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The Role of B-1 Cells in Mucosal Immune Responses

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I. INTRODUCTION

The intestinal lamina propria of the gut contains numerous plasma cells that characteristically secrete IgA. This IgA is transported across the epithelium into the gut lumen to prevent invasion of microorganisms. The high level of IgA in mucosal secretions testifies to its importance as part of the first line of defense of an animal. In fact, approximately 15 million IgA-secreting cells are found in the intestine of the mouse, which is 10 times more than the total number of IgM-secreting cells in lymphoid tissues (Van der Heijden *et al.*, 1987). Given the estimated half-life of 5 days for the vast majority of intestinal IgA plasma cells (Mattioli and Tomasi, 1973), many IgA plasma cells must develop daily from B cells to guarantee a continuous supply of cells. According to the prevailing paradigm, intestinal IgA plasma cells are derived from conventional B cells located mainly in the Peyer's patches of the small intestine (for review, see Phillips-Quagliata and Lamm, 1988; Tseng, 1988).

In this chapter, we review analyses of irradiated and nonirradiated B-cell lineage chimeras and μ,κ transgenic mice from our laboratories that indicate that many intestinal IgA plasma cells arise from Ly-1 B cells that predominate in the peritoneal cavity (Ly-1 B cells are now designated B-1 cells; Kantor *et al.*, 1991). Data from several other laboratories that support this finding are also discussed. Finally, the mucosal IgA plasma cells are considered in the context of the layered immune system (Herzenberg and Herzenberg, 1989).

II. CURRENT VIEW ON THE ORIGIN OF INTESTINAL IgA PLASMA CELLS

Peyer's patches (and appendix) are well known to contain enriched numbers of precursor cells for intestinal IgA plasma cells (Craig and Cebra, 1971,1975; Rudzik *et al.*, 1975; Tseng, 1981,1984). This conclusion was reached mainly after short-term (up to 2 weeks) transfer experiments with various cell populations to X-irradiated animals, and was initiated in the early 1970s by the classic rabbit experiments by Craig and Cebra (1971,1975). Collectively, these (and other) repopulation studies led to the following working model (for reviews, see Phillips-Quagliata and Lamm, 1988; Tseng, 1988). Antigen probably enters the Peyer's patches through specialized epithelial cells (M cells) with short microvilli and an intracellular vesicular transport system, located in the epithelium covering each follicle (Owen, 1977). In the Peyer's patches, antigen is processed by macrophages and presented to T helper cells, leading to the activation of B cells. Committed B cells leave the Peyer's patches, migrate to the mesenteric lymph nodes, and enter the blood circulation by way of the thoracic duct. These cells may expand and differentiate further in the spleen and, after some time, migrate into the gut lamina propria, where they mature to IgA-producing plasma cells. This differentiation process from Peyer's patch precursor B cell to mature IgA-secreting plasma cell in the intestinal lamina propria takes approximately 1 week.

Analysis of the phenotype of the IgA precursor cells located in the murine Peyer's patch shows that the majority of them express sIgM, sIgD, and complement (C3) receptor (Tseng, 1984). Tissue-section staining indicates that these cells are located in the lymphocyte corona of the lymphoid follicle (Butcher *et al.*, 1982). A second but minor population of IgA plasma cell precursors consists of surface/cytoplasmic IgA$^+$ blastoid cells that are likely to be derived from the IgM$^+$IgD$^+$ cells, and are found in the Peyer's patches and, in higher numbers, in the mesenteric lymph nodes and thoracic duct lymph (Guy-Grand *et al.*, 1974; McWilliams *et al.*, 1975; Pierce and Gowans, 1975; Roux *et al.*, 1981). In repopulation studies, this last population of IgA precursors homes immediately after transfer to the gut lamina propria (<24 hr). In Peyer's patches, sIgA$^+$ cells are located almost exclusively in the germinal centers of the lymphoid follicles (Butcher *et al.*, 1982). In the germinal centers, antigen-triggered B cells expand, undergo isotype switching, introduce somatic mutations in their V_H genes, and differentiate into memory cells (for review, see Kroese *et al.*, 1990). Therefore, the (conventional) IgA plasma cell precursors in the Peyer's patches may acquire somatic mutations in their V_H genes, resulting in high affinity antibodies with a narrow specificity.

III. B-1 CELL LINEAGE

B-1 cells (Ly-1 B cells) constitute a small but functionally very important subset of murine B cells that produces much serum immunoglobulin, including autoreactive and antibacterial antibodies. B-1 cells are distinguished from conventional B cells by phenotype, development, anatomical localization,

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and function (for reviews, see Hardy and Hayakawa, 1986; Herzenberg *et al.*, 1986; Hayakawa and Hardy, 1988; Kipps, 1989; Kantor and Herzenberg, 1993). B-1 cells are virtually absent from lymph nodes and Peyer's patches and are present at low frequency in spleen; however, they constitute a major fraction of the B cells in the peritoneal and pleural cavities. B-1 cells express high levels of sIgM but low levels of sIgD and B220 (RA3-6b2; Coffman and Weissman, 1981). Figure 1 shows the predominance of B-1 cells in the BALB/c peritoneal cavity and the absence of these cells in the Peyer's patches. Conventional B cells, which are dull for IgM and bright for IgD, predominate in Peyer's patches and other secondary lymphoid organs. In the peritoneal and pleural cavities, B-1 cells also express CD11b (Mac-1). B-1 cells can be divided into two independently self-replenishing populations: B-1a cells, which express detectable levels of CD5, and B-1b (formerly called "sister" cells), which do not. Both populations are present in the peritoneal and pleural cavities.

Extensive adoptive transfer studies have shown that B-1 cells and conventional B cells have different developmental pathways and have distinct progenitor cells that develop independently of each other. In essence, B-1 cells arise early during ontogeny from progenitor cells located in the fetal omentum (Solvason *et al.*, 1991) and fetal liver (Solvason *et al.*, 1991; Hardy and Hayakawa, 1992; Kantor *et al.*, 1992). After weaning, B-1 cells maintain themselves by self-replenishment and are independent of the bone marrow. Conventional B cells, in contrast, arise later in ontogeny and are replenished by *de novo* production from adult bone marrow.

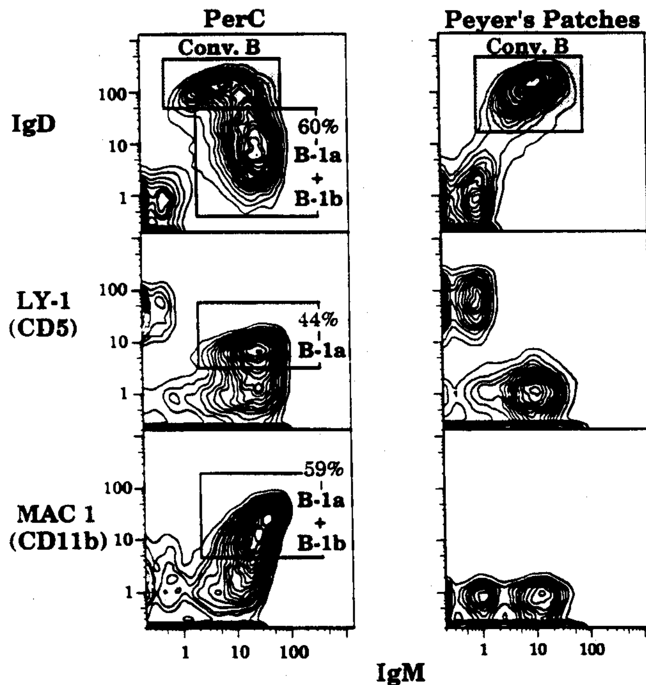

Figure 1 Comparison of Peyer's patch (*right*) and peritoneal cavity (*left*) B cells. Representative plots are shown for untreated Balb/c mice. The FACS phenotype of conventional B, B-1a, and B-1b cells are indicated. Reagents: fluorescein conjugated anti-IgM (DS1); allophycocyanin-conjugated anti-CD5 (53-7); and anti CD11b (M1/70); and biotin-conjugated anti-IgD (AMS9.1) followed by Texas Red–Avidin. The percentages of cells within the boxed populations are given per total number of live lymphocytes after gating on forward and side scatter and propidium iodide. All the plots presented have 5% probability contours.

Although their overall numbers are low (<5% of the peripheral B cells), B-1 cells produce much of the serum Ig, including half the IgM and IgA in radiation chimeras (Kroese *et al.*, 1989); however, they express a limited repertoire (Förster *et al.*, 1988a; Pennel *et al.*, 1988; Tarlinton *et al.*, 1988) including near exclusive use of V_H11 and V_H12, which are specific for phosphatidylcholine (PtC; Hardy *et al.*, 1989; Pennell *et al.*, 1989; Carmack *et al.*, 1990). B-1 cells produce autoantibodies to Fcγ (rheumatoid factor; Casali *et al.*, 1987; Hardy *et al.*, 1987; Burastero *et al.*, 1988) and thymocytes (Hayakawa *et al.*, 1990), and antibodies that react with microorganismal coat antigen such as α1-3 dextran (Förster and Rajewsky, 1987), PtC, and undefined determinants on *Escherichia coli* (Pennell *et al.*, 1985; Mercolino *et al.*, 1986). These B-1 cell antibodies tend to have low affinity and broad specificity (Lalor and Morahan, 1990). In contrast, B-1 cells respond poorly to commonly used exogenous antigen such as sheep erythrocytes and TNP-KLH; Hayakawa *et al.*, 1984). The majority of B-1 cell antihapten antibodies does not show the high affinity binding or fine specificity of conventional B cells (Lalor and Morahan, 1990). Thus, an apparent dichotomy exists in the expressed repertoire of conventional and B-1 cells. Little is known about the subdivision between B-1a and B-1b cells.

Although mature B-1 cells are only a minor population of B cells, their functional properties suggest that these cells play a crucial role in the first line of protection of the animal against common microorganisms, including those that are ubiquitous in the gut. This hypothesis raises the question of whether B-1 cells could be involved in mucosal IgA immune responses.

IV. PERITONEAL RESERVOIR OF PRECURSORS FOR GUT IgA PLASMA CELLS

We examined the possible role of B-1 cells in the mucosal immune response by using stable long-term B lineage chimeras (Kroese *et al.*, 1989,1992). These chimeras exploit the self-replenishing properties of B-1 cells and their poor repopulation by adult bone marrow, especially in the presence of mature B-1 cells. Lethally irradiated mice are reconstituted with syngeneic bone marrow (BM) and peritoneal washout cells (PerC) from immunoglobulin (Ig) allotype (Igh-C) congeneic donors (Figure 2). In these mice, B cells, plasma cells, and serum immunoglobulins derived from either donor population can be distinguished on the basis of the allotype they express.

Multiparameter flow cytometry (fluorescence-activated cell sorting; FACS) analysis of these chimeras (>2 months after transfer) demonstrates that essentially all B cells in Peyer's patch (Figure 3), spleen, and lymph node are conventional B cells derived from the bone marrow donor (b allo-

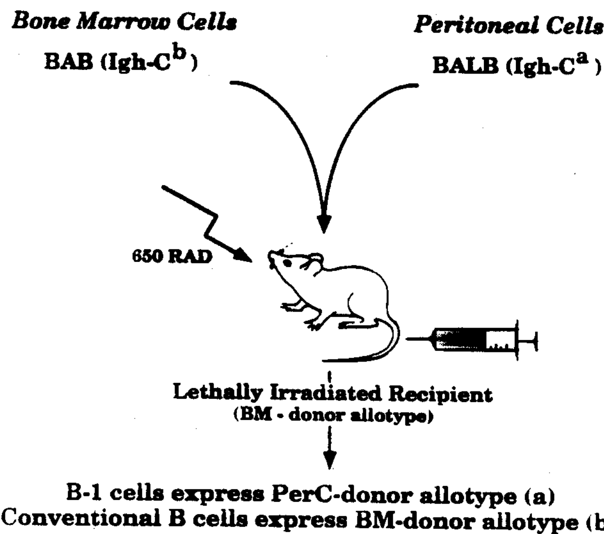

Figure 2 Preparation of radiation chimeras. Recipients [here, b allotype BAB/25 (Igh-C^{b})] mice are X-irradiated and reconstituted with peritoneal cells from an a allotype Balbc/Hz and syngeneic (BAB) bone marrow.

type). B cells derived from the PerC donor (a allotype) belong exclusively to the B-1 cell lineage. PerC-derived B-1a and B-1b cells are abundant again in the peritoneal (Figure 3) and pleural cavities of the recipient mouse and are found only at low frequencies in peripheral lymphoid tissues.

Immunohistological analysis of lymphoid organs confirms the FACS data. Specifically, in Peyer's patches only rare surface IgM-, IgD-, or IgA-positive cells of PerC donor allotype are detected in the lymphocyte corona of the lymphoid follicle. However, despite the low overall numbers of PerC-derived B cells in these PerC/BM chimeras, approximately 40% of the IgA plasma cells in the gut lamina propria and half the IgM plasma cells in the spleen are derived from PerC donor cells, even up to 1 year after transfer. Consistently, half the IgA and IgM in the serum are of the PerC donor allotype (Kroese *et al.*, 1989).

Similar data are obtained with nonirradiation B lineage chimeras (Kroese *et al.*, 1989). For example, neonatal chimeras were prepared by transfer of PerC into Igh-C congeneic neonatal homozygotes treated from birth with anti-IgM allotype-specific antibodies (Lalor *et al.*, 1989b). This procedure depletes host B cells but does not affect the injected donor B cells. After stopping the antibody treatment, development of host B-1 cells is suppressed permanently, although host conventional B cells return to normal levels. In these chimeras, B-1 cells are derived exclusively from the PerC donor, and many IgA plasma cells in the gut express the PerC donor Ig allotype (Kroese *et al.*, 1989).

The production of IgA plasma cells from B-1 cells in the nonirradiated chimeras indicates that the repopulation of the lamina propria by PerC-derived cells in the irradiation chimeras is not likely to be the result of nonspecific and immediate homing of cells. Such an aberrant migration pattern could, for example, be induced by the X-irradiation procedure, which may lead to short-term inflammation of the intestine. Immediate nonspecific homing is also unlikely, since PerC-derived IgA plasma cells appear in the intestinal lamina propria of the recipients at least 1–2 weeks after transfer (Kroese *et al.*, 1989).

These findings challenge the prevailing view that the vast majority of IgA plasma cells in the intestinal lamina propria originates from (conventional) B cells located in the Peyer's patches. Instead, the data demonstrate that the murine peritoneal cavity may serve as a huge reservoir of B cells that are capable of differentiating into IgA-secreting plasma cells. Further, given the observation that, even up to 1 year after transfer, PerC-derived IgA plasma cells are seen in the intestinal lamina propria, these PerC-derived plasma cells must be either extremely long-lived (which is rather unlikely; Mattioli and Tomasi, 1973) or derived from self-replenishing precursor cells. Since FACS analysis shows that all PerC-derived sIgM^{+} cells in these long-term stable chimeras are self-replenishing B-1 cells, the data strongly indicate that B-1 cells present in the peritoneal cavity are responsible for high numbers of intestinal IgA plasma cells.

V. IgA PLASMA CELLS IN μ,κ TRANSGENIC MICE BELONG TO THE B-1 CELL LINEAGE

In a second distinct approach, we examined the lineage origin of intestinal IgA plasma cells in IgM transgenic mice. The introduction of a functionally rearranged immunoglobulin μ heavy-chain transgene interferes with the normal development of the B-cell pool and antibody repertoire (Herzenberg *et al.*, 1987; Herzenberg and Stall, 1989; Müller *et al.*, 1989; Forni, 1990; Grandien *et al.*, 1990; Iacomini *et al.*, 1991). These transgenic mice have severely reduced numbers of B cells. As expected by the principle of allelic exclusion, the rearranged transgene inhibits further endogenous immunoglobulin heavy-chain gene rearrangements and subsequent expression. This inhibition, however, does not occur in all cells. Some B cells express endogenous IgM molecules, often concomitant with the transgenic IgM. FACS analysis and transfer studies with the transgenic mouse lines M54 and M95 have shown that the transgene selectively affects the two B cell lineages: only conventional B cells are depleted in these mice and expression of endogenous immunoglobulins is restricted totally to the B-1 cell lineage (Herzenberg *et al.*, 1987; Herzenberg and Stall, 1989). Endogenous immunoglobulin and transgenic IgM can be distinguished from each other by Igh-C allotype. Despite the loss of endogenous conventional B cells, these mice have almost normal levels of endogenous serum immunoglobulin. Most significantly, serum IgA is near normal in these animals (Grandien *et al.*, 1990).

Similar to the findings with the M54 mice, we have shown that the majority of B cells in the peripheral lymphoid organs of B6-SP6 (μ,κ transgenic) mice expresses only transgenic IgM, whereas many peritoneal B cells express endogenous IgM (either alone or in combination with transgenic IgM; Kroese *et al.*, 1992). These endogenous IgM^{+} peritoneal B cells nearly all belong to the B-1 cell lineage as determined by FACS phenotype. Furthermore, transgenic BM poorly

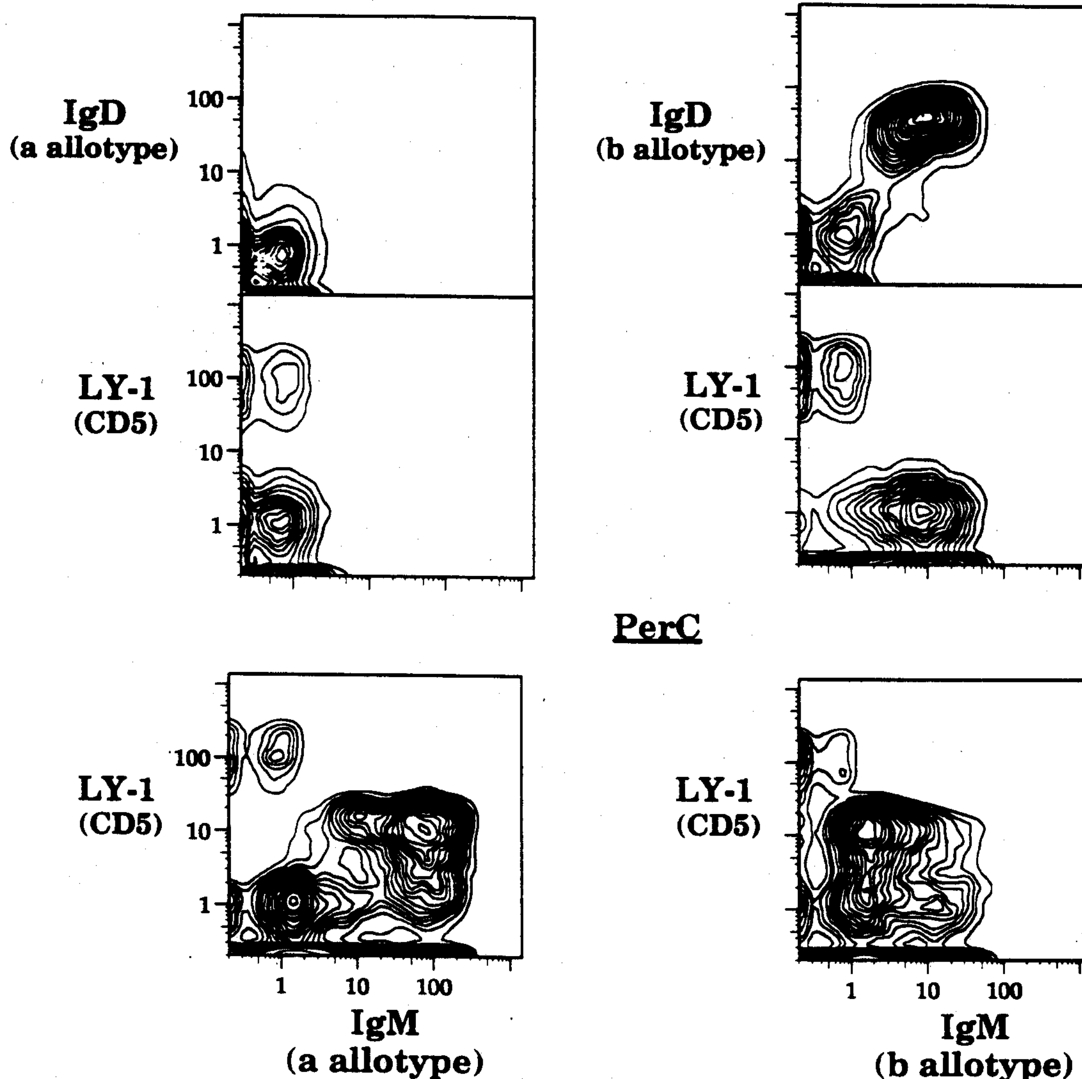

Figure 3 FACS analysis of radiation chimeras. BAB recipients of Balb/c PerC and BAB bone marrow were analyzed 10 wk after transfer. PerC-derived (a allotype) B-1a and B-1b cells are found in the recipient peritoneal cavity (PerC) but not Peyer's patches. Bone marrow-derived (b allotype) conventional B cells predominate in the Peyer's patches and other secondary lymphoid organs. Allotype specific reagents: fluorescein conjugated anti-Igh-6a (IgM of the a allotype, DS1); fluorescein conjugated anti-Igh-6b (IgM of the b allotype, AF6-78); biotin-conjugated anti-Ig5a (IgD of the a allotype, AMS9.1); and biotin-conjugated anti-Ig5b (IgD of the b allotype, AF6-122) (b allotype Mab).

reconstitutes endogenous IgM$^+$ B cells, just as adult BM from normal mice poorly reconstitutes B-1 cells. Most significantly, IgA plasma cells are found in the intestinal lamina propria of these B6-Sp6 mice and approximately 25% of them also contain transgenic IgM in their cytoplasm. Since the constant part of this IgA can be derived only from endogenous heavy-chain genes in these mice and the majority of IgA positive cells do not express the transgenic idiotype, these IgA plasma cells must be the result of an isotype switch from an endogenous IgM-expressing cell. Endogenous IgM is expressed almost exclusively by B-1 cells in these transgenics, suggesting that essentially all IgA plasma cells in the gut lamina propria of these mice belong to the B-1 cell lineage.

VI. ROLE FOR B-1 CELLS IN MUCOSAL IMMUNITY

The two different sets of experiments described earlier (i.e., B lineage chimeras and μ,κ transgenic mice) strongly indicate that B-1 cells generate many IgA plasma cells in the lamina propria. Several other lines of evidence also support

our notion that B-1 cells contribute significantly to the gut IgA response. (1) Elegant experiments by Solvason *et al.* (1991) have shown that grafts of fetal omentum into adult severe combined immunodeficiency (SCID) mice reconstitute B-1 cells (both B-1a and B-1b) but not conventional B cells. These grafts also lead to the appearance of IgA plasma cells in the gut and IgM plasma cells in the spleen. (2) Several B-1 cell lymphomas (CH12.LX, CH27, and I.29) preferentially switch *in vitro* from IgM expression to IgA expression (Stavnezer *et al.*, 1985; Arnold *et al.*, 1988; Whitmore *et al.*, 1991). (3) Virtually all B cells in autoimmune viable motheaten mice are B-1 cells; the serum of these mice contains extremely high levels of IgM, IgG3, and IgA (Sidman *et al.*, 1986). (4) CBA/N Xid mice almost completely lack B-1 cells (Herzenberg *et al.*, 1986) and have a defective immune response to certain antigens such as phosphorylcholine and *Salmonella typhimurium* (Mond *et al.*, 1977; O'Brien *et al.*, 1979). However, CBA/N Xid mice populated with B-1 cells from the peritoneum of responsive CBA/CA mice have strong serum and mucosal IgA responses after oral immunization with *S. typhimurium* (Pecquet *et al.*, 1992).

Collectively, these findings are reconciled most easily with the hypothesis that B-1 cells play a significant role in the generation of IgA plasma cells in the intestinal lamina propria. This view is also consistent with the finding that intraperitoneal priming of animals is the most effective route to enhancing mucosal IgA immune responses (Pierce and Gowans, 1975; Pierce and Koster, 1980). In addition, this hypothesis also may explain how surgical removal of Peyer's patches and prolonged thoracic duct cannulation (to deplete Peyer's patch-derived cells) fail to result in a significant drop in numbers IgA plasma cells in the gut lamina propria (Mayrhofer and Fisher, 1979; Heatley *et al.*, 1981; Enders *et al.*, 1988).

Formal proof for a role of B-1 cells in the mucosal immune response requires further study. Rare sIgA$^+$ memory cells in the PerC may be responsible for the repopulation of gut IgA plasma cells after transfer (Kroese *et al.*, 1989). Transfer experiments with sorted B-cell populations into allotype congeneic hosts could clarify this point, as well as distinguish the relative roles of B-1a and B-1b cells.

VII. MUCOSAL IgA RESPONSES AND THE LAYERED IMMUNE SYSTEM

The developmental pattern of the B and T cell lineages can be considered in the context of an evolutionarily layered immune system (Herzenberg and Herzenberg, 1989; Herzenberg *et al.*, 1992; Kantor *et al.*, 1992). In this view, the lymphocyte lineages that develop at different overlapping times during ontogeny of mammals reflect the progressive emergence of layers of hematopoietic progenitor cells during evolution. B-1 cells and some $\gamma\delta$ T cells that arise earliest in ontogeny (Havran and Allison, 1988; Ito *et al.*, 1989) would represent the evolutionarily most primitive layer of the vertebrate immune system. This view is supported by evidence showing that some $\gamma\delta$ T cells (Tonegawa, 1989) and B-1 cells share many characteristics, such as predominant localization outside lymphoid tissues including the mucosal epithelium and restricted repertoires. Conventional B cells and $\alpha\beta$ T cells arise later in ontogeny and are considered more recent evolutionary developments.

Functional considerations indicate that B-1 cells and early $\gamma\delta$ T cells, by nature of their repertoire and anatomical location, may create a first line of defense against invading pathogens. B-1 cells tend to produce a restricted set of low-affinity broad-specificity germ-line antibodies that react with common antigens on ubiquitous microorganisms. In contrast, conventional B cells produce a larger more diverse set of antibodies capable of specific high-affinity interactions with particular pathogens.

The B-1 cell repertoire is established shortly after birth, in part by positive selection, at a time when the animal encounters common exogenous (microorganismal) antigens for the first time. Feedback inhibition from mature B-1 cells (Lalor *et al.*, 1989a,b) and decreased progenitor activity (Hayakawa *et al.*, 1985; Kantor *et al.*, 1992) restrict changes in the B-1 cell antibody repertoire, beginning near adolescence. Thus, the B-1 cell antibody repertoire provides an efficient and early first line of defense against many common bacteria that challenge the animal at birth and throughout life.

Conventional B cells produce a larger more diverse set of antibodies because of continuous generation from stem cells in bone marrow throughout life. These antibodies are capable of specific high-affinity interactions with particular pathogens. The conventional B cell repertoire thus is potentially able to combat any microorganisms, including those that are less common, that escape through the barrier created by the B-1 cell antibodies.

The mucosae are attacked continuously by numerous microorganisms and require a sophisticated immune system that benefits from both layers of the immune system. The importance of a mucosal immune system is indicated by the extremely high numbers of IgA plasma cells in the lamina propria and by the fact that the mucosal immune system is evolutionarily old (Phillips-Quagliata and Lamm, 1988). The layered immune system is clearly evident in the mucosae, as reflected by differentiation into IgA plasma cells from both B-1 cells located in the peritoneal cavity and conventional B cells located in the Peyer's patches. Antigen can be processed by macrophages and presented to T helper cells; the T cells, with antigen, activate the conventional Peyer's patch B cells. These cells leave the patches by way of the mesenteric lymph nodes and thoracic duct into the circulation to migrate back to the gut lamina propria for their final differentiation to IgA-producing plasma cells.

As we have shown in this chapter, some of the gut IgA plasma cells belong to the B-1 cell lineage. B-1 cells reside primarily within the peritoneal and pleural cavities and have not been demonstrated in sizable numbers in the intestinal lamina propria (F. G. M. Kroese, A. B. Kantor, and A. M. Stall, unpublished observations). Where and how these cells receive their initial antigenic stimulus, where these cells switch from IgM expression to IgA expression, and where these cells expand is virtually unknown. Cell kinetics data demonstrate that B-1 cells within the peritoneal cavity do

not divide extensively and turn over only approximately 1% per day (Förster *et al.*, 1988b; Deenen and Kroese, 1993). Thus, proliferation of antigen-triggered B-1 cells must take place outside the peritoneal cavity before they differentiate into IgA-secreting cells to give rise to the large number of IgA plasma cells required every day. Perhaps B-1 cells leave the peritoneal cavity through draining lymph nodes or the pleural cavity into the circulation (Kroese *et al.*, 1992).

The relative contribution of the two lineages to the IgA response in normal animals is not known, nor do we know whether both B-1a and B-1b cells could be involved in the production of IgA plasma cells. Importantly, whether the two types of IgA-producing plasma cells exert different functions and have different antibody repertoires remains to be determined. For example, the IgA plasma cells from the B-1 cell lineage might produce low-affinity multireactive IgA to commensal microorganisms and give the animal a baseline level of protection by these antibodies. This IgA might play a role in the maintenance of the gut flora. IgA produced by plasma cells derived from the Peyer's patch, in contrast, could be responsible for producing specific high-affinity antibodies with somatic mutations that are essential to combatting more pathogenic bacteria in the gut. An advanced and layered humoral immune system such as this would be highly practical because it would combine the effective components common to more primitive vertebrates, which are provided by B-1 cells, with the more recently evolved and sophisticated components that are provided by conventional B cells.

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