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Maintenance of Peritoneal B-1a Lymphocytes in the Absence of the Spleen

Karsten Kretschmer,* Jana Stopkowicz,* Stephan Scheffer, † Tim F. Greten, † and Siegfried Weiss*

Positive selection by autoantigens is believed to play an important role in the generation/maintenance of B-1a cells. Recently, it has been described that splenectomy results in the loss of an already established B-1a cell pool. To elucidate whether the spleen influences the peritoneal B-1a repertoire, we have analyzed the consequences of splenectomy in the recently established IgL-transgenic L2 mouse model. L2 mice are characterized by a severe block of B-2 development and predominance of B-1a cells, which exhibit a pronounced IgH oligoclonality, presumably due to positive selection by autoantigens. In this study, we show that, in striking contrast to splenectomized normal mice, L2 mice exhibit unchanged frequencies of peritoneal B-1a cells. The IgH repertoire of these B-1a cells, however, was severely perturbed in that the previously described predominant B-1a H chains were no longer present. The repertoire changes were partial since phosphatidylcholine-specific B-1a cells were present in similar numbers before and after splenectomy. Thus, splenic Ags appear to act as “survival factors” for major subsets of peritoneal B cells. The loss of B-1a cells in the absence of such factors is compensated by repertoire changes among B-1a cells in B cell lymphopenic L2 but not normal mice. The Journal of Immunology, 2004, 173: 197–204.

The generation of mature B lymphocytes from pluripotent hemopoietic stem cells of fetal sources and adult bone marrow (BM) results in the establishment of a heterogeneous adult B cell compartment consisting of two distinct B cell subsets, commonly referred to as B-1 and B-2. They differ in surface marker expression, anatomical location, developmental and maintenance requirements as well as in functional properties (1–4).

Within the B-2 compartment additional subpopulations can be defined: follicular (FO) and marginal zone (MZ) B cells. In the adult BM, B-2 precursors continuously develop into immature B cells which still have to undergo a series of sequential maturation steps to become mature B cells of either the FO or MZ B cell phenotype. This process was suggested to take place in the spleen (5).

B-1 cells can be subdivided into B-1a cells expressing moderate levels of CD5 and the CD5−B-1b sister population. B-1a cells can be found in the peritoneum as well as in the spleen. Although they represent a minor population of the splenic B cell compartment, the absolute number is similar to the number of B-1a cells found in the peritoneum. However, striking differences between splenic and peritoneal B-1a subpopulations in terms of gene expression, physiology, and Ab repertoire indicate that both B-1a subpopulations differ in their developmental program and function (6–9). B-1a cells are believed to develop mainly in early life from fetal sources as a distinct lineage and are maintained in the adult by self-replenishment (10). Evidence was also provided that B-1a cells may develop from the same progenitor as B-2 cells but the specificity of the receptor expressed by a given cell would determine whether it differentiates into a lymphocyte displaying a B-1a or B-2 phenotype (11). The contribution of the respective developmental pathways to the mature B-1a pool remains to be determined. Nevertheless, both models agree in that the development and/or maintenance of B-1a cells is strongly influenced by their Ag specificity and signals via the BCR, which is consistent with continuous positive selection by autoantigens (1, 2).

Compared with B-2 cells, the terminal differentiation processes of immature cells to mature B-1 cells are less well defined with respect to intermediate developmental stages and place of differentiation. Interestingly, peritoneal B-1a cells appear to be strongly dependent on the presence of the spleen (12). Congenitally asplenic Hox11-null mice exhibited substantially reduced frequencies of peritoneal B-1a cells. In addition, splenectomy of wild-type mice resulted in the rapid loss of an already established B-1a cell pool, whereas peritoneal B-1b and B-2 cells were not affected. Congenitally, asplenic and splenectomized (SP) mice exhibited a diminished IgM-mediated immune response against phosphorylcholine and bacterial polysaccharides. This appears to be consistent with the fact that the B-1a lineage contributes most of the natural IgM in the serum of naive animals and preferentially produces Abs against certain thymus-independent Ags like phospholipids and polysaccharides (13). Such findings might at least partly explain recurent and invasive infections with encapsulated bacteria in humans with anatomical or functional asplenia (14).

The mechanistic reason for the dependence of peritoneal B-1a cells on the presence of the spleen is not known (12). The spleen could play an important role in the generation of peritoneal B-1a cells. B-1a cells might be continuously generated from precursors...
Advantages compared with normal mice: 1) The homeostasis of removal of the spleen. The L2 mouse model exhibits two major influences of environmental changes potentially affecting the selection of B-1a cells from L2 mice allows us to study the environment of immunocompetent wild-type mice populate the peritoneum of recipients in substantial numbers but do not enter the splenic B-1a compartment (8, 9).

To elucidate the mechanistic reason for the loss of peritoneal B-1a cells in the absence of the spleen, we made use of the recently established L2 mouse model (16). B cells present in the peripheral lymphoid organs of L2 mice almost exclusively express the Tg λ(23) L chain. A severe block of B cell development in the adult BM results in the predominance of CD5+ B-1 cells, whereas B-2 (FO B) cells are completely absent (9). In accordance with continuous positive selection by autoantigens, B-1a cells of L2 mice exhibit a pronounced oligoclonal IgH chain repertoire, which is characterized by a high frequency of reoccurring identical amino acid sequences mainly due to independent rearrangement events (17). We now investigated to what extent the IgH chain repertoire of the peritoneal B-1a cell pool from L2 mice is influenced by the removal of the spleen. The L2 mouse model exhibits two major advantages compared with normal mice: 1) The homeostasis of B-1a cells can be studied in the absence of continuous influx of adult BM derived B cells. 2) The extensively characterized IgH chain repertoire of B-1a cells from L2 mice allows us to study the influence of environmental changes potentially affecting the selection of B-1a cells.

Matherial and Methods
Mice
Heterozygous L2 mice used for the experiments were crossed on the BALB/c background for 20–25 generations. RAG-1−/− mice were on the BALB/c background.

For surgery, mice were anesthetized using Metofane (Jannsen-Cilag, NJ). After shaving, a small incision was made in the skin at the left flank and the peritoneum was opened. For splenectomies the spleen was removed. For detection of in vivo proliferation, mice were pulse labeled by two i.p. injections of 1 mg of BrdU in a 4-h interval and sacrificed 3 wk thereafter. Cells were first stained for surface Ags and then incorporated BrdU treatment was revealed by an anti-BrdU Ab according to the manufacturer’s recommendations (BD PharMingen). Since some cytotoxic effects of BrdU treatment have been reported (19), treated and untreated mice were compared for the frequency of cells present in the peritoneum. No effect due to BrdU administration was observed.

Amplification of \( V_\mu D_1 J_\mu C_\mu \) chain transcripts, sequencing, and sequence analysis
RT-PCR amplification of \( \mu \) chain transcripts using a \( V_\mu \) gene consensus oligonucleotide (\( V_\mu \) cons) in combination with a \( C_\mu \) constant region primer (\( C_\mu 1 \)) from total RNA of bulk-sorted CD19+ PtC-liposome binding peritoneal cells was done as described previously (17).

The single-cell RT-PCR procedure was previously described in detail (9). In brief, CD5+ IgM+ peritoneal B-1 cells were first bulk sorted and then sorted again to deposit single cells into strips of eight PCR tubes. This two-step sorting process ensures deposition of single cells by exclusion of doublets. As a negative control, one of eight tubes did not receive a cell but was otherwise processed like tubes containing single cells. First round of single-cell RT-PCR was done using the Qiagen OneStep RT-PCR kit (Valencia, CA) and the \( V_\mu \) cons and \( C_\mu 1 \) oligonucleotides. For the second amplification, the same \( V_\mu \) cons oligonucleotides were used in combination with an internal constant region primer (\( C_\mu 2 \)). PCR products were gel purified and then sequenced directly using the oligonucleotide \( C_\mu 2 \). Sequence analysis and comparison were done as described previously (9).

Results
Removal of the spleen does not reduce the peritoneal B-1a cell pool of L2 mice
Normal mice show a rapid loss of peritoneal B-1a cells following splenectomy (12). Consistent with this finding, an ~2-fold reduction of CD5+ IgM+ B-1 cells was observed in the peritoneum of normal control mice 1 wk after splenectomy, mainly affecting the IgM+ CD5+ B220low C-1 compartment (4- to 5-fold reduction). The IgM+ CD5+ B220− population appeared to be unaffected or only slightly reduced up to 3 wk after surgery (Fig. 1A and data not shown). However, B220+ and B220low− B-1a cells were similarly reduced 6 wk after splenectomy, resulting in an ~4- to 5-fold reduction of CD5+ IgM+ B-1 cells (Fig. 1B). In contrast, SP L2 mice did not differ in the frequency of B-1a cells compared with SO controls even 13 wk after splenectomy (Fig. 1, C and D). Importantly, we did not observe any changes in the frequency of B-1b cells following splenectomy in non-Tg mice nor did we find changes in the frequency of CD5− B cells in L2 mice under these circumstances. In addition, the level of CD5 expression on B-1a cells following splenectomy of L2 mice suggests a stable CD5 surface expression (Fig. 1, A–C, and data not shown).

In both L2 and non-Tg mice, total peritoneal cell numbers were unaffected by the surgical procedure. SO and SP non-Tg and L2 mice exhibited total peritoneal cell numbers of 3–4 × 106 cells. In conclusion, in contrast to non-Tg mice, the frequency of peritoneal B-1a cells in L2 mice does not appear to be affected by the removal of the spleen.
Surgery induces proliferation of peritoneal B cells

Both sham operation and splenectomy of normal mice leads to a reduction in the frequency of peritoneal B-1a cells during the first 2 days and only in SO mice B-1a cell numbers begin to recover thereafter (12). To determine whether the peritoneal B cell compartment of L2 mice is affected by loss in cellularity and induction of proliferation following surgery, L2 and non-Tg mice were i.p. pulse labeled with BrdU at day 2 after sham operation or splenectomy. BrdU incorporation would not be expected in untreated mice (20) and was only detected in ~1% of peritoneal B cells from operated L2 and non-Tg mice, irrespective of whether the spleen was removed or not (Fig. 2). Thus, the proliferative response in L2 mice was indistinguishable from that of normal mice, suggesting a similar surgery-mediated B cell loss shortly after operation. Since the adult BM of L2 mice does not produce CD5⁺ B cells (16), proliferation of the remaining B-1a cell population most likely accounts for the compensation of the partial cell loss after sham operation and splenectomy.

Removal of the spleen dramatically changes the selection of peritoneal B-1a cells in L2 mice

Splenectomy might result in changes within the B-1a compartment that are not discernible by monitoring the frequency of such cells. To determine whether the loss of the spleen has any effect on the peritoneal B-1a cell population of L2 mice, sorted peritoneal B-1a cells from pools of three SP or SO L2 mice were used 6 wk after surgery for the amplification of IgH chain transcripts by single-cell RT-PCR. Obtained IgH chain sequences were then compared with IgH chain data sets of peritoneal B-1a cells from unmanipulated L2 mice generated by independent experiments using different techniques (hybridomas, n = 113; single-cell RT-PCR, n = 55; bulk sorting and cloning, n = 59) (9, 17).
The data set of IgH chains of B-1a cells from SO L2 mice resembles the characteristic oligoclonal IgH chain repertoire of untreated L2 mice to a large extent. Of IgH chain amino acid sequences from SO mice, 42% (15 of 34 sequences) were identical to sequences described for the B-1a repertoire of untreated L2 mice (Fig. 3A, SO1–8). From these, three sets of sequences were isolated repeatedly (SO1, SO7, and SO8). Certain IgH chain amino acid sequences were recovered from SO L2 mice at frequencies described for untreated L2 mice. A VH Q52-DFL16.2-J4 H-encoded amino acid sequence dominating the data set from SO L2 mice (SO1: 17.6%, 6 of 34) was isolated from untreated L2 mice at similar high frequencies before (hybridomas: 14.4%; 17 of 118 sequences; single-cell RT-PCR: 14.8%; 8 of 54) (9, 17). Four variants of this IgH chain have been detected so far, differing in their encoding nucleotide sequences but resulting in the same amino acid sequence (9, 17). Furthermore, certain sequences that were found in SO mice, e.g., the V\textsubscript{H}7183-DSP-J\textsubscript{H}1 nucleotide sequence (SO7: 5.9%, 2 of 34), were previously reproducibly recovered from untreated L2 mice, although at low frequencies (hybridomas: 0.9%; 1 of 118; single-cell RT-PCR: 1.9%; 1 of 54; bulk sorting and cloning: 1.7%, 1 of 59).

In striking contrast, peritoneal B-1a cells of SP L2 mice exhibited a severe perturbation of the IgH chain repertoire. No overlapping IgH chain sequences could be recovered from SP and SO mice. Furthermore, only a single IgH chain from B-1a cells of SP mice was identical to an IgH chain observed in previous experiments with untreated L2 mice (Fig. 3A, SP1). Interestingly, this sequence belongs to a group of IgH chains that was shared between both the splenic and peritoneal B-1a compartment (9).

A second IgH chain nucleotide sequence was recovered repeatedly from the peritoneum of SP mice (Fig. 3B, SP2). Two putative D gene segments that are separated from each other by a GC-rich stretch, presumably N nucleotides, could be identified within the CDR3 region of this IgH chain. The repeated recovery of such an extensively diversified IgH chain can only be explained by proliferative expansion in vivo.

The IgH chain CDR3 length comparison between the three data sets revealed additional substantial differences. Peritoneal B-1a of SO and untreated L2 mice showed a similar CDR3 length distribution (Fig. 3C). In both groups, a CDR3 length of 14 aa was...
Splenectomy does not affect phosphorylcholine-specific B-1a cells

Anti-PtC Abs represent a well-documented specificity to common bacterial and autoantigens that, in normal mice, is only produced by B-1a cells (21). The IgH and IgL chains of anti-PtC Abs from normal BALB/c mice are restricted to a few V gene families, mainly V_H Q52, V_H 11, and V_H 12, that are combined with certain κL chains (18). However, in mice exclusively expressing AL chains, the PtC specificity is compensated (9).

The frequency of PtC-specific cells in the peritoneal B-1a compartment of Tg L2 and non-Tg mice was unaffected by the absence of the spleen (Fig. 4). This is in clear contrast to the Ab response against pneumococcal polysaccharides and phosphoryl-choline (12).

It was shown before that an increase of junctional diversity by Tg TdT expression resulted in the loss of protection against pneumococci, demonstrating that the lack of N additions is essential for the generation of protective anti-phosphorylcholine Abs (22). In contrast, the ~3-fold increase of IgH chain N nucleotide insertions after splenectomy (Table I) does not reduce the frequency of PtC-specific B-1a cells. Consistently, preliminary data indicate that Tg expression of TdT does not abrogate the generation of PtC-specific peritoneal B-1a cells (data not shown).

Since in normal mice B-1a cells mediating this specificity are characterized by the frequent occurrence of independent identical IgH and IgL chain rearrangements (18), the unaffected PtC-specific B-1a compartment of SP L2 mice appears to be at odds with the loss of reoccurrent oligoclonal IgH chain sequences. However, the characterization of PtC-specific peritoneal B-1a cells in untreated A2 Tg L2 mice revealed a significantly more heterogeneous IgH chain repertoire compared with non-Tg mice. In agreement with previous observations, IgH chain rearrangements of PtC-specific B-1a cells from non-Tg BALB/c mice most frequently involved V_H 11 and V_H 12 gene segments in combination with J_H 4 and V_H Q52 in combination with J_H 4 (data not shown). Several identical IgH chain sequences could be recovered from independent experiments and some were identical to IgH chains described by others to be involved in the PtC specificity (18). In contrast, PtC-specific peritoneal B-1a cells of L2 mice exhibit a restricted gene segment usage with a preference for VH_Q52, V_H 11, and V_H 12 gene segments in combination with J_H 1 and V_H Q52 in combination with J_H 4 (data not shown).

Several identical IgH chain sequences could be recovered from independent experiments and some were identical to IgH chains described by others to be involved in the PtC specificity (18). In contrast, PtC-specific peritoneal B-1a cells of L2 mice exhibit a restricted gene segment usage with a preference for VH_Q52 in combination with J_H 4 (single cell RT PCR: 56.4%, 22 of 39 sequences; data not shown). No evidence for oligoclonality of such Ag-specific B-1a cells was observed. Only 2 of 39 IgH chains from the single-cell RT-PCR experiment were identical in nucleotide sequence. Different nucleotide sequences resulting in the same amino acid sequence were not observed. In summary, the unaltered presence of PtC-specific B-1a cells following splenectomy appears to be compatible with the loss of the recurring oligoclonal IgH chain sequences.

Table I. Comparison of N nucleotide insertions among peritoneal B-1a cells from L2 mice

<table>
<thead>
<tr>
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<th>No N Nucleotides in</th>
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<th>N Nucleotides in</th>
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<tr>
<td></td>
<td>V_H-D and D-J_H</td>
<td>V_H-D but not D-J_H</td>
<td>V_H-D and D-J_H</td>
<td>V_H-D and D-J_H</td>
</tr>
<tr>
<td>Untreated (54)</td>
<td>61.1% (33)</td>
<td>5.6% (3)</td>
<td>20.3% (11)</td>
<td>7.4% (4)</td>
</tr>
<tr>
<td>Sham-operation (35)</td>
<td>62.9% (22)</td>
<td>8.6% (3)</td>
<td>11.4% (4)</td>
<td>17.1% (6)</td>
</tr>
<tr>
<td>Splenectomy (34)</td>
<td>20.6% (7)</td>
<td>8.8% (3)</td>
<td>35.3% (12)</td>
<td>32.4% (11)</td>
</tr>
<tr>
<td>PtC-liposomes (39)</td>
<td>41.0% (16)</td>
<td>15.4% (6)</td>
<td>23.1% (9)</td>
<td>15.4% (6)</td>
</tr>
</tbody>
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a Frequency of IgH chain transcripts which a particular D gene segment could not unambiguously assigned to.
b Number in parentheses, number of sequences analyzed.
Migratory capacity of B-1a cells that were maintained in the absence of the spleen.

Intraperitoneal reconstitution experiments using either a congenic IgH allotype system or CFSE labeling revealed that peritoneal exudate cells from SO or SP non-Tg and L2 mice were i.p. transferred into RAG-1−/− mice. Recipient mice were analyzed 4 wk after transfer. A, Analysis of peritoneum, blood and spleen of transferred mice for the presence of CD5+ IgM+ B-1 cells. B, Frequency of PtC-specific B-1a cells in peritoneum and spleen. Splenic B cells were enriched by magnetic cell sorting based on the expression of CD19. CD19+ cells were 50–60% CD5+.

FIGURE 5. Migratory capacity of B-1a cells that were maintained in the absence of the spleen. Four weeks after surgery, 2 × 10⁶ cells of peritoneal exudates from SO or SP non-Tg and L2 mice were i.p. transferred into RAG-1−/− mice. Recipient mice were analyzed 4 wk after transfer. A, Analysis of peritoneum, blood and spleen of transferred mice for the presence of CD5+ IgM+ B-1 cells. B, Frequency of PtC-specific B-1a cells in peritoneum and spleen. Splenic B cells were enriched by magnetic cell sorting based on the expression of CD19. CD19+ cells were 50–60% CD5+.

Migratory capacity of B-1a cells that were maintained in the absence of the spleen

Intraperitoneal reconstitution experiments using either a congenic IgH allotype system or CFSE labeling revealed that peritoneal B-1a cells derived from either Tg or non-Tg mice have the potential to populate the peritoneum of immunocompetent recipients but do not enter the splenic B cell compartment (our unpublished observations and Refs. 8 and 9). Nevertheless, transfer of peritoneal exudate cells from L2 and normal mice into the peritoneum of immunodeficient RAG-1−/− mice indicated that B-1a cells have the capacity to enter the spleen in substantial numbers under such conditions (9). To analyze whether peritoneal B-1a cells, which were maintained in the absence of the spleen, still exhibit the potential to enter the splenic compartment in RAG-1−/− mice, peritoneal exudate cells of SO and SP L2 or non-Tg mice were isolated 4 wk after surgery and transferred into the peritoneum of RAG-1−/− mice. Flow cytometric analysis 4 wk after transfer demonstrated that transferred cells of all experimental conditions populate the peritoneum, blood and spleen at similar frequencies (Fig. 5A). Thus, B-1a cells from SO and SP L2 and non-Tg mice were not distinguishable by their capacity to enter the spleen of RAG-1−/− mice based on the frequency of such cells.

Interestingly, peritoneal B-1a cells expressing high levels of surface IgM acquire low surface IgM levels after migration to the spleen, which resembles the phenotype of splenic B-1a cells in unmanipulated mice (our unpublished observation and Ref. 8). Thus, this phenotypical difference between splenic and peritoneal B-1a cells is determined by the particular environment even in RAG-1−/− mice.

In unmanipulated L2 mice, the splenic B-1a compartment exhibits at least a 60- to 70-fold lower frequency of PtC binders compared with the peritoneum (9). This is comparable to the situation in normal mice (9, 23). One might expect that such biased distribution of PtC-specific B-1a cells between both compartments is not maintained after transfer into RAG1−/− mice. Therefore, the frequency of PtC-liposome-binding B-1a cells in peritoneum and spleen of RAG-1−/− mice, i.e., reconstituted with peritoneal exudate cells of SO or SP L2 and non-Tg mice, was analyzed (Fig. 5B). Surprisingly, although
PtC-specific B-1a cells were detectable in the peritoneum of recipients 4 wk after transfer, such cells were below detection level in the spleen. Thus, the anatomical distribution of PtC-specific B-1a cells is maintained even under severe lymphopenic conditions. This is consistent with our previous interpretation that splenic and peritoneal B-1a cells represent two specialized and distinct lineages (9).

Discussion
To further the influence of the spleen on peritoneal B-1a cells, we have analyzed the consequences of splenectomy in IgL chain Tg L2 mice. In this study, we show that in striking contrast to normal mice the same frequency of peritoneal B-1a cells can be maintained in L2 mice in the absence of the spleen for long periods of time. Why does splenectomy differentially affect the frequency of peritoneal B-1a cells in L2 and normal mice? Gene expression profiling interrogating ~12,000 transcripts revealed ~25 transcripts to be 5-fold or more regulated between peritoneal B-1a cells from L2 and normal mice (9). In contrast, the comparison of B-1a cells and B-2 cells revealed >600 genes that are at least 5-fold differentially expressed (9). Thus, by gene expression profiling, peritoneal B-1a cells from L2 mice appear closely related to B-1a cells from normal mice.

The major difference between L2 and normal mice appears to be the absence of a continuous influx of BM-derived B-2 cells into the periphery of L2 mice as revealed by BM chimeras (16). Thus, in normal mice B-1a cells may have to compete with the compensatory influx of B-2 cells into the peritoneum. In contrast, in B cell lymphopenic, L2 mice B-1a cells have the potential to compensate the surgery-mediated loss of cellularity.

The loss of peritoneal B-1a cells under such circumstances could be explained by the production of a survival factor in the spleen that either acts on migrating B-1a cells or on cells residing in the peritoneal cavity. The general migratory capacity of peritoneal B-1a cells was demonstrated with parabiosed mice (24). Whether the peritoneal and splenic B-1a compartments are also interrelated by exchange was not addressed in this study. We and others described that peritoneal B-1a cells transferred into the peritoneal cavity of immunocompetent mice, although capable of populating the peritoneum, do not enter the splenic B-1a compartment even after long time periods posttransfer (8, 9). In contrast, after i.p. transfer into RAG-1−/− mice, peritoneal B-1a cells do enter the spleen of such mice (9). The assumption that B-1a cells, depending on the presence of the spleen, have to circulate continually through this organ implies that peritoneal B-1a cells that are still present following splenectomy are not migratory. However, peritoneal B-1a cells from either SO or SP L2 or non-Tg mice did not differ in their potential to enter the spleen after i.p. transfer into RAG-1−/− recipients. Thus, they retain their capacity to migrate under such experimental conditions.

It seems reasonable to postulate that the survival factor, which is lost following splenectomy, represents Ags. Such Ags appear to mediate the continuous positive selection of B-1a cells, which is required for the maintenance of the self-renewing B-1a cell pool in the adult. Removal of splenic autoantigens results in the failure of continuous positive selection. Although such Ags are associated with the spleen, the entry of B-1a cells into the splenic B cell compartment might not be required. In the absence of competing B-2 cells, like in L2 mice, the loss of such cells might be quickly compensated by proliferation of B-1a cells that are independent of the spleen and splenic autoantigens. In contrast, in normal mice such compensation is not seen since B-2 cells might be migrating to and/or proliferating in the peritoneum. Whether B-1a cells are able to reconquer this space eventually needs to be shown. Further studies should be directed toward the identification of the survival factors, i.e., the autoantigens mediating the maintenance of the natural peritoneal B-1a repertoire.

Alternatively, the presence of a B-1a precursor in the spleen could readily explain the loss of peritoneal B-1a cells after removal of the spleen. However, no evidence exists for undifferentiated splenic B-1a cell precursors, i.e., precursors that have not yet completed their H and L chain rearrangements. Consequently, Clarke and colleagues (15) suggested the presence of a surface IgM+ B-1a precursor residing in the spleen. They described CD5+ CD43+ CD23inhB220inh IgMinh B cells in the spleen of mice carrying the anti-PtC IgH and IgL chain genes Vιι12 and Wιι4 as transgenes. This surface marker phenotype was considered to be indicative of an intermediate developmental stage at the transition from B-2 cells (CD5+ CD43+ CD23+ B220+ IgM+) to B-1a cells (CD5+ CD43+ CD23− B220inh IgMinh). However, transfer of peritoneal B-1a cells from either L2 or normal mice (CD5+ CD43+ CD23− B220inh− IgMinh) into the peritoneum of RAG-1−/− mice demonstrated that, after migration to the spleen, such cells are still CD5+ CD43+ but acquire a B220inh IgM phenotype and segregate into CD23− and CD23inh subpopulations (our unpublished data). Thus, such phenotypic differences are dependent on local environmental influences rather than on their developmental program. In addition, we show here that the PtC-specific peritoneal B-1a cell pool in both L2 and normal mice does not require continuous influx from the spleen. In striking contrast to phosphorylcholine-specific B-1a cells (12), even 13 wk after removal of the spleen we found the frequency of PtC-specific B-1a cells not to be affected by splenectomy in L2 and non-Tg mice. Our findings are therefore not compatible with the requirement of splenic surface IgM+ B-1a precursors to maintain the bulk population of PtC-specific B-1a cells. Although we cannot exclude this for Vιι12/Vιι4 B-1a cells, we feel that a putative B-1a precursor in the spleen does not account for the rapid loss of peritoneal B-1a cells after splenectomy.

Independent of the frequency, dramatic changes took place in the peritoneal B-1a compartment of L2 mice after splenectomy, as revealed by the loss of the predominating B-1a specificities, changes in the pattern of CDR3 lengths, and the increase of CDR3 diversity. The unexpected loss of the 14-aa CDR3 length predominating in unmanipulated and SO L2 mice remains puzzling. It could only partially be explained by the loss of the dominating VιιQ52-DFL16.2-4Jιι4e-encoded clones. Structurally similar Ags might be involved in the positive selection process of such additional IgH chain specificities leading to similar sequences and CDR3 length. However, with the exception of the clonal amino acid sequence mentioned above, the IgH chains exhibiting a 14-aa CDR3 length in the presence of the spleen appear to be heterogeneous with respect to their CDR3 amino acid sequence (data not shown).

The dependence of the oligoclonal sequences dominating the IgH chain repertoire of unmanipulated and SO L2 mice on the spleen and the dramatic perturbation of the repertoire after splenectomy clearly indicates changes in the selective forces acting on the B-1a cells that were maintained in the absence of the spleen. Substantial evidence for positive selection mechanisms essential for the generation of B-1a cells exists (1, 2). The most clear-cut example for the necessity of an interaction with autoantigen to generate B-1a cells is the natural serum Ab specific for the Thy-1 glycoprotein (CD90). B-1a cells expressing the anti-Thy-1 BCR as a Tg could only be demonstrated when the autoantigen was present in these mice (25). However, the role of autoantigen in the survival of mature B-1a cells remains to be determined. The same is true for the multitude of genetic events known to alter the number of B-1a cells in the adult mouse (1, 2).
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References