Commentary

Bar Mitzvah for B-1 Cells: How Will They Grow Up?

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The discovery of $CD5^+$ B cells by Hayakawa et al. (1) initiated a 13 year-long discussion about the origin and functional properties of these cells. The $CD5^+$ B cells (B-1a) and their phenotypic $CD5^-$ "twins" (B-1b cells) differ from conventional peripheral B cells (B-2) by anatomical location, developmental origin, surface marker expression, antibody repertoire and growth properties (2–4). B-1 cells form a dominant population of B lineage cells in the peritoneal cavity, but are rare in the spleen and lymph nodes of adult mice (3–6). The progenitors of B-1a cells are abundant in the fetal omentum and liver but in contrast to the progenitors of conventional B-2 cells, are missing in the bone marrow of adult mice (3, 7–9). The progenitors of B-1b cells are present in the fetal omentum and liver as well as in the bone marrow of adult mice (10, 11). However, a supply of B-1b cells from the bone marrow appears to be restricted by a negative feedback mechanism according to which the entrance of newly generated cells into the adult peripheral pool of B-1 cells is prevented by the presence of the mature B-1 population (12). In the absence of a continuous supply of bone marrow–derived B-1 cells, the size of the B-1 cell population is kept constant due to the self-renewal capacity of B-1 cells (3, 13). Due to their ability to produce large quantities of multireactive IgM, IgG3 and IgA, B-1 cells are considered carriers of "natural immunity" (7). It seems that the maintenance of B-1 cell population at a stable level might be necessary to control the level of antibody production by these cells. Thus the anti–IL-10 antibody-induced ablation of B-1 cells is accompanied by a drastic reduction of serum immunoglobulin titers (14). On the other hand, the expansion of autoreactive B-1 cells is associated with the development of autoimmunity in mice and man (15).

The unusual properties of B-1 cells suggest two major questions: (*a*) what signals control the proliferation and survival of B-1 cells? (*b*) what factors control antibody production by B-1 cells?

Under conditions which induce proliferation of B-2 cells, cross-linking of surface IgM on B-1 cells leads not to proliferation, but to death by apoptosis (16–18). These results suggest the existence of mechanisms selectively restricting the antigen receptor-mediated proliferation of B-1 cells. This idea is supported by the recent results of Bikah et al. (19) which demonstrate the negative role of CD5 in antigen receptor-mediated proliferation of B-1 cells. In these experiments the intraperitoneal injection of wild-type mice with high doses of anti-IgM induced the apoptosis of B-1 cells, while the same treatment of CD5-deficient mice resulted in the proliferation of B-1 cells. The modulatory role of CD5 in IgM-mediated activation of B-1 cells is not completely unexpected in view of the negative role of CD5 in control of T cell receptor mediated signaling in developing thymocytes (20). The CD5-mediated negative regulation of TCR signaling may occur due to the recruitment of tyrosine phosphatase SHP-1 by CD5 to the TCR complex (21). SHP-1 is also known as a negative regulator of B cell receptor (BCR)-mediated signaling in B-2 cells (22). Furthermore, the negative role of SHP-1 in B-1 cell activation has been supported by the observation of B-1 cell accumulation and appearance of circulating autoantibodies in *moth-eaten* mice carrying the mutated SHP-1 gene (23, 24). Given that the association of CD5 with BCR was found in human B lymphoma cells (25), it seems likely that one of the mechanisms of the negative regulation of BCR-mediated signaling could well lie in the recruitment of tyrosine phosphatase SHP-1 by CD5 to BCR complex. Since B-1b lymphocytes do not express CD5, the function of CD5 in these cells might be substituted by another negative regulator of B cell signaling such as CD22 protein (26, 27). Indeed an increased antigen receptor-mediated signaling in the absence of CD22 was found to be accompanied by an enlargement of the population of B-1 cells and the appearance of autoantibodies in the serum of mutant mice (28).

If antigen receptor-mediated signals do not induce growth of B-1 cells, alternative mechanisms must contribute to their growth. In this issue Karras et al. explains growth properties of B-1 cells as a consequence of constitutive activation of STAT3 protein (29). The term STAT stands for signal transducers and activators of transcription and defines a family of structurally related cytoplasmic proteins that are phosphorylated and rapidly translocated to the nucleus after receptor engagement (30). Therefore, the continuous presence of phosphorylated STAT3 protein in nuclear extracts of non-manipulated B-1 cells was taken by the authors as evidence of the constitutive activation of this protein. In lymphocytes the activation of STATs is traditionally associated with cytokine receptor signaling (31). STAT3 can also be activated by anti-IgM antibodies in B-2 cells (29). In B-1 cells, however, the pattern of STAT3 phosphorylation argues against STAT3 activation either by cytokine or by antigen stimulation. Therefore, it may well be that the constitutive STAT3 activation in B-1 cells may substitute for the antigen receptor-mediated proliferative signal. STAT3 expression has been associated with the neoplastic transformation of cells induced by Abl, Src and HTLV-1 viruses (33–35) and therefore might contribute to the growth factor–inde-

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/97/03/981/04 $2.00
Volume 185, Number 6, March 17, 1997 981–984

pendent proliferation of these cells. In a similar way, the presence of constitutively active STAT3 in B-1 cells may abrogate the dependence of proliferation of these cells on antigen receptor signaling.

An apparent independence of B-1 cell proliferation from antigen receptor-mediated signaling raises questions about the role of the antigen receptor in B-1 cell function. B-1 cells are virtually absent in the peritoneal cavity of mice deficient for CD19 or CD21 proteins (32, 33), both of which are known to amplify IgM-mediated signaling (34, 35). Furthermore, a negligibly low level of antigen receptor-mediated activation of B cells in Xid- or Btk-deficient mice as well as in PKCβ-deficient mice is associated with the virtual absence of B-1 cells in the peritoneal cavity of these mice (36, 37, 38).

A potential model to account for the antigen receptor–dependent maintenance of B-1 cells is that CD5 and/or CD22-associated SHP-1 keep the threshold of the antigen receptor–mediated activation at a level insufficient to induce the proliferation of B-1 cells, but sufficient to provide the signals that promote the survival of B-1 cells. The expression of CD19 and CD21 might be essential for the amplification of the survival signal, which is transmitted to the nucleus through Btk/PKCβ-containing signal transducing chain. The likelihood of the existence of such a signaling pathway for survival is supported by the physical interaction between Btk and PKC proteins (39) and the similarity of immunodeficiencies observed in Btk- and PKCβ-deficient mice (38). Notably, both Btk and PKC are known to be involved in the regulation of survival of B cells (40, 41). The situation where antigen receptor–mediated stimulation fails to induce proliferation of B-1 cells, but necessary to support survival of these cells, looks paradoxically static and contradicts the existing explanation for continuous B-1 cell renewal. It seems, however, that constitutive expression of STAT3 in B-1 cell could take these cells out of limbo and allow their untriggered growth.

Another important aspect of B-1 lymphocyte function is the control of antibody production by these cells. Antibody production by B-1 cells could be induced by some multivalent T cell–independent antigens, especially in connection with the production of autoreactive and anti-bacterial specificities (42–46). However, the exact origin of naturally occurring antigens which stimulate the differentiation of B-1 cells into antibody-producing cells remains elusive. In this issue Murakami et al. (47) demonstrates the critical role of the microbial microenvironment in the activation of antibody production by B-1 cells. Using transgenic mice carrying immunoglobulin genes encoding the anti–red blood cells autoantibody (anti-RBC Ab) they demonstrated a correlation between the microbial colonization of gut and the activation of autoreactive B-1 cells. Thus, the anti-RBC Ab transgenic mice which were kept under either germ-free or specific pathogen-free conditions did not develop the autoreactive antibody-induced hemolytic anemia, whereas the colonization of gut of mice with various microorganisms in a conventional breeding environment resulted in a rapid onset of the disease in ~50% of animals. The activation of autoreactive cells in the anti-RBC transgenic mice might be induced by bacteria-derived antigens that cross-react with surface anti-RBC–specific immunoglobulins. However, given the known ability of peritoneal B-1 cells to be activated by LPS in vivo (17, 48), it seems likely that the antibody production by B-1 cells in anti-RBC mice is induced polyclonally by bacteria-derived LPS and that the "autoreactive" quality of the receptor was relevant to the induction of disease but not to the induction of the antibody response itself.

The emerging picture of B-1 cell activation is far from completion. It remains to be understood how the cross-talk among various signaling pathways keeps the potentially dangerous B-1 cells under control at a place where these cells should be, in the peritoneum.

I thank J. Howard, N. Killeen, K-P. Lam, K. Rajewsky, M. Thomas, N. Wagner, and U. Weiss for the discussion and critical reading of the manuscript.

This work was supported by the Deutsche Forschungsgemeinschaft through SFB 243.

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Received for publication 16 January 1997.

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