B-1a B Cells that Link the Innate and Adaptive Immune Responses Are Lacking in the Absence of the Spleen

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Abstract

Splenectomized individuals are prone to overwhelming infections with encapsulated bacteria and splenectomy of mice increases susceptibility to streptococcal infections, yet the exact mechanism by which the spleen protects against such infections is unknown. Using congenitally asplenic mice as a model, we show that the spleen is essential for the generation of B-1a cells, a B cell population that cooperates with the innate immune system to control early bacterial and viral growth. Splenectomy of wild-type mice further demonstrated that the spleen is also important for the survival of B-1a cells. Transfer experiments demonstrate that lack of these cells, as opposed to the absence of the spleen per se, is associated with an inability to mount a rapid immune response against streptococcal polysaccharides. Thus, absence of the spleen and the associated increased susceptibility to streptococcal infections is correlated with lack of B-1a B cells. These findings reveal a hitherto unknown role of the spleen in generating and maintaining the B-1a B cell pool.

Key words: B-1a cells • asplenia • natural antibodies • Streptococcus pneumoniae • Hox11

Introduction

Absence of the spleen predisposes individuals to a risk of overwhelming infection, most often due to the encapsulated bacterium Streptococcus pneumoniae (1). Various hypotheses have been proposed to account for this increased infection risk in asplenic individuals, including lack of opsonins or marginal zone (MZ)* macrophages, altered T cell function, impaired clearance by the reticuloendothelial system, and mechanical filtration of pathogens by the spleen (2). Congenital asplenia in humans is often associated with complex heart defects and abdominal heterotaxy resulting from a laterality defect that begins to develop before spleen fate is specified (3). While cases of isolated congenital asplenia with no other obvious abnormalities have been reported (4, 5), such individuals have seldom been documented. This may reflect that congenital asplenia is a rare occurrence but, alternatively, it has been proposed that such individuals die early in childhood from overwhelming sepsis, without an autopsy being performed (6–8).

There are, broadly speaking, two major branches of the immune response to invading microorganisms. The innate immune system provides immediate protection by promptly initiating a local inflammatory reaction. The adaptive immune system expands antigen-specific T and B cells in the lymphoid organs and provides long-lasting protection against subsequent infections by the generation of memory T and B cells. In the mouse, a population of B cells, known as B-1a, cooperates with the innate immune system at the onset of bacterial and viral infections (9). B-1a cells produce natural antibodies, mostly IgM, assumedly without prior exposure to antigen. Such antibodies recognize thymus-independent antigens, such as the polysaccharides of encapsulated bacteria (10). By opsonizing invading pathogens, they play a crucial role in the activation of the complement cascade and the amplification of early innate defense mechanisms (11).

To understand the role of the spleen in the response to bacterial polysaccharides, we have used the Hox11-null mouse as a model of congenital asplenia (12, 13). We show that the spleen is indispensable for the generation and maintenance of the B-1a pool. Congenitally asplenic and splenectomized mice have reduced B-1a cells and a diminished IgM-mediated immune response against bacterial polysaccharide antigens.
Materials and Methods

Mice. C57BL/6, Hox11-null, Rag-2-null, and SCID mice were bred and housed in specific pathogen-free conditions in the institute’s animal facility. All mice used were aged 6–10 wk. The Hox11-null mice were on a mixed genetic background of MF1 (12.5%) and C57BL/6 (87.5%). For splenectomies and sham-operations, C57BL/6 mice were anesthetized and after shaving, a large incision was made in the skin at the left flank. The peritoneum was opened and the spleen and vein ligated with catgut (Ethicon). The spleen was removed and the incision closed with surgical silk-thread (Ethicon). For sham-operated mice, the peritoneum was opened and surgically closed.

FACS® Analysis. Antibodies used for lymphocyte stainings were anti–mouse CD5-Biotin (clone 3A–7; BD Pharmingen), anti–mouse B220–PE (clone RA3–6B2; Southern Biotechnology Associates), anti–mouse IgM-Cy5 (Jackson ImmunoResearch Laboratories), anti–mouse IgD-FITC (clone 12.26c2a; Southern Biotechnology Associates), anti–Mac-1 (clone M1/70, own), anti–mouse CD23–Biotin (clone B3B4; BD Pharmingen), and anti–mouse CD21–FITC (clone 7G6, own). Biotinylated antibodies were detected with Streptavidin–Red670 (GIBCO BRL and Life Technologies). Mouse peritoneal cells were isolated by peritoneal lavage with 5 ml PBS containing 0.05% (vol/vol) Tween 20 (Sigma–Aldrich) in DEAE buffer were added. Emitted light was measured at 405 and 490 nm at various time-points afterwards. Total serum IgM antibodies were detected similarly except that plates were first coated with 50 μl 1 μg/ml goat anti–mouse IgM (Southern Biotechnology Associates) followed by addition of diluted serum. For measurements of total IgM sera were diluted between 1:500 and 1:8,000. The 1:1,000 dilution is shown. Mouse IgM (Roche, Biocelantic) was used as a standard control.

Results

Hox11-Null Mice Lack B-1a Cells. We have previously shown that the spleen plays a major role in the late phases of B cell development. Transitional 1 (T1) B cells, recently emigrated from the bone marrow, accumulate in the spleen and here differentiate to transitional 2 (T2) and mature B cells (19). To further study the function of the spleen in the development of B cells, we analyzed congenitally asplenic Hox11-null mice (12, 13). Of the major B cell and T cell populations from bone marrow, lymph node, and thymus analyzed by FACS®, we could not detect any gross phenotypic abnormalities. A minor increase in the number of T1 B cells in bone marrow and peripheral blood was observed, perhaps reflecting a shortage of space, as these cells normally collect in the spleen. Reductions were also observed in the proportion of mature B cells in blood, lymph node, and bone marrow (data not shown). This confirms the role of the spleen in the generation of normal numbers of mature B cells. The most striking finding, however, was a large reduction in the total cell number in the peritoneal cavity of Hox11-null mice (8.9 ± 2.0 × 10⁶ in C57BL/6, 5.1 ± 1.0 × 10⁶ in Hox11-null, Fig. 1 A). However, in the peritoneal lymphocyte population there was no significant difference in the proportion of total B cells between C57BL/6 and Hox11-null (59.5% ± 5.7% in C57BL/6, 69.1% ± 6.6% in Hox11-null). Thus, nonB cells including T cells but also myeloid cells are reduced in the peritoneal cavity of Hox11-null mice.

To identify the different populations of B cells in the peritoneal cavity we used antibodies against IgM, IgD, B220, and CD5. This antibody combination allows the dis-
The relative expression of IgM and IgD can be used to discriminate IgM^{pos}IgD^{dull} B-1 (gate 1) from IgM^{dull}IgD^{pos} B-2 cells (gate 2, left panels). Based on the expression of IgM and IgD, B-1 and B-2 cells were gated as indicated and analyzed for the expression of B220 and CD5 to further distinguish between CD5^{pos}B220^{dull} B-1a cells (top region, bottom right panels) and the CD5^{pos}B220^{pos} B-1b population (bottom region, bottom right panels). While B-1b cells are normal in Hox11-null mice, the B220^{dull}CD5^{pos} B-1a population is absent. B-2 cells (gate 2) are CD5^{neg}B220^{pos} mature B cells (top right panels). The CD5^{pos}B220^{pos} B-1a population that remains in Hox11-null mice is indicated with an arrow. (C) The proportion of B-1a cells in the peritoneal cavity of C57BL/6 (n = 8) and Hox11-null (n = 8) are shown. Peritoneal B cells (IgM^{pos} and/or IgD^{pos}) were measured by flow cytometric analysis and IgM^{dull}IgD^{pos}CD5^{pos}B220^{dull} were classified as B-1a cells. (D) Relative serum IgM levels in blood of C57BL/6 (n = 10) and Hox11-null mice (n = 9) were determined by anti-IgM ELISA. Each data point represents the relative serum IgM level of one mouse measured at a 1:1,000 dilution.

Several other markers can be used to discriminate between B-1 and B-2 cells: B-1 cells are Mac-1^{pos}, CD43^{pos}, and CD23^{neg}, whereas B-2 cells are CD23^{pos}, Mac-1^{neg}, and CD43^{neg} (15–17). Stainings with combinations of these antibodies verified that the B-1a population was reduced in the peritoneal cavity of Hox11-null mice (data not shown).

As B-1 cells are known to be the major source of natural antibodies and most of the circulating IgM is contributed by B-1a cells in the absence of previous antigen stimulation (10), serum IgM levels in the blood of mutant and C57BL/6 mice were compared, revealing significantly lower levels in Hox11-null mice (Fig. 1 D). B-1a cells are not only present...
within the peritoneal cavity but can also be detected in spleen and to very low amounts in lymph nodes (18). We found that B-1a cells are also present in the blood. We stained peripheral blood with antibodies against IgM, IgD, CD5, and either B220 or Mac-1. The IgM^posIgD^dull population includes T1 B cells, recently emigrated from the bone marrow, B-1a, and B-1b cells (Fig. 2 A and B). In the peripheral blood lymphocyte population, the proportion of total B cells was not significantly different between C57BL/6 and Hox11-null mice (Fig. 2 C).

B-1a cells represent a significant proportion of B cells in the peripheral blood of C57BL/6 mice. B220^dull B-1a cells were absent from the blood of Hox11-null mice (Fig. 2 A and C) but, as in the peritoneal cavity of Hox11-null mice, a few CD5^posB220^pos cells could be detected (indicated by the arrow in Fig. 2 A). To distinguish between T1 and B-1b cells we analyzed the expression of CD5 and Mac-1 on gated IgM^posIgD^dull cells. Transitional B cells do not express CD5 and Mac-1. In normal mice, they exit the bone marrow and collect in the spleen (19), but in asplenic mice they accumulate in the blood (Fig. 2 B and C). There was no change in the B-1b cell population (CD5^posMac-1^pos) but a slight reduction in the proportion of mature B cells in asplenic mice (Fig. 2 B and C).

Hox11 is expressed in the branchial arches, the developing hindbrain, the spinal cord, the pharynx, the outflow tracts of the heart, the external auditory meatus, the neurons of the developing cranial sensory ganglia, and in the spleen capsule and trabeculae, but not in hematopoietic cells during fetal life (12, 13). Extensive analysis of the spleen, bone marrow, and peritoneal cavity of adult and neonatal mice did not reveal any evidence of Hox11 expression in B cells (data not shown). To formally exclude the possibility that the reduction of the B-1a cell pool in Hox11-null mice was...
due to an intrinsic hematopoietic defect, rather than due to the absence of the spleen, we transferred \textit{Hox}11-null fetal liver cells into SCID mice, which lack both B and T cells. This protocol allows the reconstitution of all lymphocyte populations including B-1a and B-1b cells with cells of donor origin (18). 4 wk after transfer, B-1a cells were detectable in the peritoneal cavity of SCID mice reconstituted with \textit{Hox}11-null fetal liver cells (Fig. 3 A). Thus, \textit{Hox}11-null progenitors retain the ability to generate B-1a cells, but are unable to do so in the absence of the spleen. A control transfer of C57BL/6 fetal liver cells into irradiated \textit{Hox}11-null mice was also performed (Fig. 3 B). The majority of B-1a cells was still absent although a small number of B220$^{\text{dull}}$ B-1a cells was present. This indicates that there may be a limited capacity to generate some B-1a cells from wild-type precursors in the absence of the spleen.

\textbf{Removal of the Spleen Depletes the B-1a Pool.} To investigate whether the spleen plays a role in the maintenance of an already established B-1a cell pool, we splenectomized normal C57BL/6 mice and determined the frequency of all peritoneal B cell populations at various time points thereafter. Cells were stained with antibodies against IgM, CD5, B220, and Mac-1 and the proportion of IgM$^{\text{pos}}$Mac-$^{\text{1 pos}}$CD5$^{\text{pos}}$B220$^{\text{dull}}$ B-1a cells was determined at various time points thereafter (Fig. 4). There was a similar reduction in the number of B-1a cells in splenectomized and sham-operated mice in the first 2 d. However, after day 3, the proportion of B-1a cells in sham-operated mice began to recover whereas in splenectomized mice it declined further. 6 d after the removal of the spleen, CD5$^{\text{pos}}$B220$^{\text{dull}}$ B-1a cells were $\sim 1/4$ of those in sham-operated mice and appeared to stabilize at this level as further measurements at 10, 14 and 26 d after splenectomy revealed little change (Fig. 4 A and B). The frequency of B-1b remained stable after splenectomy (Fig. 4 C). Therefore, the spleen is indispensable for the maintenance of an already established B-1a cell pool.

Despite the significant reduction, a fraction of B-1a cells survived in splenectomized mice. Our observations suggest that $\sim 75\%$ of the peritoneal B-1a cells require the spleen...
for their generation and survival while the rest can survive without the spleen for at least 26 d. B-1a cells are considered a self-replenishing population, originally derived from fetal liver progenitors (18). The depletion of the majority of B-1a cells after splenectomy suggests that the spleen is indispensable either for their self-renewal or for their selection into the B-1a pool. We have previously shown that the spleen plays a role in the differentiation of transitional into mature B cells (19). We now demonstrate that the spleen is also required for a normal B-1a cell pool. In contrast, the spleen is not necessary for the development and survival of B-1b cells.

Asplenic Mice Do Not Respond to Vaccination with Streptococcal Polysaccharides. B-1a cells are responsible for the production of IgM against thymus-independent antigens such as polysaccharides and phospholipids of the bacterial capsule (20–22) and are known to participate in the early response to bacterial and viral infection (9, 10, 23). The polysaccharide bacterial capsule is the major virulence factor of S. pneumoniae. Protective IgM antibodies have the function to opsonize the bacteria and facilitate their phagocytosis. Using mice challenged with a noncapsular strain of S. pneumoniae, it has been well documented that the major protective immune response is directed toward phosphorylcholine (24–26). The response against phosphorylcholine is also T cell independent. Levels of natural antibody against phosphorylcholine and pneumococcal polysaccharide were reduced in Hox11-null mice (18.2% + 17.4% in Hox11-null mice, n = 16, compared with 100.0% + 69.6% in C57BL/6, n = 14, for antiphosphorylcholine; 20.4% + 27.2% in Hox11-null mice compared with 100.0% + 108.8% in C57BL/6 for antipneumococcal polysaccharide). To determine if the absence of the spleen affected B-1a–dependent immune responses, Hox11-null, splenectomized, sham-operated, and control mice were immunized subcutaneously, intramuscularly, intraperitoneally, or intravenously with Pneumovax® 23, a vaccine composed of polysaccharides from the 23 most common types of Streptococcus pneumoniae (27). Control and sham–operated mice rapidly produced Pneumovax®–specific IgM antibodies via all four injection routes, while Hox11-null (Fig. 5 A) and splenectomized mice (Fig. 5 B) failed to respond.

The Spleen, in the Absence of B-1a Cells, Is Not Sufficient for a Normal Immune Response to Bacterial Polysaccharides. To exclude the possibility that the absence of the spleen itself, rather, or in addition to, the lack of B-1a cells, was responsible for the failure of the immune response, we generated mice lacking B-1a cells but possessing a spleen. To do this,
bone marrow or fetal liver cells were transferred into RAG2-null mice (Fig. 6). It has been reported that transfer of bone marrow cells repopulates all major B cell populations with the exception of B-1a cells, whereas transfer of fetal liver cells also repopulates the B-1a population (18). 4 wk after transfer the major B cell populations in bone marrow (pro-B and pre-B, immature, transitional, and recirculating), spleen (T1, T2, and mature), and lymph nodes (mature) were analyzed. All these populations were present (data not shown). However, as expected, the peritoneal CD5<sup>+</sup>B220<sup>+</sup> B-1a population was lacking in the mice transferred with bone marrow but present in the mice that had received fetal liver cells (Fig. 6 A). Interestingly CD5<sup>+</sup>B220<sup>+</sup> B cells (indicated by the arrow in Fig. 6 A) phenotypically identical to residual CD5<sup>+</sup> B cells of Hox11-null mice (see also Fig. 1 B) were detectable after bone marrow transfer, suggesting they are not only phenotypically but also ontogenetically distinct from the CD5<sup>+</sup>B220<sup>+</sup> B-1a cells.

To confirm that the B220<sup>-</sup> B-1a cells are indeed the cells responsible for the production of IgM against bacterial polysaccharides, the reconstituted mice were immunized subcutaneously, intramuscularly, intraperitoneally, or intravenously with Pneumovax<sup>®</sup> 23 and the production of specific IgM was measured. Mice transferred with fetal liver cells, and, thus, possessing B-1a cells, produced specific IgM, regardless of the route of immunization. In contrast, mice transferred with bone marrow and, thus, lacking B-1a cells failed to respond (Fig. 6 C and D). Therefore, the presence of B-1a cells is required to induce an immune response to streptococcal polysaccharides. In contrast, the presence of the spleen, B-1b, and B-2 cells in the absence of B-1a cells is not sufficient.

The importance of MZ B cells in response to T cell-independent antigens has been recognized (28–30). Mice transferred with bone marrow cells possessed normal numbers of CD23<sup>-</sup>CD21<sup>+</sup>IgM<sup>+</sup> MZ B cells (reference 19; Fig. 6 B), but their inability to respond to bacterial polysaccharides indicates that in the absence of B-1a cells, MZ B cells are not sufficient for a response to Pneumovax<sup>®</sup> 23. The impaired response to streptococcal polysaccharides in mice lacking B-1a cells may indicate that B-1a and B-1b cells are not only ontogenetically (31), but also functionally, distinct. Thus, in Hox11-null, splenectomized and bone marrow reconstituted mice, there is a direct correlation between absence of B-1a cells and lack of immune response to streptococcal polysaccharides.

**Discussion**

Using asplenic Hox11-null mice, we have shown that the spleen is required for the normal development of the B-1a population. The origin and differentiation of B-1a cells is controversial. At one time, B-1a cells were assumed to be ontogenetically distinct from B-2 cells, based on the observation that they are derived from fetal liver but not adult bone marrow (26). The absence of N nucleotide addition and the preferential usage of proximal V gene segments in fetal precursors are possible reasons for the generation of high numbers of B-1 cells from fetal liver but not from adult bone marrow (32). More recent data show that B-1a cells may develop from conventional B cell precursors as a consequence of positive selection after encounter with antigen (33, 34). It is conceivable that this selection occurs in the spleen and the presence of B-1a precursor cells in the spleen has been described previously (35). Therefore, the spleen may offer a specialized microenvironment where

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Figure 6. Mice lacking B-1a cells but possessing a spleen fail to respond to immunization with streptococcal polysaccharides. (A) Fetal liver (FL) or bone marrow (BM) cells from C57BL/6 mice were injected intraperitoneally into irradiated Rag2-null mice and four weeks later reconstituted mice were analyzed. A representative FACS<sup>®</sup> analysis of total un gated peritoneal cells stained for CD5 and B220 from a FL- and a BM-transferred mouse is shown above. The CD5<sup>+</sup>B220<sup>+</sup> B-1a cell population is boxed. The CD5<sup>+</sup>B220<sup>+</sup> B cells phenotypically identical to residual CD5<sup>+</sup> B cells of Hox11-null mice are indicated by a box and arrow. (B) Representative FACS<sup>®</sup> analyses of CD23<sup>-</sup>- gated spleen cells stained for CD21 and IgM from a FL- and a BM-transferred mouse are shown. The normal position of the MZ B cell population (MZ) is boxed. (C) Specific IgM antibodies in sera of transferred mice. Pneumovax<sup>®</sup> 23 was injected intraperitoneally 20 d after transfer. Antibodies were measured by ELISA 1 d before and 6 d after Pneumovax<sup>®</sup> 23 injection. The optical density (OD<sub>405</sub>) measured with a 1:80 serum dilution is shown. Each data point indicates the relative antibody level of a single mouse. (D) The response to Pneumovax<sup>®</sup> 23 of mice transferred with either bone marrow (BM) or dE14.5 fetal liver cells (FL) is shown as mean fold-increase of specific serum IgM as detected by ELISA. The optical density (OD<sub>405</sub>) was measured with a 1:80 serum dilution. For each route of injection, five mice were transferred with bone marrow and three with fetal liver cells.
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ligand-dependent positive selection of B-1a precursors occurs. In addition, the spleen may function as a reservoir of fetal liver-derived B-1a precursors in adult life.

A few CD5pos B-1a cells were still present in the peritoneal cavity and the blood of Hox11-null mice and could also be detected in the peritoneal cavity of irradiated Rag2-null mice 4 wk after transfer of C57BL/6 bone marrow. However, these cells express higher levels of B220 and slightly lower levels of CD5 as compared with the CD5pos B220null B-1a cells of C57BL/6 animals. This suggests that CD5pos B cells might consist of two distinct subpopulations. B220null CD5 cells, corresponding to B-1a B cells, are strictly dependent on the spleen for their generation, and develop rapidly in high numbers from fetal liver precursors. In contrast, B220pos CD5 cells can be generated in the absence of the spleen, can be derived from both fetal liver and adult bone marrow, and in these respects are very similar to B-1b cells. Our results demonstrate that the former population is required for natural IgM production and the response to streptococcal polysaccharide antigens.

Splenectomy of C57BL/6 mice leads to a rapid loss of ~75% of the peritoneal B-1a cell pool. It is unknown whether the reduction in B-1a cells in splenectomized mice reflects a defect in their generation, their survival, or a combination of both. B-1a cell precursors may home to the spleen from the liver during the fetal life and B-1a B cells may be continuously generated from these precursors in the adult. Alternatively, B-1a cells may be long-lived and their rapid loss upon splenectomy would suggest that they are unable to survive in the absence of the spleen. This could reflect the requirement of a factor generated in the spleen that is essential to B-1a survival. It is possible that B-1a cells continually circulate through the spleen, where the interaction with a specialized microenvironment supports their survival.

B-1a cells were originally identified based on the expression of CD5 (36), a glycoprotein expressed by all T cells after positive selection (37, 38). In the B cell compartment, only B-1a cells are CD5pos. However, CD5 can be induced on conventional B cells after B cell antigen receptor (BCR) cross-linking (39) and low levels of CD5 are expressed by chronically stimulated, anergic B cells (40). Therefore, it has been proposed that expression of CD5 is indicative of a prior interaction of B-1a cells with antigen. The BCRs of B-1a cells comprise not only polyreactive but also autoreactive specificities and it has been demonstrated that autoantigens can be responsible for their positive selection (33). Given that CD5 is a negative regulator of BCR signaling in B-1a cells (41) and that tight regulation of the activation of B-1a cells specific for autoantigens is essential, low expression of B220 in addition to up-regulated CD5 expression might downmodulate BCR signals after positive selection by antigen and thus prevent autoimmunity (42, 43).

It has been demonstrated that fetal liver- and bone marrow-derived B cells have different signaling properties (34). The CD5pos B220pos B cells present in Hox11-null, Rag2-null reconstituted, and C57BL/6 mice may be B-1a precursors that do not, for some reason, downmodulate B220 after interaction with ligand in the spleen. The reduced threshold for signaling in such cells may contribute to autoimmune phenomena attributed to CD5pos B cells. Alternatively, these cells may be ontologically distinct from typical B-1a cells. Their presence after C57BL/6 bone marrow transfer into Rag2-null mice indicates that they are a bone marrow-derived population.

At the outset, bacterial infections are often localized and are initially exposed to components of the innate immune system. Natural IgM antibodies secreted by B-1 cells are part of this system and play a crucial role in the activation of the complement cascade and the amplification of early innate defense mechanisms (11). Hox11-null mice exhibit significantly reduced levels of serum IgM and of natural antibodies. In addition, lack of B-1a cells in Hox11-null and splenectomized mice correlates with an inability to produce specific IgM in response to T cell-independent streptococcal polysaccharides. We observed that Pneumovax®-specific IgM was produced regardless of the route of inoculation. Although B-1a cells were originally described in the peritoneal cavity, we found B-1a cells in the peripheral blood. They have also been detected in the spleen and 50% of the IgA-secreting plasma cells in the mucosa of the bowel are B-1 derived (44, 45). Furthermore, B-1a cells can be detected in lungs of mice infected with influenza virus (46), suggesting that they may be able to migrate to sites of infection. Circulation through the body via the blood could be a mechanism by which B-1a cells rapidly reach infected and inflamed tissues. Therefore, this might be important to limit dissemination of invading pathogens. Thus, the presence of B-1a cells in the blood might explain the production of Pneumovax®-specific IgM in C57BL/6 mice regardless of the route of immunization while Hox11-null mice, lacking peritoneal and blood B-1a cells, did not respond.

MZ B cells of the spleen contribute to the early immune response to thymus-independent (TI)-2 antigens (28–30). However, though MZ B lymphocytes were reconstituted to normal amounts after transfer of bone marrow cells into Rag2-null mice, these animals did not produce specific IgM in response to immunization with streptococcal polysaccharides. Presence of the spleen including MZ B cells is, therefore, not sufficient for an adequate response to TI-2 antigens if B-1a cells are absent. It has recently been demonstrated that B-1 cells dominate the response to TI antigens in the peritoneum, while MZ B cells contribute to the early response to blood-borne particulate TI antigens (47). Nevertheless, mice possessing B-1a cells but lacking MZ B cells show an impaired response to the TI-2 antigen TNP-Ficoll after intraperitoneal administration (28). Hence, it is possible that both B-1a and MZ cells are required for the production of specific IgM in response to different repetitive polysaccharide antigens. While MZ B cells may be important in the late phases of infections when pathogens can be found in the blood and are filtered into the MZ of the spleen, B-1a B cells may play a role in the limitation of early bacterial growth at the site of infection (47).
In summary, we have demonstrated that the spleen is indispensable for the generation and survival of B-1a cells in the mouse. This reveals an essential role of the spleen in producing these cells, and their absence may account for the concomitant infections that occur in asplenic individuals. Future experiments will focus on determining how the spleen functions in the generation and survival of these cells.

We thank Horst Mossmann, Norbert Joswig, Uta Stauffer, and colleagues in the central animal facility for maintenance of the mouse colony and assistance with surgery. Hox11-null mice were provided by T.H. Rabbitts, Laboratory of Molecular Biology, Cambridge, UK. We thank Holger Weber for helpful discussion.

Accepted: 8 February 2002
Submitted: 2 July 2001
Revised: 12 December 2001

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