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Partial splenectomy but not total splenectomy preserves immunoglobulin M memory B cells in mice

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Abstract

Purpose: The mechanism by which partial splenectomy preserves splenic immune function is unknown. Immunoglobulin (Ig) M memory B cells are critical for the immune response against encapsulated bacteria and are reduced in asplenic patients, although it is unknown whether partial splenectomy can preserve memory B cells. We hypothesized that IgM memory B cells (murine B-1a cells) would be preserved after partial splenectomy but not after total splenectomy in mice.

Methods: We performed total splenectomy (n = 17), partial splenectomy (n = 10), or sham laparotomy (n = 16) on C57BL/6J mice. Mice were killed on postoperative day 10 or 30, and peritoneal washings were analyzed by multiparameter flow cytometry for expression of murine B-1a cells ($IgM^{pos}IgD^{dull}CD5^{pos}B220^{dull}$).

Results: We found that B-1a cells were significantly reduced after both total and partial splenectomies compared with sham laparotomy in the early postoperative period, although normal levels of B-1a cells returned by postoperative day 30 in mice undergoing partial splenectomy but not total splenectomy.

Conclusion: Partial splenectomy but not total splenectomy preserves the B-1a B-cell population in mice within 30 days after surgery. Maintenance of these critical B cells may contribute to the preservation of a splenic-dependent immune response after partial splenectomy.

For more than 50 years, it has been recognized that a total splenectomy places children at increased risk for overwhelming postsplenectomy sepsis from encapsulated bacteria [1-4]. More recent studies have suggested that the spleen may have a critical role in protecting against common childhood diseases, including respiratory tract infections by encapsulated bacteria [5]. Interest in partial splenectomy as an alternative to total splenectomy in children for a number of clinical indications, including various congenital hemolytic anemias, has increased. However, the mechanism by which partial splenectomy may contribute to the maintenance of an intact immune response remains unknown.

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Memory immunoglobulin (Ig) M B cells, the B-cell population that produces natural antibodies and antibodies against T-independent antigens such as pneumococcal polysaccharides, have been recently proposed as one critical mechanism by which the spleen can contribute to the protection against encapsulated pathogens [6-9]. The spleen appears to play an important role in the maintenance of normal IgM memory B cells in postnatal life. Children and adults without a spleen have a significantly reduced memory IgM B-cell population [8,9]. Similarly, mice with congenital asplenia lack peritoneal B-1a cells, the murine equivalent of blood IgM memory B cells in humans [8-10]. The B-1a cell population is also reduced in mice after total splenectomy [11].

The effect of partial splenectomy on the B-1a cell population in mice and the IgM memory B cell population in humans is not known. We hypothesized that partial splenectomy but not total splenectomy would preserve normal memory B cells in mice. To test this hypothesis, we examined the effects of partial splenectomy and total splenectomy on the B-1a cell population in mice.

1. Methods

1.1. Surgical procedures

We performed total splenectomy, partial splenectomy, or sham laparotomy on 6- to 10-week-old C57BL/6J mice that had been bred and raised in pathogen-free conditions. For all procedures, mice were anesthetized then sterilely prepared and draped. We made a 1-cm paramedian incision over the left upper quadrant. For total splenectomy, the spleen was identified, small clips were applied to the vascular pedicles, and the spleen was removed. For partial splenectomy, the medial vascular pedicle was clipped and divided. Another clip was placed transversely across the main body of the spleen toward the upper pole, such that 85% to 90% of the spleen was resected, leaving approximately 10% to 15% of splenic tissue preserved by upper pole vessels. For sham operations, a laparotomy incision was made; but no splenic tissue was resected. Mice were recovered and then sacrificed at either postoperative day 10 or 30 for analysis. All research in this study was approved by the Duke University Institutional Animal Care and Use Committee.

1.2. Data collection and analysis

Peritoneal washings were obtained by irrigating the peritoneal cavity with 5-mL wash solution containing phosphate-buffered saline (Gibco, Invitrogen, Carlsbad, CA, USA) with 2% fetal calf serum (Gibco) and 0.01% NaN_3. The washings were filtered through a 40-nm filter (BD Bioscience, San Jose, CA, USA) and depleted of erythrocytes by lysis with fluorescence-activated cell sorting (FACS) Lysis Solution (Becton Dickinson, San Jose, CA, USA). Cells were washed, resuspended, and incubated at a concentration of 1 × 10e6 cells per sample with the following antibodies at manufacturer-recommended concentrations: IgM-FITC

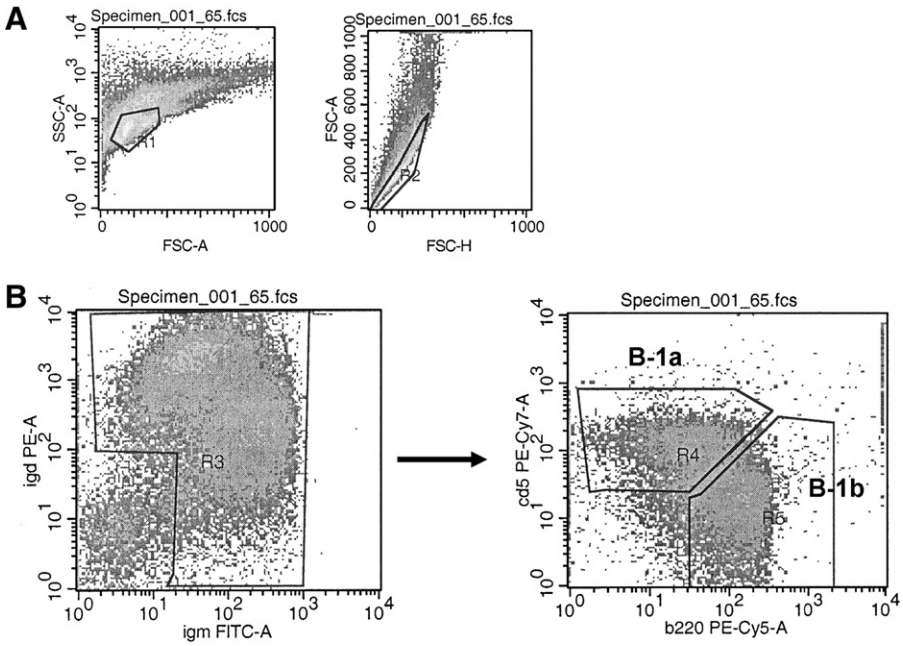

Fig. 1 Representative flow cytometric (FACS) analysis of C57BL/6J mouse peritoneal B cells isolated 30 days after sham operation demonstrating our gating strategy. Cells were stained with antibodies against IgM, IgD, CD5, and B220. A, Based on forward and side scatter, cells were gated as above to isolate the lymphocyte population. B, Based on the relative expression of IgM and IgD, B cells were gated (R3 gate, $IgM^{pos}IgD^{dull}$) and analyzed for the expression of B220 and CD5 to further distinguish the $B220^{dull}CD5^{pos}$ B-1a cells (R4 gate) from the $B220^{pos}CD5^{neg}$ B-1b and B-2 cells (R5 gate).

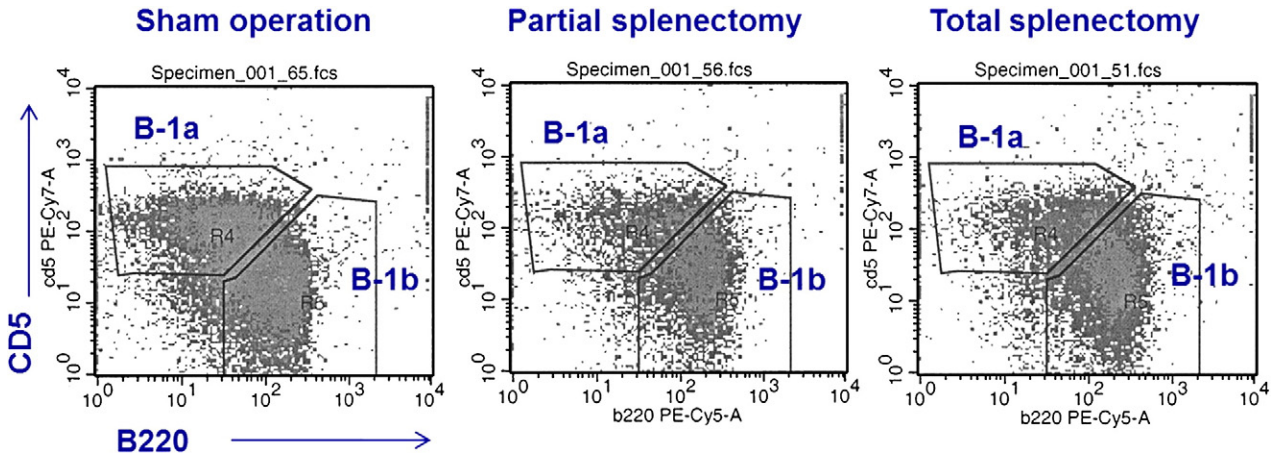

Fig. 2 Representative FACS analysis of mouse peritoneal B cells isolated 10 days after sham operation, partial splenectomy, or total splenectomy and gated for $IgM^{pos}IgD^{dull}$ population. At postoperative day 10, the peritoneal B-1a cell population ($B220^{dull}CD5^{pos}$) was significantly reduced in both the partial splenectomy and total splenectomy groups compared with the sham operation group.

(clone 11/41; BD Pharminogen, San Jose, CA, USA), IgD-PE (clone 11-26c.2a; BD Pharminogen), CD5-PE-cy7 (clone 53-7.3; BD Pharminogen), B220-PE-cy5 (RA3-6B2; BD Pharminogen). Controls were prepared from pooled peritoneal washings of normal mice with appropriate isotype controls. Four-color flow cytometry was performed on an FACS CANTO II flow cytometer (Becton Dickinson). Flow cytometric profiles were analyzed using CELLQuest software (Becton Dickinson). The B-1a cell population within the experimental and control groups were compared using unpaired Student *t* test analysis.

2. Results

We analyzed the peritoneal cell population of experimental and control mice by multiparameter flow cytometry. Within the B-cell population ($IgM^{pos}IgD^{dull}$), we were able to distinguish among previously described subpopulations of B cells by differential expression of CD5 and B220 (Fig. 1) [11]. We found that the B-1a cell population ($CD5^{pos}B220^{dull}$) was reduced after either partial splenectomy (n = 4) or total splenectomy (n = 8) compared with mice undergoing sham laparotomy (n = 7) at postoperative day 10 (Fig. 2; Table 1) (control, 16.38% ± 2.9% of lymphocytes; partial splenectomy, 8.10% ± 3.2% [$P < .01$]; total splenectomy, 9.27% ± 3.6% [$P < .01$]). However, by postoperative day 30, the B-1a cell population had recovered in the partial splenectomy group (n = 6), with B-1a cell levels that were equivalent to mice undergoing sham laparotomy (n = 9) (partial splenectomy, 19.12% ± 3.1%; control, 25.47% ± 6.7% of lymphocytes; *P*, nonsignificant). In contrast, the B-1a cell population remained decreased in the total splenectomy group (n = 9) compared with sham laparotomy group (total splenectomy, 10.93% ± 4.4%; partial splenectomy, 25.47% ± 6.7% of lymphocytes; $P < .01$) (Fig. 3, Table 1).

3. Discussion

Despite increasing interest in the use of partial splenectomy for children, the mechanism by which partial splenectomy may contribute to the maintenance of an intact immune response is unknown. Recent interest has been directed to the role of memory B cells in the maintenance of an immune response, and several lines of evidence suggest that the spleen is critical to the production and maintenance of these cells [7-13]. In this animal study, we confirmed our hypothesis that after partial splenectomy but not total splenectomy, murine B-1a cells are preserved compared with mice undergoing sham laparotomy. This finding supports the mounting evidence that partial splenectomy may preserve critical factors for an intact immune response.

Early B-cell development is similar in mice and humans, with B-cell production occurring in the bone marrow, then migrating to lymphoid follicles and the spleen [8]. Two distinct types of B cells have been

Table 1 Murine B-1a population as a percentage of lymphocytes in early and late postoperative period

Early postoperative period (postoperative day 10)		
Sham operated (n = 7)	16.38% ± 2.9 %	–
Partial splenectomy (n = 4)	8.10% ± 3.2 %	$P < .01$
Total splenectomy (n = 8)	9.27% ± 3.6 %	$P < .01$
Late postoperative period (postoperative day 30)		
Sham operated (n = 9)	25.47% ± 6.7 %	–
Partial splenectomy (n = 6)	19.12% ± 3.1 %	*P*, NS
Total splenectomy (n = 9)	10.93% ± 4.4 %	$P < .01$

Mean values of B-1a cells as a percentage of lymphocytes for each experimental group are shown above. *P* values are calculated by comparing each experimental cohort to the sham-operated cohort at that time point using unpaired Student *t* test; *P* value is significant at $P < .05$. NS indicates nonsignificant.

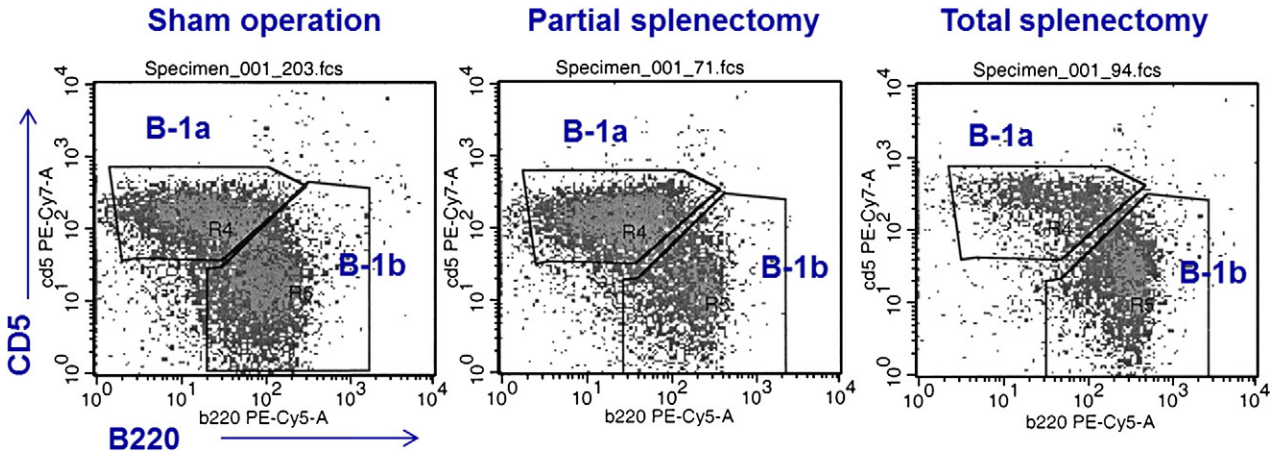

Fig. 3 Representative FACS analysis of mouse peritoneal B cells isolated 30 days after sham operation, partial splenectomy, or total splenectomy and gated for $IgM^{pos}IgD^{dull}$ population. At postoperative day 30, the peritoneal B-1a cell population ($B220^{dull}CD5^{pos}$) had recovered in the partial splenectomy group to levels similar to the sham operation group, whereas that population remained significantly reduced in the total splenectomy group compared with the sham operation group.

characterized in mice: mature B cells and B-1a cells. Mature B cells, which produce monospecific, high-affinity antibodies with the help of T cells, are critical for the adaptive immune response. In contrast, B-1a cells produce natural antibodies of the IgM subtype, which bind several antigens with low affinity and are T cell independent. These natural antibodies are immediately primed to target foreign pathogens before specific antibodies are produced, which is particularly useful against encapsulated bacteria. In humans, IgM memory B cells (which express IgM but are IgD^{dull}) are analogous to the murine B-1a cell population [8]. These B cells are distinct from the mature B cells, which express isotypes other than IgM, and may be a link between the innate and adaptive immune systems in humans. In addition, memory IgM B cells are known to be reduced in asplenic/splenectomized patients [8,11]. However, given the complexity of the human immune response, the functional significance of a reduced memory IgM B-cell population is not known. Human clinical trials are needed to further assess the memory B-cell population in humans after partial and total splenectomies and to determine the effects on clinical end points such as Overwhelming postspelenctomy sepsis (OPSI).

We have found that murine B-1a cells are preserved after partial splenectomy but not total splenectomy. In this small animal study, we assayed peritoneal washings to assess the murine B-1a population. Prior studies have established that the murine B-1a cell population is the major source of natural antibodies (including the majority of circulating IgM) in mice [11]. Most studies have relied on peritoneal washings to assay the murine B-1a population, given the ease of collecting large numbers of lymphocytes via this route. The peritoneal B-1a population provides an accurate representation of the B-1a population in peripheral blood and the small numbers of B-1a cells in murine lymph nodes [9,11]. Although peritoneal washings have been shown to provide an accurate representation of the murine B-1a cell population, future studies that include peripheral blood and lymph node assays to assess the B-1a population might yield additional insights.

Although in this small animal study, we demonstrated that the murine B-1a cell is preserved after partial splenectomy but not total splenectomy, we did not challenge partial and total splenectomy mice with an antigen challenge. In studies of functionally asplenic mice, others have found that the reduced B-1a cell population in these mice correlates with low levels of IgM natural antibodies and inadequate immune response to *Streptococcal* polysaccharides [11]. We suspect that partial splenectomy may preserve a similar ability to respond to *Streptococcal* antigens. Further antigen challenge studies using encapsulated bacteria may provide additional evidence of the significance of the B-1a cell population.

Similarly, we did not measure the B-1a population at time points beyond 30 days, and the long-term ability of partial splenectomy to preserve these cells is unknown. Further experiments to determine the cell count, B-cell subpopulations, and splenic architecture of the splenic remnant in partially splenectomized animals might yield more answers.

In summary, we have shown that peritoneal B-1a cell population is preserved in mice after partial, but not total, splenectomy. This preliminary animal study suggests that partial splenectomy might preserve the analogous cell population of peripheral blood IgM memory B cells in humans. Given the current interest in maintaining immune function in children who require splenic resection for hematologic disease or hypersplenism [12-16], preservation of memory IgM B cells would support use of partial splenectomy. Further studies in animal models and human trials will better define immunologic benefits of partial splenectomy.

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