*, we used anti Ig allotype reagents to stain frozen sections taken from lymphoid organs from stable chimeric mice, i.e., 2-7 months after reconstitution. Within the spleen, (immunoperoxidase) staining for IgM of the PerC-donor (Igh-6a) revealed the presence of high numbers of intensely stained cells (presumably cytoplasmic IgM) with typical plasma cell morphology, that were localized in the red pulp. Only few $Igh\text{-}6a^+$ lymphocyte-like cells were found. These cells were predominantly localized in the lymphocyte corona of the follicles.

In Peyer's patches, as in spleen, most of the B cells expressed Ig of the bone marrow donor allotype. Rare, scattered cells in the lymphocyte corona expressed IgM, IgD or IgA of PerC donor allotype (see fig. 3). Very few IgA or IgM plasma cells of PerC donor allotype were present. Thus, the vast majority of follicular IgM^+IgD^+ B cells in the Peyer's patches are derived from bone marrow precursor cells.

Dendritic staining (anti Igh-6a) was present in most follicles of spleen and Peyer's patches, apparently reflecting the presence of antigen-antibody complexes trapped on the surface of the follicular dendritic cells [24].

Most of the IgA containing cells in the lamina propria of the small intestine are derived from peritoneal donor cells in chimeric mice. The IgM^+IgD^+ in the Peyer's patches are thought to be precursors of most of the IgA plasma cells in the lamina propria of the gut [5]. Nevertheless, we surprisingly found that many IgA containing cells in

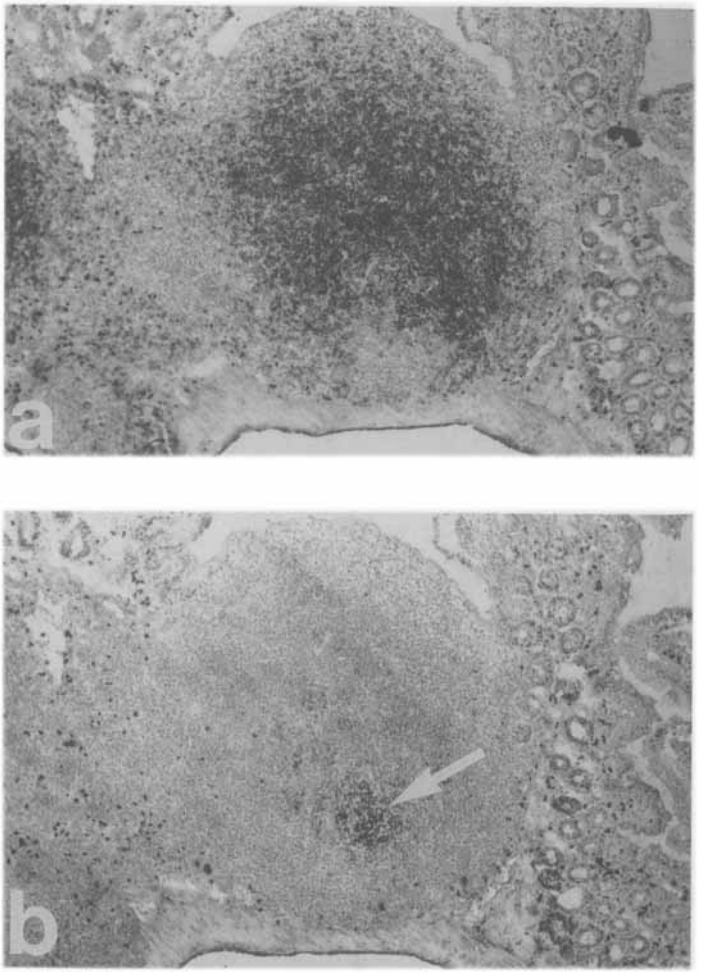

Figure 3. Peyer's patch from an irradiation chimera, 7 months after transfer of Igh^a PerC and Igh^b bone marrow cells. Serial sections were stained by immunoperoxidase using anti Igh-6b (IgM of bone marrow donor) (a) or anti Igh-6a (IgM of PerC donor) (b). Virtually all IgM^+ cells in the Peyer's patch are of bone marrow origin; only rare scattered cells are stained with anti Igh-6a (most of the black dots seen on photograph b are due to endogenous peroxidase staining). Note the dendritic staining in the germinal center (arrow), due to immune complexes trapped by the follicular dendritic cells.

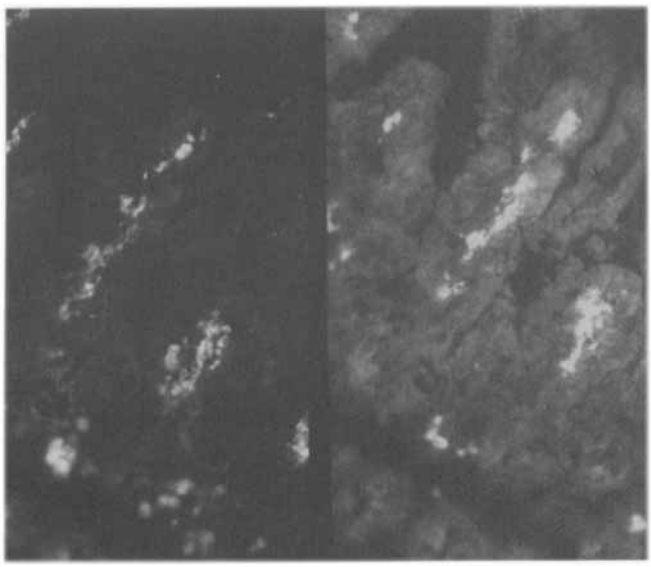

Figure 4. Double immunofluorescence staining of the lamina propria of the small intestine (from the same chimera shown in figure 3) Staining with fluoresceinated anti-IgA (left panel) and biotinylated anti Igh-2a (IgA of PerC donor), followed by avidin conjugated to Texas Red (right panel). The majority of the IgA containing cells in the lamina propria react with anti Igh-2a and thus are derived from PerC.

the lamina propria of the gut are derived from PerC donor cells (see fig. 4). Double immunofluorescence staining with an anti IgA isotype reagent (which detects both allotypes equally) and an anti Igh-2a reagent (which detects the PerC donor IgA allotype) demonstrated that the PerC-derived IgA plasma cells represent a major proportion of the IgA-containing cells in the lamina propria. Preliminary studies with single cell suspensions prepared from lamina propria indicate that roughly 50 per cent of the IgA cells in the gut are of PerC origin.

DISCUSSION

Studies here demonstrate that a major proportion of the IgA plasma cells in the gut in PerC/bone marrow chimeras are derived from precursor cells resident in the peritoneal cavity (of the PerC donor). In contrast, Peyer's

patch B cells in these animals are almost exclusively derived from precursors resident in bone marrow. Very few PerC-derived B cells are detectable in these Peyer's patches. Thus, our data raise serious questions about conclusions from earlier studies suggesting that IgM+IgD+ B cells in Peyer's patches are the precursors of most or perhaps all of the IgA plasma cells in the gut [2-5]. In essence, although Peyer's patches may contain some precursors of intestinal IgA plasma cells, they clearly do not contain all such precursors.

Two previous findings agree with this conclusion: 1) the numbers of intestinal IgA containing cells are either not reduced [8] or only partially reduced [9] following surgical removal of the Peyer's patches in rats; and, 2) prolonged thoracic duct cannulation, which would be expected to deplete IgA plasma cell precursors originating in Peyer's patches, only partially reduces the numbers of IgA plasma cells in the gut [7]. Thus, there is good reason to believe that results from our studies using allotype-marked cells to determine precursor/progeny relationships in irradiation chimeras provide a valid indication of the normal origins of intestinal plasma cells.

We have not as yet established the lineage to which the PerC-derived IgA precursor cells belong; however, several lines of evidence suggest that they derive from Ly-1 B cells or from the related "sister" population:

First, all evidence to date indicates that while (self-replenishing) precursors for Ly-1 B cells are abundant in PerC, precursors for conventional B cells are not present [11,15];

Secondly, preliminary studies with mice reconstituted with sorted IgM+Ly-1+ (typical Ly-1 B cells) and IgM+Ly-1- (sister cells and conventional B cells) indicate that both sorted populations give rise to significant numbers of intestinal IgA plasma cells; and,

Finally, precursors for the PerC-derived IgA plasma cells appear to be self-replenishing (like Ly-1 B), since the half-life of the intestinal IgA plasma cell has been estimated to be 4-5 days [25] and the IgA plasma cells to which they give rise are present in chimeras even 7 months after irradiation and transfer.

To conclude, studies presented here shed new light on the origin and function of IgA producing cells involved in mucosal immunity These studies demonstrate the existence of a bone marrow independent reservoir of IgA plasma cell precursors that is present in the peritoneal cavity .

ACKNOWLEDGEMENTS

F.G.M.K. is a recipient of a NATO science fellowship from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.). Supported by NIH grants AI19957 (E.C.B.) and HD01287 and CA42509 (L.A.H.). The interest and help of Dr. Leonard A. Herzenberg is appreciated.

REFERENCES

1. P. Brandtzaeg, *Scand J Gastroenterol [Suppl]*, **114**, 137-156 (1985)
2. S.W. Graig and J.J. Cebra, *J Exp Med,* **134**, 188-200 (1971)
3. A.J. Husband and J.L. Gowans, *J Exp Med*, **148**, 1146-1160 (1978)
4. J. Tseng, *J Immunol*, **127**, 2039-2043 (1981)
5. J. Tseng, *J Immunol,* **132**, 2730-2735 (1984)
6. E.C. Butcher, R.V. Rouse, R.L. Coffman, C.N. Nottenburg, R.R. Hardy and I.L. Weissman, *J Immunol*, **129**, 2698-2707 (1982)
7. G. Mayrhofer and R. Fisher, *Eur J Immunol*, **9**, 85-91 (1979)
8. R.V. Heatly, J.M. Stark, P. Horsewood, E. Bandouvast, F. Cole and J. Bienenstock, *Immunology*, **44**, 543-548 *(1981)*
9. G.A. Enders, S. Balhaus and W. Brendel, *Immunology*, **63**, 411-414 (1988)
10. K. Hayakawa, R.R. Hardy, D.R. Parks and L.A. Herzenberg, *J Exp Med,* **157**, 202-218 (1983)
11. K. Hayakawa, R.R. Hardy, L.A. Herzenberg and L.A. Herzenberg, *J Exp Med*, **161**, 1554-1568 (1985)
12. K. Hayakawa, R.R. Hardy, M. Honda, L.A. Herzenberg, A.D. Steinberg and L.A. Herzenberg, *Proc Natl Acad Sci USA,* **81**, 2494-2498 (1984)
13. K. Hayakawa, R.R. Hardy and L.A. Herzenberg, *Eur J Immunol*, **16**, 450-456 (1986)
14. I. Förster and K. Rajewsky, *Eur J Immunol*, **17**, 521-528 (1987)

15. K. Hayakawa, R.R. Hardy, A.M. Stall, L.A. Herzenberg and L.A. Herzenberg, *Eur J Immunol*, **16**, 1313-1316 (1986)
16. A.M. Stall and M.R. Loken, *J Immunol*, **132**, 787-795 (1984)
17. A.M. Stall, F.G.M. Kroese, F.T. Gadus, D.G. Sieckmann, L.A. Herzenberg and L.A. Herzenberg, *Proc Natl Acad Sci USA*, **85**, 3546-3550 (1988)
18. P.W. Kincade, G. Lee, L. Sun and T. Watanabe, *J Immunol Methods*, **42**, 17-26 (1981)
19. J.A. Ledbetter and L.A. Herzenberg, *Immunol Rev*, **47**, 63-90 (1979)
20. T. Springer, G. Galfre,D.S. Secher and C. Milstein, *Eur J Immunol*, **9**, 301-306 (1979)
21. R.R. Hardy, K. Hayakawa, D.R. Parks, L.A. Herzenberg and L.A. Herzenberg, *J Exp Med*, **159**, 1169-1188 (1984)
22. R.R. Hardy, in The Handbook of Experimental Immunology, 4 th edition, D.M. Weir, L.A. Herzenberg, C.C. Blackwell and L.A. Herzenberg, eds, Blackwell Scientific Pub., Ltd., Edinburgh
23. F.G.M. Kroese, A.S. Wubbena, D. Opstelten, G.J. Deenen, E.H. Schwander, L. de Leij, H. Vos, S. Poppema, J. Volberda and P. Nieuwenhuis, *Eur J Immunol*, **17**, 921-928 (1987)
24. G.G.B. Klaus, J.H. Humphrey, A. Kunkl and Dongworth, *Immunol Rev*, **53**, 3-28 (1980)
25. C.A. Mattioli and T.B. Tomasi, *J Exp Med*, **138**, 452-460 (1973)