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Contribution of B-1 Cells to Intestinal IgA Production in the Mouse

Frans G. M. Kroese, John J. Cebra,* Maarten J. F. van der Cammen,
Aaron B. Kantor,[†] and Nicolaas A. Bos

*Department of Histology and Cell Biology, University of Groningen, Groningen, The Netherlands; *Department of
Biology, University of Pennsylvania, Philadelphia, Pennsylvania; and [†]Department of Genetics, Stanford University
Medical School, Stanford, California*

In this paper we review the existing evidence that peritoneally derived B-1 cells may contribute significantly to the generation of IgA-secreting plasma cells in the murine intestinal lamina propria. The evidence is based upon a variety of experimental approaches performed in our laboratory and others and include (i) transfer studies of (sorted) B-1 cells into B-cell-depleted mice either experimentally (X-irradiation, anti-μ treatment) or genetically (SCID), (ii) analysis of genetically modified or manipulated mice (motheaten mice, CBA/N Xid mice, μ,k transgenic mice), and (iii) transplantation studies of fetal omentum. The data thus support the view that in addition to conventional B cells (B-2 cells) located in the Peyer's patches (PP), B-1 cells contribute to the pool of IgA-containing cells in the gut. Indeed, cotransfer of PP cells and peritoneal cells (PerC), which contain largely B-1 cells, into SCID recipients demonstrates that both PP and PerC contribute in a balanced fashion to the pool of IgA-containing cells in the gut lamina propria over long periods. Most likely IgA-positive (memory) B cells in PP are responsible for the long-term generation of IgA-producing cells derived from the PP inoculum. The potency of B-1 cells to contribute to mucosal IgA responses is also illustrated in adoptive transfer experiments in which PerC B-1 cells (or sorted B-1 cells) are adoptively transferred into untreated, Ig allotype congenic, SCID mice. These studies show that 6 months after injection of a few million PerC, almost all B cells in spleen and the recipient's peritoneal cavity have the B-1 cell phenotype, while approximately 40 million PerC donor-derived IgA-producing cells can be detected in the gut lamina propria by allotype-specific ELISA spot assays. In conclusion, the data presented here show that, in principle, B-1 cells located in the peritoneal cavity may be an important source of precursors for intestinal IgA plasma cells in the mouse. However, the experiments performed so far do not allow us to draw definitive conclusions yet on the physiological contribution (in terms of numbers) and function (in terms of their specificity repertoire) of both B-1 cells and Peyer's patch-derived B-2 cells to the pool of IgA-containing cells in the gut lamina propria in normal, unmanipulated animals. © 1995 Academic Press, Inc.

Along with numerous other differences (such as phenotype, origin, and tissue distribution), the suggested difference in antibody repertoire between the bone marrow-derived population of conventional B cells (or B-2 cells) and the small population of self-replenishing B-1 cells (previously called Ly-1 B cells) is one of the most intriguing features (for review see 1). B-1 cells generally appear to produce antibodies directed to autoantigens and microorganism-related antigens such as phosphatidylcholine (PtC) (2, 3), α (1 → 3) dextran (4), lipopolysaccharide (5), phosphorylcholine (T15 idiotype) (6, 7), and determinants on *Salmonella typhimurium* (8). This biased repertoire is also reflected by the observation that B-1 cells appear to have a distinct usage of particular V_H genes, like their frequent usage of the $V_H 11$ gene (9), as well as the fewer N-region insertions detected in these (B-1a) cells (10). B-1 cells contribute significantly to the levels of serum IgM and are believed to be the main source of natural antibodies in the mouse (3, 11). Current knowledge about the origin of B-1 cells suggests that the skewed antibody repertoire of B-1 cells is the result of positive selection mechanisms that operate early in neonatal life, i.e., in the period that these cells are generated and they encounter most common environmental antigens for the first time. Once these cells have been formed, they are maintained largely (or solely) by self-replenishment throughout the life of the animal and may provide a first line of defense to "natural" (self-) antigens (1, 11–13).

In this context, it is not a surprise that B-1 cells also may play a role in mucosal immune responses, as we reported previously (14–17). Immediately after birth the gut is invaded by numerous bacteria and the gut flora is responsible for the bulk load of microbial antigens of the animal. Throughout the life of the animal the composition of the gut flora is relatively constant. Thus, most of the natural antigens encountered by an animal during its life are provided by the microflora in the gut. In order to combat the potential danger of bacterial infections from the gut lumen, extremely high numbers of antibody-secreting

cells are located in the gut wall. At these sites 15–50 million antibody-secreting cells are present in the connective tissue layer (lamina propria) underlying the epithelium of the murine intestine, and nearly all of these cells secrete IgA antibodies (18, 19). The importance of the mucosal immune system is reflected by the fact that these IgA-secreting cells account for approximately 90% of the overall number of antibody-producing cells in the mouse (18).

Initially, these IgA-secreting cells were thought to be derived from (conventional) B cells located in the Peyer's patches (PP) (20). At these sites B-1 cells are undetectable (14). Follicular type B cells (IgM and IgD positive), triggered by intestinal-derived antigens, leave these anatomical sites, migrate via mesenteric lymph nodes and thoracic duct into the circulation, and home back to lamina propria of the mucosa to differentiate to IgA-secreting cells (21, 22). IgA is then transported into the gut lumen to exert its function by binding to microbial and food antigens present in the gut. We have challenged the view that (conventional) B cells in the PP are the sole source of IgA precursor cells. In this paper we review the data to support the hypothesis that in addition to B cells from PP B-1 cells may also be an important source of intestinal IgA precursor cells, albeit the contribution of both B-cell populations in normal animals still remains to be established.

EVIDENCE THAT B-1 CELLS CAN DIFFERENTIATE TO IgA SECRETING CELLS

There is accumulating evidence from our laboratory and others, using a number of different experimental approaches, that B-1 cells can readily switch *in vivo* to IgA expression-secreting cells and contribute to the IgA response of the gut lamina propria. The variety of methods used are based on the differential properties of B-1 cells: their unique phenotype, fetal development, (fixed) repertoire, and self-replenishment capabilities during adult life. In this section we summarize these experiments and also present some novel findings, collectively showing that B-1 cells can synthetize and secrete IgA.

Transfer Experiments with B-Cell-Depleted Mice

1. X-irradiated or anti-IgM-treated mice. In our initial studies we explored the potential role of B-1 cells in the mucosal immune response using B lineage chimeric mice, in which B-1 cells and B-2 cells are distinguished from each other on the basis of their Ig allotype expression. Such chimeras can be constructed by virtue of the self-replenishing capability of B-1 cells, but not of B-2 cells. To this end, lethally X-irradiated mice are reconstituted with syngeneic bone marrow (BM) followed by transfer of peritoneal cells (PerC) from Ig allotype congenic donors

(23, 24). Alternatively, Igh-C congenic PerC are transferred into neonatal allotype homozygous mice treated from birth with anti-IgM antibodies directed to IgM allotype of the recipient (25). After stopping the anti-IgM treatment, recipient B-1 cells are permanently depleted, while their B-2 cells develop to normal levels. Flow cytometry analysis of both types of stable chimeric mice (>3 months after transfer) show that B cells carrying IgM of the PerC-donor allotype are exclusively B-1 cells (both B-1a cells and B-1b cells) and are enriched in the peritoneal cavity of the host. Conventional B cells (B-2 cells) express the BM donor allotype. We observed that, as expected, PP, which are almost completely composed of B-2 cells, contained only rare PerC-donor-derived cells. By contrast, in the gut lamina propria of these chimeras a large proportion (up to 50%) of the IgA-containing cells expressed the PerC-donor allotype, even 1 year after transfer (14). Figure 1 shows an example of the gut of a PerC/BM chimeric mouse simultaneously stained for PerC donor and BM donor IgA. The majority of IgA-containing cells in the gut are probably short-lived (26) and require a continuous supply from precursor cells. Since, in these B lineage chimeras the only cells identifiable with the PerC donor allotype are B-1 cells, we concluded that also the PerC-donor derived IgA cells in the gut originated from the B-1 cell population (14). Indeed, transfer experiments with sorted B-1 cells, and sorted B-1a and B-1b cells from BALB/c mice (Igh-C^a) into irradiated recipients (BAB/25; Igh-C^b) have recently shown that both B-1a and B-1b cells can develop into IgA-secreting cells (F. G. M. Kroese and A. B. Kantor, unpublished observations). These cells probably do not express IgA at time of transfer, since sorted sIgA-negative B-1 cells (IgM^{bright}IgD^{dull}) are also able to give rise to intestinal IgA cells upon transfer into irradiated recipient mice.

2. CBA/N mice. CBA/N Xid mice are deficient in B-1 cells (11) and do not mount a mucosal IgA response after oral inoculation with live *Salmonella typhimurium* or phosphorylcholine (PC) (8). Transfer of B-1 cell-containing cell suspensions (PerC) from CBA/Ca donors results in strong mucosal and serum IgA responses to both *Salmonella* and PC in the reconstituted Xid recipients. By contrast, donor cell suspensions in which B-1 cells are lacking are ineffective in the induction of a *Salmonella* or PC response upon transfer into Xid mice.

3. SCID mice. We have found previously that transfer of Ig allotype congenic PerC into untreated SCID mice results in the long-term reconstitution of B-1 cells, but not of B-2 cells, along with the presence of IgA-producing cells in the gut lamina propria (15). More recent experiments confirmed and extended these findings (19). BrdUrd incorporation studies first suggested a role for mesenteric lymph nodes in the expansion of the PerC-donor-derived IgA population and second allowed us to generate a panel of eight IgA-producing hybridomas de-

rived from the PerC-donor (thus, probably B-1 cell derived) in these chimeras (19). Excitingly, with a flow cytometric method developed by Van der Waaij *et al.* (27), all hybridomas produce antibodies that appear to bind to a certain proportion of the (anaerobic) bacteria present in the normal fecal flora (19). Furthermore, when mixtures of allotypically marked B cells from PP and PerC (PP to PerC ratios varying between 2:1 and 2:3) are injected intraperitoneally into these SCID mice, donor PerC are retained over long periods in the peritoneal cavity, while PP cells disappear quickly from these sites (28). However, 3 months after transfer, each cell source contributes about half of the IgA-producing population in the gut. The long-term reconstitution of PP-derived B-2 cells might be (entirely) due to long-lived sIgA$^+$ memory B cells present in the PP inocula, since transfer of sorted IgA-positive B cells (along with C.B17 thymocytes) from PP also results in long-term donor-derived IgA in the gut lamina propria (28).

Taki *et al.* (7) transferred (sorted) B-1a cells into SCID mice either alone or in the presence of syngeneic, unprimed T cells. They found that B-1a cells can "spontaneously" secrete IgA (and other isotypes) into the serum of the recipients (2–4 weeks after transfer), even in the absence of T cells. The presence of T cells, however, results in a 10-fold increase in serum IgA levels in these mice compared to SCID mice repopulated with only B-1a cells. Interestingly, in their experiments levels of IgA production were higher when B-1a cells (and T cells) were derived from animals reared under conventional conditions, compared to SPF donors.

Experiments with Genetically Altered Mice

Nearly all B cells in autoimmune mice with the viable motheaten mutation are B-1 cells. Concomitantly, the serum of these mice contains extremely high levels of IgM, IgG3, and IgA (29).

We conducted a series of experiments with μ,k transgenic mice to demonstrate the B-1-cell origin of IgA-secreting cells in the gut (17). The transgenic mouse line B6-Sp6 carries a fully rearranged, TNP-specific IgM transgene derived from a Balb/c (Igh-C^a allotype) on a C57bl (Igh-C^b) background (30). As expected, most of the peripheral B cells in these mice express transgenic IgM, while endogenous IgM expression is largely suppressed. However, as seen in several other IgM transgenic mouse lines, a small fraction (<10%) of the B6-Sp6 B cells in peripheral lymphoid organs clearly expresses endogenous IgM molecules on their cell surface (17, 31–33). Evidence presented in detail elsewhere (17) shows by three criteria

FIG. 1.   Section of the gut taken from a B lineage chimeric animal, 3 months after reconstitution. Chimeric mice were constructed by reconstituting lethally irradiated C.B17 mice (Igh-C^b) with syngeneic BM and PerC from Ig congenic mice (BALB/c; Igh-C^a). Frozen gut sections were simultaneously stained for BM-derived IgA, using mAb HIS-M2 (red fluorescence), and for PerC donor-derived IgA, using mAb HY16 (green fluorescence). Note the balanced proportions of both BM- and PerC-derived IgA plasma cells in the lamina propria of the villi.

that the majority of endogenous IgM-expressing cells in the B6-Sp6 mice are B-1 cells, viz., (i) they are enriched in the peritoneal cavity, where they (ii) exhibit the typical phenotype of B-1 cells (i.e., all of them express Mac-1/ CD11b and many of them also CD5), and (iii) BM from these mice repopulate endogenous IgM-expressing cells poorly in a similar fashion as does normal BM (1, 34). In the guts of these mice many (transgenic-idiotype negative) IgA-secreting cells, of which the Cα chains must be derived from endogenous C-region genes, are found. Consequently, the intestinal IgA-containing cells are the result of differentiation and isotype switching from B cells expressing endogenous IgM. Given the notion that most endogenous IgM-bearing cells in B6-Sp6 mice are B-1 cells, we concluded that most IgA-containing cells in the gut lamina propria of these mice also belong to the B-1-cell lineage.

Transplantation Experiments with Fetal B-1 Cell Progenitors

Direct support for the observation that B-1 cells may differentiate to IgA-secreting cells comes from elegant studies by Kearney and co-workers (35). In their experiments, fetal omentum was transplanted under the kidney capsule of SCID mice (35). The only B cells that develop after transplantation from the sIg-negative progenitor cells present in the omentum are B-1 cells (both B-1a and B-1b cells); B-2 cells were never observed in these recipients. Immunohistological staining with IgA allotype-specific antibodies clearly demonstrated the presence of fetal omentum (and thus B-1 cell)-derived IgA in the gut lamina propria, as well as the presence of IgA in the serum. T cells did not develop from this tissue. Apparently, and in line with the aforementioned transfer experiments by Taki et al. (7), even in the absence of T-cell help B-1 cells can differentiate to at least some IgA-producing cells.

In Vitro Studies with B-1 Cell Lines

Isotype switching of B-1 cells to IgA has been demonstrated in vitro at the clonal level using the B-1 lymphoma cell line CH12.LX as a model system. This cell line preferentially and readily switches to IgA-producing cells in cultures containing cytokines like IL-4 (36).

TOWARD THE QUANTIFICATION OF THE CONTRIBUTION OF B-1 CELLS IN MUCOSAL IgA PRODUCTION

To unravel the question to what extent various B-cell subsets are involved in the generation of IgA plasma cells in the gut, it is essential to address this issue in terms of absolute numbers of IgA-producing cells rather than estimating the frequencies of donor- and host-derived IgA cells. A simple method to determine numbers of actively antibody-secreting cells is the ELISA-plaque assay. Using this technique we estimated that in the lamina propria of the small intestine from BALB/c mice approximately 50 million IgA-secreting cells are located. This number is in the same order of magnitude as the 15 million antibody-secreting cells in the gut of another mouse strain (C$_3$H) using a reversed plaque assay, as described previously (18). In one experiment we transferred approximately 1 million B cells (BALB/c) from various sources into untreated C.B17 SCID mice. In these mice T-cell help for isotype switching and differentiation was provided by injection of 5 million thymocytes (from C.B17 mice) 1 month before transfer of B cells. B-cell donor-derived IgA-secreting cells were determined by allotype-specific ELISA-plaque assays. As shown in Table 1, transfer of approximately 1 million PerC B cells (largely B-1 cells) results in the generation of 40 million IgA-secreting cells in the gut, 6 months after transfer. After transfer of PerC, B cells responsible for this long-term IgA production are probably B-1 cells: only cells with the B-1-cell phenotype could be detected in such reconstituted SCID mice (both in spleen and in the peritoneal cavity) (15, 19). Furthermore, in a distinct set of experiments we observed recently that transfer of ~1 million sorted B-1 cells (IgM^{br}IgD^{du} from PerC) gave rise to approximately 6–12 million B-1 donor allotype IgA-producing cells in the gut lamina propria of the SCID recipient mouse, 6 months after injection ($n = 3$). Apparently, the B-1 cells proliferate extensively in the SCID recipients on their way to become IgA-secreting cells.

This long-term repopulation of IgA cells in the gut is seen not only in the case of transfer of PerC, but also

TABLE 1

Number of IgA-Secreting Cells in the Small Intestine of Reconstituted SCID Mice

Source of B cells[a]	No. mice tested	Number ($\times 10^6$) of donor-derived IgA[a]-secreting cells[b]
Peritoneal cavity	2	40 ± 24
Spleen	5	51 ± 22
Peyer's patches	2	40 ± 24
Lymph nodes	2	40 ± 8
PBS control	4	0
BALB/c mice[c]	4	46 ± 19
C.B17 mice	4	0

[a] All C.B17-SCID recipient mice received 5×10^6 C.B17-derived thymocytes 1 month before injection of 1×10^6 living B cells derived from BALB/c mice. Gut lamina propria cells of the reconstituted mice were tested for donor-derived IgA-secreting cells 6 to 8 months after injection of B cells. The ELISA-plaque assay was performed with HY16 (anti-IgA^a) as catching antibody and developed with GAM-IgA-alkaline phosphate conjugate.

[b] Numbers represent the mean ± SEM of individually tested recipient mice.

[c] For comparison the numbers of control BALB/c and C.B17 mice are given.

when B cells from other locations (LN, spleen, and PP) are injected. Although it is well known that (conventional) B cells from PP are an important source of IgA-secreting cells in the gut, the identity of the B cells in these inocula responsible for the long-term reconstitution of IgA-secreting cells in the SCID recipient mice remains to be established. Except for spleen, LN and PP are almost exclusively composed of B-2 cells. These B cells are not able to maintain themselves (as $IgM^{dull}IgD^{bright}$ cells) by self-replenishment (11, 37), although they are long-lived (37–39), in particular after transfer into SCID mice (40). However, the increased numbers of IgA-secreting cells in the gut long after transfer of the various B-2 cell inocula are hard to reconcile with this longevity of these cells in the absence of self-replenishment. A likely possibility is therefore that B-2 cells from spleen and LN differentiate (possibly via the PP microenvironment) and with T-cell help to IgA-memory cells. Under influence of the antigens in the gut lumen and in the absence of any other IgA precursor cell population, these memory cells are maintained in the mouse and serve as a continuous source of mucosal IgA plasma cell precursors.

In the transfer studies presented before, donor-derived IgA could be detected using monoclonal antibodies [e.g., HY-16 (41)] that bind to IgA encoded by Igh-Cα^a genes (Igh-2a), but not to the IgA encoded by Igh-Cα^b genes (Igh-2b) of the host. To our best knowledge, no monoclonal antibody recognizing Igh-2b, but not Igh-2a, is available, allowing the positive and simple detection of both IgA allotypes at the same time. Such antibody would greatly facilitate studies concerning the origin and function of IgA in chimeric animals. For this reason we have developed recently a mAb, HIS-M2 (IgG_1 isotype), that binds to Igh-2b, but not to Igh-2a. This antibody is specific for IgA and does not react with other isotypes. The reactivity pattern of HIS-M2 and HY16 with several mouse strains is shown in Table 2. In Fig. 1, we show an example of the simultaneous detection of BM-derived and PerC-derived IgA containing cells in the gut lamina propria of a B lineage chimera.

DISCUSSION: THE PHYSIOLOGICAL CONTRIBUTION OF B-1 CELLS IN MUCOSAL IgA PRODUCTION

The data summarized in the previous sections provide evidence that, in principle, B-1 cells can switch *in vivo* toward IgA expression and subsequently differentiate to (mucosal) IgA plasma cells. However, although the observations also suggest that B-1 cells may contribute significantly in these IgA responses, the type of experiments performed so far does not allow us to draw definite conclusions on the relative contribution of B-1 cells and B-2 cells in the generation of IgA plasma cells in normal,

unmanipulated animals. Furthermore, the relative involvement of either B-1a and/or B-1b cells in this response is also not known.

The *in vivo* experiments used were generally carried out in animals in which B cells and plasma cells were absent either due to depletion by irradiation or anti-IgM treatment or due to a genetic defect (SCID). It could be that in these situations any B cell (B-1 or B-2) specific for intestinal antigens in the presence of T-cell help is driven to switch and differentiate to IgA plasma cell. Whether such a relative "empty" lamina propria thus results in a disturbed contribution of certain B-cell subpopulations to participate in the formation of IgA plasma cells is not known.

We also cannot exclude the possibility that once formed, switched B cells persist in the animal (at mucosal sites?) as $sIgA^+$ memory cells that continue to divide and give rise to IgA plasma cells. It is even possible that these IgA plasma cell precursors are seeded into the gut early during ontogeny, when the majority of B cells in the animal are B-1 cells. In this scenario, most of the IgA plasma cells in a normal unmanipulated animal would then be of B-1 cell origin. A recent experiment by Schroff *et al.* (42) may support the suggestion that (self-maintaining) memory cells might play a role in the long-term intestinal IgA plasma cell formation. Germfree mice, which characteristically have only very few IgA-secreting cells in the gut, were monoassociated with the murine commensal organism *Morganella morganii*. In these mice, anti-PC IgA-secreting cells are present in the lamina propria for more than 300 days postmonoassociation, which is long after cessation of the germinal center reaction (as the source of IgA plasma cell precursors) in the PP. This experiment indicates that memory type B cells (in this case B-2 cells)

TABLE 2

Reactivity Pattern of HIS-M2 and HY16 with IgA from Various Mouse Strains

Strain	Igh-C allotype	Reactivity with	
		HIS-M2	HY-16
BALB/c	a	−^a	+
B6C20	a	−	+
C57b1	b	+	−
C.B17	b	+	−
DBA/2J	c	−	−
AKR/5	d	+	−
CA21	e	+	−
CE/J	f	+	+
RIIIS/J	g	−	+

^a Reactivity was established both by immunohistological staining of tissue sections from gut and by ELISA after coating microtiter plates with serum from various mouse strains using monoclonal anti-IgA as catching antibody.

derived from the germinal centers in the PP are present for almost 1 year and generate IgA-secreting cells.

Long-term (in the order of months) repopulation studies assume that IgA cells in the gut are short-lived and thus need a continuous input from dividing precursor cells (either located inside or outside the gut). Tritium incorporation studies have shown that the vast majority of IgA-containing cells in the murine intestine are short-lived with a "half-life" of approximately 5 days (26); a minor fraction of these cells appears, however, to be replaced at a much slower rate and they were considered to be long-lived. It should be noted here that these kinetic studies were performed in very young animals (3 weeks old), when the intestinal IgA plasma cell population is being built up. Whether in adult mice the majority of IgA-secreting cells also have a short life-span might be expected, but is not exactly known. We speculate here that it is even possible that these long-lived and short-lived IgA-secreting cells are derived from distinct precursor populations (B-1 versus B-2 cells?). Furthermore, if and how the life-span of B cells and their descendants is affected in the type of transfer experiments described before also remains to be seen. Sprent et al. have already demonstrated that lymph node B cells injected into SCID mice appear to have a prolonged life-span (40). Thus, we cannot rule out formally the possibility that in the transfer experiments, the IgA-containing cells in the gut lamina propria are extremely long-lived and do not need to be supplied by their precursor cells.

Future experiments to address the issue on the origin of IgA plasma cells should therefore aim at studying the role of allotypically marked B-1 cells in a relatively unaffected environment, i.e., in the presence of normal numbers of B-2 cells and an intestinal lamina propria "filled" with IgA-secreting cells. To achieve this situation, the B-1-cell population of the recipient mouse should be replaced by allotypically marked B-1 cells, leaving the B-2-cell population unaffected. That these experiments are possible was demonstrated by Pecquet et al. (8) in immunodeficient mice. As mentioned before, they showed that unirradiated CNA/N Xid mice (which lack B-1 cells due to a genetic defect) injected with PerC are able to mount a mucosal IgA response specific for *Salmonella* and PC. This response is probably associated with the presence of B-1 cells. Alternatively, lineage chimeras might be constructed, e.g., by (repeated) peritoneal washings (43) to remove the majority of the B-1-cell population or by selective removal of B-1 cells using certain drugs or agents. We have shown recently that treatment of mice with a few ip injections of a low dose of the cytostatic drug adryamicin selectively depletes for long periods of time the peritoneal B-1-cell population (F. G. M. Kroese, T. de Boer, and C. Smit, unpublished). Transfer of PerC cells or sorted B-1 cells (B-1a and/or B-1b) from Igh-C allotypic congenic mice into these mice may result in B lineage chimeras in which the B-2-cell population remains intact.

In addition, injected B-1 cells block the development of the host's B-1 cells by virtue of feedback inhibition (25). These types of experiments may give some clues to whether under these probably more physiological situations, B-1 cells also (continue to) play a role as a source for IgA plasma cells.

Together, all data strongly indicate that both B-1 and B-2 cells are involved in the generation of mucosal IgA in normal animals, albeit that their relative contributions are not exactly known. Final proof of the contribution of B-1 cells in the mucosal IgA response may await the discovery of lineage-specific genes, which are expressed from the B-1-cell stage (in the peritoneal cavity) throughout the plasma cell stage (in the gut). The differentiation and migration pathway of peritoneal sIgM-positive B-1 cells to the IgA-secreting plasma cells in the gut lamina propria is also not known. However, flow cytometry analysis did not reveal the presence of typical B-1 cells in the gut lamina propria (F. G. M. Kroese and A. M. Stall, unpublished observations), suggesting that isotype switching possibly takes place elsewhere. It is possible that this may occur to some extent in the peritoneal cavity, since some peritoneal B cells express IgA (14), albeit that the vast majority of these cells do not express IgM, IgD, or CD5, but are B220 positive. PCR analysis has now confirmed the presence of IgA mRNA among PerC (F. G. M. Kroese, R. de Waard, P. Dammers, and N. A. Bos, unpublished). Another important question that needs to be addressed is whether B-1 cell derived IgA and B-2 cell derived IgA differ in repertoire and exert different functions. The observation that hybridomas derived from SCID mice repopulated with PerC produce IgA antibodies reactive with gut flora (19) may confirm the biased repertoire to microorganism of IgM antibodies secreted by B-1 cells. Possibly, B-1-derived IgA provide the animal with a first line of humoral defense mechanism to most commonly encountered antigens, while PP B-2 cell derived IgA plays a role in high-affinity responses to (pathogenic) bacteria, when the IgA produced by B-1 cells is not sufficient to combat the infection.

ACKNOWLEDGMENTS

REFERENCES

1. Kantor, A. B., and Herzenberg, L. A. (1993) *Annu. Rev. Immunol.* **11,** 501–538.

2. Mercolino, T. J., Arnold, L. W., Hawkins, L. A., and Haughton, G. (1988) *J. Exp. Med.* **168,** 687–698.

3. Hays
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3. Hayakawa, K., Hardy, R. R., Honda, M., Herzenberg, L. A., Steinberg, A. D., and Herzenberg, L. A. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2494–2498.

4. Förster, I., and Rajewsky, K. (1987) *Eur. J. Immunol.* **17**, 521–528.

5. Su, S. D., Ward, M. M., Apicella, M. A., and Ward, R. E. (1991) *J. Immunol.* **146**, 327–331.

6. Masmoudi, H., Mota-Santos, T., Huetz, F., Coutinho, A., and Cazenave, P. A. (1990) *Int. Immunol.* **2**, 515–520.

7. Taki, S., Schmitt, M., Tarlington, D., Förster, I., and Rajewsky, K. (1992) *Ann. N.Y. Acad. Sci.* **651**, 328–335.

8. Pecquet, S. S., Ehrat, C., and Ernst, P. B. (1992) *Infect. Immun.* **60**, 503–509.

9. Carmack, C. E., Shinton, S. A., Hayakawa, K., and Hardy, R. R. (1990) *J. Exp. Med.* **172**, 371–374.

10. Gu, H., Förster, I., and Rajewsky, K. (1990) *EMBO J.* **9**, 2133–2140.

11. Herzenberg, L. A., Stall, A. M., Lalor, P. A., Sidman, C., Moore, W. A., Parks, D. R., and Herzenberg, L. A. (1986) *Immunol. Rev.* **93**, 81–102.

12. Hardy, R. R., Carmack, C. E., Li, Y. S., and Hayakawa, K. (1994) *Immunol. Rev.* **137**, 91–118.

13. Herzenberg, L. A., Kantor, A. B., and Herzenberg, L. A. (1992) *Ann. N.Y. Acad. Sci.* **651**, 1–9.

14. Kroese, F. G. M., Butcher, E. C., Stall, A. M., Lalor, P. A., Adams, S., and Herzenberg, L. A. (1989) *Int. Immunol.* **1**, 75–84.

15. Kroese, F. G. M., Ammerlaan, W. A. M., and Deenen, G. J. (1992) *Ann. N.Y. Acad. Sci.* **651**, 44–58.

16. Kroese, F. G. M., Kantor, A. B., and Herzenberg, L. A. (1993) *In* Handbook of Mucosal Immunology (Ogra, P. L., Mestecky, J., Lamm, M. E., Strober, W., McGhee, J. R., and Bienenstock, J., Eds.), pp. 217–224, Academic Press, Orlando, FL.

17. Kroese, F. G. M., Ammerlaan, W. A., and Kantor, A. B. (1993) *Int. Immunol.* **5**, 1317–1327.

18. Van der Heijden, P. J., Stok, W., and Bianchi, A. T. J. (1987) *Immunology* **62**, 551–555.

19. Bos, N. A., Bun, J. C. A. M., Bijma, H., Cebra, E. R., Cebra, J. J., Deenen, G. J., van der Cammen, M. J. F., and Kroese, F. G. M. (1994) *in* In Vivo Immunology: Regulatory Processes during Lymphopoiesis and Immunopoiesis (Heinen, E., Ed.), pp. 265–269, Plenum, New York.

20. Craig, S. W., and Cebra, J. J. (1971) *J. Exp. Med.* **134**, 188–200.

21. Phillips-Quagliata, J. M., and Lamm, M. E. (1988) *In* Migration and Homing of Lymphoid Cells (Husband, A. J., Ed.), Vol. II, pp. 53–75, CRC Press, Boca Raton, FL.

22. Tseng, J. (1988) *In* Migration and Homing of Lymphoid Cells (Husband, A. J., Ed.), Vol. II, pp. 77–97, CRC Press, Boca Raton, FL.

23. Hayakawa, K., Hardy, R. R., Stall, A. M., Herzenberg, L. A., and Herzenberg, L. A. (1986) *Eur. J. Immunol.* **16**, 1313–1316.

24. Hayakawa, K., Hardy, R. R., Herzenberg, L. A., and Herzenberg, L. A. (1985) *J. Exp. Med.* **161**, 1554–1568.

25. Lalor, P. A., Herzenberg, L. A., Adams, S., and Stall, A. M. (1989) *Eur. J. Immunol.* **19**, 507–513.

26. Mattioli, C., and Tomasi, T. (1973) *J. Exp. Med.* **138**, 452–460.

27. Van der Waaij, L. A., Mesander, G., Limburg, P. C., and Van der Waaij, D. (1994) *Cytometry* **16**, 270–279.

28. Cebra, J. J., Bos, N. A., Cebra, E. R., Cuff, C. F., Deenen, G. J., Kroese, F. G. M., and Shroff, K. E. (1994) *in* In Vivo Immunology: Regulatory Processes during Lymphopoiesis and Immunopoiesis (Heinen, E., Ed.), pp. 255–259, Plenum, New York.

29. Sidman, C. L., Shultz, L. D., Hardy, R. R., Hayakawa, K., and Herzenberg, L. A. (1986) *Science* **232**, 1423–1425.

30. Rusconi, S., and Köhler, G. (1985) *Nature* **314**, 330–334.

31. Forni, L. (1990) *Eur. J. Immunol.* **20**, 983–989.

32. Grandien, A., Coutinho, A., and Andersson, J. (1990) *Eur. J. Immunol.* **20**, 991–998.

33. Rabin, E., Yingzi, C., Imanishi-Kari, T., and Wortis, H. H. (1992) *Eur. J. Immunol.* **22**, 2237–2242.

34. Kantor, A. B., Stall, A. M., Adams, S., Herzenberg, L. A., and Herzenberg, L. A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3320–3324.

35. Solvason, N., Lehuen, A., and Kearney, J. F. (1991) *Int. Immunol.* **3**, 543–550.

36. Mercolino, T. J., Arnold, L. W., and Haughton, G. (1986) *J. Exp. Med.* **163**, 155–165.

37. Kantor, A. B., Stall, A. M., Adams, S., Watanabe, K., and Herzenberg, L. A. (1995) *Int. Immunol.* **7**, 55–68.

38. Förster, I., and Rajewsky, K. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4781.

39. Deenen, G. J., and Kroese, F. G. M. (1993) *Eur. J. Immunol.* **23**, 12–16.

40. Sprent, J., Schaefer, M., Hurd, N., Surh, C. D., and Ron, Y. (1991) *J. Exp. Med.* **174**, 717–728.

41. Butcher, E. C., Rouse, R. V., Coffman, R. L., Nottenburg, C. N., Hardy, R. R., and Weissman, I. L. (1982) *J. Immunol.* **129**, 2698–2707.

42. Schroff, K. E., Meslin, K., and Cebra, J. J. (1995) Submitted for publication.

43. Marcos, M. A. R., Gaspar, M. L., Malenchere, E., and Coutinho, A. (1994) *Eur. J. Immunol.* **24**, 1033–1040.