The Initiation of B Cell Clonal Expansion Occurs Independently of Pre-B Cell Receptor Formation

Gregory H. Kline, Tracy A. Hayden and Patricia Riegert

J Immunol 2001; 167:5136-5142; doi: 10.4049/jimmunol.167.9.5136
http://www.jimmunol.org/content/167/9/5136

References
This article cites 37 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/167/9/5136.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
The Initiation of B Cell Clonal Expansion Occurs Independently of Pre-B Cell Receptor Formation

Gregory H. Kline, Tracy A. Hayden, and Patricia Riegert

Current models of B cell development posit that clonal expansion occurs as a direct result of Ig H chain expression. To test this hypothesis, we isolated a population of early B cells in which H chain recombination is initiated and assessed \( V_{H}DJ_{H} \) rearrangements in both cycling and noncycling cells. We found that actively dividing cells within this population are enriched for H chain rearrangements that are productive when compared with their counterparts in G0/G1, apparently supporting a role for H chain expression in initiating early B cell division; entrance into the cell cycle was accompanied by \( V_{H} \) gene-dependent H chain selection. However, we also identified a phenotypically identical population of actively cycling early B cells in the absence of H chain expression in recombination activating gene knockout mice. In addition, actively cycling early B cells could be detected in pre-B cell receptor (pBCR)-negative A5 knockout mice, but we found no evidence for \( V_{H} \)-dependent H chain selection in this population. Given these results, we suggest that the initiation of clonal expansion, at this early stage in B cell development, occurs independently of H chain expression. Although the cycling cell pool is enriched for pBCR-positive cells in mice expressing surrogate L chain, pBCR formation is not required for the initiation of cell division.


Several groups have defined discrete stages in the B cell developmental pathway by differential expression of cell surface markers (1–3). Two commonly applied methods use the differential expression of either CD43 (leukosialin) or CD117 (c-kit) in combination with cell size in defining the pro-B cell stage of development (1, 2). The DNA recombination events necessary to produce an H chain occur in pro-B cells, which express both leukosialin and c-kit. Hardy and coworkers (1) have further delineated pro-B cells based on the expression of CD24 (heat-stable Ag, or HSA)\(^{3}\) and aminopeptidase A, recognized by the BP-1 Ab. According to this scheme, cells in the earliest stage of B cell differentiation do not express CD24 or aminopeptidase A and are designated fraction A, whereas those in the next stage, fraction B, express CD24 but not aminopeptidase A. Fraction C contains the most mature pro-B cells, which express both CD24 and aminopeptidase A on the cell surface. CD19 first can be detected in cells in fraction B and is present thereafter during B cell development (4). DNA content analyses of these populations indicate that the majority of pro-B cells are in G0/G1, although a sizeable proportion of cells within fraction C are actively cycling (1). All of the cycling cells within fraction C belong to a subpopulation, fraction C\(^{\prime}\), defined by relatively high levels of CD24 expression.

During B cell development, the transition from pro-B to pre-B (fraction D) cell is marked by four to six rounds of cell division (5, 6). The initiation of cell division at this stage in B cell development has been ascribed to Ig H chain formation. Support for the role of H chain in this process has come from studies using animals in which the H chain is not produced. Recombination activating gene (RAG) 1 or 2 knockout mice lack one of the proteins required for DNA recombination (7, 8). In these animals, B cells fail to form at sites where B cell lymphopoiesis normally occurs. B cell development in RAG knockout (RAG\(^{−/−}\)) mice is arrested in fraction C, and the HSA high-expressing fraction C\(^{\prime}\) cells are absent. By crossing a H chain transgene onto the RAG knockout background, fraction C\(^{\prime}\) is restored (9). This result has been used to support the supposition that H chain expression initiates B cell clonal expansion.

The presence of a productive H chain, one from which a H chain protein can be expressed, culminates in the assembly of the pre B cell receptor (pBCR). The pBCR complex is formed by the interaction of Ig H chain with the surrogate light chain (SLC), itself a heterodimer consisting of the \( \lambda \)5 and VpreB proteins (10, 11). Mice lacking the \( \lambda \)5 protein (A5 knockout, A5\(^{−/−}\)) fail to form SLC and pBCR and harbor profoundly reduced B cell numbers. However, some B cells do mature in these animals, unlike the complete block observed in RAG\(^{−/−}\) mice. These mature B cells may arise as a result of “premature” L chain recombination, whereby L chain acts as a “surrogate” for SLC, allowing continued B cell differentiation (12, 13). Alternatively, in the absence of SLC, B cell maturation may be severely hindered but not absolutely prevented; that, is, SLC expression and consequent pBCR formation may greatly increase the efficiency of, but are not required for, B cell differentiation. Following this scenario, a small number of early B cells progress to fraction D, whereupon developmentally “normal” L chain recombination occurs (14). The reduction of B cell numbers seen in A5\(^{−/−}\) mice has been attributed to a lack of H chain-mediated clonal expansion, which occurs independently of B cell differentiation. However, the presence of a productive H chain rearrangement does not ensure clonal expansion. Recent studies have found that up to 50% of all productive H chain rearrangements are incapable of pairing with SLC, hence, they are “dysfunctional” (15, 16). Expression of a dysfunctional H chain as a transgene fails, on its own, to mediate B cell clonal expansion (17). Additionally, dysfunctional H chains fail to mediate allelic exclusion (15, 16).

The Basel Institute was founded and supported by Hoffman-LaRoche, Ltd.

1 The Basel Institute was founded and supported by Hoffman-LaRoche, Ltd.

2 Address correspondence and reprint requests to Dr. Gregory Kline, Basel Institute for Immunology, Grenzacherstrasse 487, Basel, CH-4005, Switzerland. E-mail address: kline@bii.ch

3 Abbreviations used in this paper: HSA, heat-stable Ag; RAG, recombination activating gene; pBCR, pre B cell receptor; SLC, surrogate L chain; slg, surface immunoglobulin; pTCR, pre TCR.

Received for publication June 18, 2001. Accepted for publication September 6, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Copyright © 2001 by The American Association of Immunologists

0022-1767/01/$02.00
Previous studies indicate that V to DJ H chain recombination occurs within fraction B (12, 13). The components of the SLC, A5, and VpreB, are also expressed, and actively dividing cells can be detected within fraction B (18). These observations, taken together, lead us to speculate that H chain-mediated clonal expansion may in fact occur earlier than previously suggested, within fraction B rather than fraction C. To test this hypothesis and the role of pBCR formation in this process, we sorted fraction B cells based on both cell surface marker phenotype and cell cycle status, isolating fraction B cells either in G1/G0 or S/G2/M of the cell cycle. We found that actively dividing cells are enriched for productive H chain rearrangements when compared with cells in G1/G0, with the same cell surface marker phenotype, supporting the view that H chain-driven clonal expansion does in fact occur at fraction B. However, a population of cycling cells with the cell surface marker phenotype of fraction B can be isolated from RAG-/- mice, which are incapable of H chain formation. In addition, analyses of A5+/-- mice demonstrated that cells within fraction B enter the cell cycle independent of pBCR formation but, perhaps as expected, productive H chains are not selected for in the absence of SLC expression. Based on these results, it appears that B cell clonal expansion, at this early stage in development, occurs independently of H chain status. In this context, pBCR formation is not required for the initiation of B cell clonal expansion, but rather acts as a means to enrich for cells that harbor functional H chain rearrangements into the cycling cell pool.

Materials and Methods

Animals

All animals used in this study were sacrificed between 6–8 wk of age. BALB/c and C57BL/6 mice were purchased from BRL (Fullinsdorf, Switzerland), BALB/c RAG-2+/-- (backcrossed 12 generations on the BALB/c background) were purchased from Taconic Farms (Terrytown, NY), and C57BL/6-A5+/-- (backcrossed 6 generations on the C57BL/6 background) were received from The Jackson Laboratory (Bar Harbor, ME).

Isolation and staining of whole bone marrow cells for cell sorting and analysis

Whole bone marrow cells were isolated by flushing the femurs and tibiae of mice with HBSS (Life Technologies, Rockville, MD) supplemented with 0.1% BSA Fraction V (HB; Sigma, St. Louis MO). Cells then were counted, pelleted by centrifugation, and resuspended to a cell density of 10^7 cells/ml in HBSS supplemented with 10% FCS and 1 mg/ml of Hoechst 33342 (Molecular Probes, Eugene, OR). The cells were incubated in the presence of this Hoechst solution in a H2O bath maintained at 37°C for 1 h. After this, the cells were pelleted by centrifugation, the Hoechst solution discarded, and the cells washed by resuspension in HB supplemented with 0.1% sodium azide (HBA). The cells then were enumerated before beginning staining with anti-CD43FITC (clone S7; BD PharMingen, San Diego, CA). After a 15-min incubation on ice, excess Ab was removed by washing the cells with HB. The cells then were resuspended in HBA supplemented with of 1 mg/ml of rat Ig (The Jackson Laboratory) and stained with goat anti-mouse IgG(‘A’M)-biotin (Zymed, South San Francisco, CA) for 15 min on ice followed by washing with HBA plus 1 mg/ml rat Ig and resuspended in HBA. The biotin was then removed by staining with SA-Cy5. Next, the cells were stained with anti-CD19 SpectraRed (Southern Biotech Associates, Birmingham, AL) and BP1 PE (BD PharMingen). After a final wash, the cell suspension was passed through nylon gauze and sorted exclusively on the basis of cell surface markers using a MoFlo (Cytometry, Fort Collins, CO) high speed sorter. Sorted surface Ig (sIg) CD19+/CD43+ BP1+ cells then were then resolved into G1/G0 and S/G2/M populations based on Hoechst 33342 staining by a second sort with a FACSVantage (BD Biosciences, Mountain View, CA) equipped with an Enterprise laser (Coherent, Santa Clara, CA).

Genomic DNA preparation, PCR amplification, and DNA sequencing

On completion of the second sort, genomic DNA was prepared from the sorted cell populations with a blood and cell culture DNA mini kit (Qiagen, Basel, Switzerland) following the manufacturer’s instructions for the isolation of genomic DNA from cultured cells. A total of 200 ng of this genomic DNA then was used as the starting template for the first round of nested PCR, with the initial reaction consisting of 5 μl of 10X Taq buffer, 2.5 mM MgCl2, 2.5 ng of VH-specific primer 1, 2.5 ng of JH-universal primer, 2.5 U of Taq polymerase (Roche Diagnostics, Basel, Switzerland), and H2O to a total volume of 50 μl. The reactions were placed into a thermal cycler (Biorad, Tampa, FL) and subject to 10 min at 95°C, followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 1.5 min. The resultant products then were extended by a 10-min incubation at 72°C followed by a 4°C soak. A total of 2 μl of this first reaction was used as the template for the second round of PCR. This reaction was conducted as above with the following exceptions: VH-specific primer 1 was replaced by VH-specific primer 2, one of four JH-specific primers was used instead of the JH-universal primer, and the thermal cycling consisted of 10 min at 72°C, 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, and an extension of products with a 10-min incubation at 72°C followed by a 4°C soak. Therefore, four independent second-round PCR amplifications were conducted to isolate VHDJH rearrangements using each JH individually. On completion of this second round of PCR, the resultant products were cloned with the TOPO-TA cloning kit (Invitrogen, San Diego, CA) following the manufacturer’s instructions. Plasmid templates were sequenced with the Big Dye Terminator Cycle sequencing kit (Applied Biosystems, Warrington, U.K.) and run on a model 377 automated sequencer (Applied Biosystems) following manufacturers instructions.

Sequences of oligonucleotide primers used for PCR

The following primers were used for PCR analysis: for VH-specific primer 1 (location number given from first coding nucleotide of the VH exclusive of leader sequence), 36–60 ctgctgtggaaaacacttcag (2–25), 81X ggag ggcttagcgacgctcagag (25–48), J58 ctctggtgagatactcgaag (47–70), Q52 cccaggtgctgaagctcag (–22); for VH-specific primer 2 (non-germline encoded A-start site is underlined), 36–60 gctctgtggacgctgagt cactg (25–48), 81X cctctgtggacgctgagt (49–72); J58 gctctgg gcctggcattacctg (69–92), Q52 gctctgtgctgaagctcag (92–122); for JH universal primer (location number given from first nucleotide of JH1), gagaactctcaacaagc (1355–1373); and for JH-specific primers JH1, acatatctgcatcagcctagg (137–160); JH2 gggggagttcataataactctg (550–573); JH3, acaaaaaaggttgtagtg (827–860); and JH4, aaaaaaaagctcg gacag (1331–1353).

Results

The initiation of early B cell clonal expansion occurs within fraction B

After H chain recombination, during the course of B cell development, clonal expansion is thought to be initiated by the expression of Ig H chain (9). To determine whether H chain-mediated clonal expansion initiates within fraction B, the earliest defined stage in B cell development in which variable (VH) gene to DJH recombination is observed, we isolated this population (sIg + CD19 + CD43 + BP1 + ) from mouse bone marrow by cell sorting as depicted in Fig. 1. Previous studies aimed at defining early B cell developmental stages have relied on relative cell size or an associated increase in HSA (CD24) expression as an indicator of cell cycle status (1, 2). Rather than depend on these indirect measures for assessing cell cycle status, we have further resolved this early B cell population with the viable DNA binding dye Hoechst 33342, which is actively transported across the cell membrane, negating the need for fixation. Using this combination of cell surface markers and Hoechst 33342 staining, it was possible to directly isolate early B cells either in G1/G0 or S/G2/M of the cell cycle. Within this isolated early B cell population, 20–25% of cells were consistently found to be actively dividing, that is, in S/G2/M of the cell cycle.

We sought to determine whether the cell division we observed in this early B cell population was mediated by the presence of H chain. We predicted that if cell division was driven by H chain, then actively dividing cells would be enriched for productive H chain rearrangements in comparison with cells within G1/G0. This was accomplished at the population level by determining the ratio of productive (capable of expressing a full-length H chain protein)
to nonproductive (incapable of expressing a full-length H chain protein due to the presence of a premature stop codon or frame shift) rearrangements for selected V\textsubscript{H} genes or gene families (Fig. 2 and Refs. 19–21). Because the majority of H chain rearrangements are nonproductive, H chain-mediated clonal expansion would be expected to result in an increased proportion of productive H chain rearrangements in cycling cells vs their noncycling counterparts. As shown in Fig. 2, the percentages of productive rearrangements of all of the H chain rearrangements isolated were found to be higher among actively cycling cells when compared with cells in G\textsubscript{0}/G\textsubscript{1} with the same cell surface marker phenotype. The enrichment of productive H chain rearrangements among cycling cells provides evidence for the initiation of H chain-mediated clonal expansion within Fraction B. Although Fraction B cycling cells were enriched for productive H chain rearrangements, it is important to note that this enrichment varied considerably among the H chain rearrangements assessed. Whereas 68% of all rearrangements using V\textsubscript{H} 36–60 were productive, only 36% of V\textsubscript{H} 81X rearrangements were productive. ten Boekel et al. (15, 16), using an in vitro assay for testing the ability of H chains to pair with SLC, demonstrated that there is a differential propensity for pBCR formation dependent on the VH gene used. In this study, it was observed that of all H chain proteins tested, those that used the VH 81X gene were the least likely to associate with SLC and form pre-BCR. The differences we observe appear to correlate with the propensity of an H chain to interact with SLC. However, V\textsubscript{H} gene use did not affect the percentages of productive rearrangements seen among G\textsubscript{0}/G\textsubscript{1} cells. In fact, productive rearrangements using V\textsubscript{H} 36–60 gene family members and V\textsubscript{H} 81X were equivalent in nondividing cells, with ~20% of all rearrangements being productive. This would be expected if H chain DNA recombination were to begin in this population, with the majority of rearrangements being nonproductive.
Although the cells we isolated have the cell surface marker phenotype ascribed to early B cells, we have found that this population can include a small fraction of slg^− B cells. These “contaminating” slg^− cells were found to represent up to 10% of the cells in G_0/G_1 of the cell cycle (data not shown). Some of these cells no longer express surface IgM, but had apparently undergone class switching. The possibility that our analyses were skewed by the presence of H chain rearrangements from mature B cells was precluded by the removal of cells bearing any H chain isotype by FACS (Fig. 1).

**Actively cycling early B cells can be isolated from recombination-deficient mice**

Our findings lead us to suppose that in a sense, fraction B mirrors fraction C, with B and B’ reflecting C and C’, with H chain-driven clonal expansion occurring in fraction B’, followed by progression to C’ and continued expansion and differentiation. To test this model, we examined the bone marrow cells from recombination-deficient RAG^-/- mice for the presence of cycling fraction B cells. In RAG^-/- mice, fraction C’, a subset of fraction C delineated on the basis of relatively high expression of CD24, is absent, and B cell development is halted within fraction C. We expected that if the initiation of cell proliferation among fraction B early B cells was dependent on the presence of H chain, then such cells would be absent in RAG^-/- mice. The loss of this population would not necessarily have been observed in previous studies because cycling fraction B cells represent only 0.2% of nucleated bone marrow cells, and because fraction B cells could enter fraction C, there is not that stark loss of future developmental stage as is seen between fractions C and D. However, it must be noted that Bosma has observed the loss of cells expressing the highest levels of CD24 among fraction B cells in SCID mice (22). Our FACS analysis of fraction B cells from RAG-deficient mice is shown in Fig. 3. Contrary to our expectations, we found that actively cycling fraction B cells could be found in RAG-deficient mice. Therefore, cell division within this population appears to occur independently of H chain expression.

**Actively dividing fraction C cells can be identified in RAG-deficient mice**

Given our finding that cell division could be initiated in the absence of H chain in fraction B, we sought to determine whether actively cycling cells could be identified among fraction C cells in RAG-2^-/- mice. As stated earlier, fraction C’, a subset of fraction C, identified by relatively high levels of CD24, is absent in RAG knockout mice. Because actively cycling cells reside in fraction C’, absence of this population in RAG^-/- mice is the basis for the supposition that B cells do not actively divide in the absence of H chain. In fact, we observed that actively cycling fraction C cells could be identified in RAG^-/- mice, although the percentage of such cells (7%) is much lower than that observed in recombination-competent control animals (30%; Fig. 4, b and e). However, comparison of the total pro-B cell compartments (CD19^+CD43^+) of control mice and RAG-2^-/- mice, not divided into fractions B or C (Fig. 4, c and f), revealed that the overall number of cycling early B cells was, on average, greater in RAG-deficient mice than in control animals (457,000 in RAG-2^-/- mice compared with 313,000 in controls).

**Analysis of B cell clonal expansion among fraction B cells isolated from λ5 knockout mice**

Our findings in RAG^-/- mice could be used to imply that initiation of early B cell clonal expansion occurs in the absence of H chain. However, this is tenuous based on this data alone, because in RAG^-/- mice, we are limited to the presence of cell surface markers in identifying B cells. Recent studies have demonstrated that expression of cell surface Ags commonly associated with the
B lineage does not necessarily mean that such cells are committed to the B cell developmental pathway (23, 24). To overcome this obstacle, we decided to investigate whether H chain rearrangements could be isolated from actively dividing fraction B cells from $\lambda^5^-/-$ mice. B cell numbers within the $\lambda^5^-/-$ mice are profoundly reduced, presumably owing to a lack of pBCR-directed clonal expansion (25). Unlike RAG$^-/-$ mice, H and L chain recombination are not prevented in $\lambda^5^-/-$ mice, and a few mature B cells do develop. Analyses of bone marrow cells isolated from $\lambda^5^-/-$ mice revealed that cells that we identified as fraction B were present (Fig. 5). The relative percentage of actively dividing cells within this population was similar to that observed in pBCR$^+$ control mice. The isolation of H chain rearrangements from these cells allowed us to assign these cells unambiguously to the B lineage. Rearrangements isolated from $\lambda^5^+/-$ mice fraction B cells sorted according to cell cycle status are depicted in Fig. 6 along with those rearrangements from pBCR$^+$ mice. Comparing the relative percentages of productive H chain rearrangements between pBCR$^+$ and pBCR$^-$ ($\lambda^5^-/-$) mice revealed a striking reduction in the percentage of productive $V_{H}36-60$ rearrangements in cycling cells from pBCR$^-$ mice. In contrast to pBCR$^+$ mice, productive $V_{H}36-60$ and $V_{H}81X$ rearrangements occurred at almost equal frequency among the actively cycling B cell pool in $\lambda^5^-/-$ mice. Therefore, pBCR formation is not required for the entrance of cells into the cell cycle, but rather facilitates the entrance and perhaps the survival of B cells expressing functional H chains into an already cycling cell pool.

Discussion

In this study, we sought to define the role of Ig H chain in mediating B cell clonal expansion. To do so, we isolated the pro-B cell population, fraction B, in which H chain expression begins. Before this, initiation of early B cell clonal expansion was thought to occur as a direct result of Ig H chain expression. Based on the comparison of H chain rearrangements isolated from fraction B cells either in G0/G1 or S/G2/M of the cell cycle, we found that actively cycling fraction B cells are enriched for productive H chain rearrangements. This finding is in apparent agreement with the widely held view that Ig H chain initiates early B cell division. Contrary to expectations, we found that a phenotypically indistinguishable population, based on cell surface marker expression, of actively cycling fraction B cells could be identified in RAG$^-/-$ mice that are incapable of forming an H chain molecule. Whether these populations are actually identical and comparable in the sense of developmental stage is unclear because in the case of “wild-type” mice, these cells would have presumably expressed RAG. We have interpreted these results to mean that early B cell division occurs as a “preprogrammed” event in the course of development rather than as a consequence of H chain formation.

Our current working model of B cell development centers on the idea that microenvironmental conditions exist that force early B cells to enter the cell cycle in the absence of H chain signaling. In this model, early B cell division occurs as a result of contact with or secretion of cytokines by surrounding cells. After this initial round(s) of cell division is completed, continued cell division and differentiation are dependent on the pBCR, which relieves the need for cell-to-cell contact and/or exogenous cytokines. Anecdotal support for the concept of a defined cycling early B cell niche is derived from our finding that even though RAG$^-/-$ mice fail to make H chain, the absolute numbers of cycling pro-B cells is equivalent to, if not greater than, that observed among recombinant-competent control mice. This model concurs with a scheme of early B cell development previously proposed by Paige and others (26–30) in which early B cell expansion and development is dependent on IL-7, along with cell-to-cell contact between pro-B cells themselves and the surrounding stromal cells. Perturbations in developing B cell numbers, as observed after vaccination or hormone treatment or in aging mice, may be the result of alterations in the size of the cycling cell niche (31–33).

If the initiation of B cell clonal expansion is not dependent on H chain expression, then why are cycling cells enriched for productive H chain rearrangements?

If early B cell clonal expansion is dependent on H chain expression, all cycling early B cells, by definition, would be required to possess a productive H chain molecule. If B cell expansion occurs independently of H chain-derived signals, then not all cycling B cells would be expected to have a productive H chain. However, we observed an enrichment of productive H chain rearrangements among cycling fraction B cells. How is this selection brought about? Taking our findings in wild-type, RAG$^-/-$, and $\lambda^5^-/-$ mice together, we propose that pBCR formation acts to enrich the cycling early B cell pool with cells that possess productive H chain

FIGURE 5. The Absence of pBCR does not prevent entrance into cell cycle, slg$^+$/CD19$^+$CD43$^-$BP1$^-$ early B cells from the bone marrow of C57BL/6 control mice and C57BL/6-$\lambda^5^-/-$ mice were sorted on the basis of cell surface marker phenotype as shown in Fig. 1. The isolated cells then were analyzed based on their cell cycle status. C57BL/6 (a) and C57BL/6-$\lambda^5^-/-$ (b) mice with the percentage of cells in G0/G1 represented by the number given on the left of each panel and the percentage of cells in S/G2/M represented by the number given on the right of each panel.

FIGURE 6. Comparison of H chain rearrangements isolated from slg$^+$/CD19$^+$CD43$^-$BP1$^-$ cells delineated on the basis of cell cycle status between C57BL/6 and C57BL/6-$\lambda^5^-/-$ mice. H chain rearrangements using either the $V_{H}81X$ gene or $V_{H}36-60$ gene family members among slg$^+$/CD19$^+$CD43$^-$BP1$^-$ cells isolated as G0/G1 or S/G2/M from C57BL/6 or C57BL/6-$\lambda^5^-/-$ mice are shown. Rearrangements isolated from cells in G0/G1 are depicted with filled bars, and the rearrangements from actively cycling cells (S/G2/M) are shown with grey bars. The $V_{H}36$ gene or $V_{H}81X$ gene family analyzed is shown below the graph with bars within the brackets representing rearrangements using the specified $V_{H}$ gene or $V_{H}$ gene family. The number given above each bar represents the percentage of productive rearrangements of either $V_{H}81X$ or $V_{H}36-60$, and the number in parentheses is the number of unique sequences analyzed.
rearrangements as outlined in Fig. 7. Accordingly, pBCR expression may render pro-B cells more sensitive to the cycling signals in this microenvironment. Therefore, B cells expressing H chains that pair with SLC are promoted into the cycling cell pool. In mice incapable of pBCR formation (A5<sup>−/−</sup>), entrance into the cycling cell pool of H chain-expressing B cells is not guaranteed. Therefore, the initiation of cell division among early B cells with productive H chains is unknown. Whether there are strain-dependent differences in the propensity of a particular V<sub>H</sub> gene to form functional H chains is unknown.

Is the initiation of clonal expansion in the absence of recombination unique to B cells?

Early B and T cell development occur in a similar fashion in that cells undergo H chain recombination (Ig or TCR-β), which, if productive then pairs with SLC or pre-T α, and possibly forms a pBCR or pre-TCR (pTCR), expression that is followed by clonal expansion and differentiation (34). Recently, Petrie and coworkers (35, 36) have investigated the role of TCR-β-chain recombination in regulating early T cell division. Before this work, TCR-β expression was thought to be required for the entrance of CD4<sup>−</sup>CD25<sup>−</sup>44<sub>low</sub> (DNIII) thymocytes into the cell cycle. Comparison of DNIII cells from RAG knockout mice and wild-type controls revealed that the initiation of cell division, at this stage in T cell development, occurred independently of TCR-β expression. Therefore, the initiation of cell division among early B and T cells appears to occur in the absence of Ig H chain or TCR-β-derived signals. However, a question not directly addressed in Tourigny’s studies (35) was the role of pTCR. It is interesting to speculate that pTCR, in T cell development, may act in a manner akin to pBCR in B cell development. It would be of interest to know whether productive TCR-β rearrangements are favored among cycling DNIII cells from pTCR<sup>−/−</sup> mice but not so from pTCR<sup>−/−</sup> pre-T α knockout mice.

Acknowledgments

We express our great appreciation to Drs. A. Rolink and S. Gillilan for helpful discussions and critical reading of the manuscript.

References


Downloaded from http://www.jimmunol.org/ by guest on April 20, 2017