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The CDR-H3 Repertoire from TdT-Deficient Adult Bone Marrow Is a Close, but Not Exact, Homologue of the CDR-H3 Repertoire from Perinatal Liver

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Compared with adult bone marrow (BM), the composition of the perinatal liver CDR-3 of the Ig H chain (CDR-H3) repertoire is marked by a paucity of N nucleotides and by enrichment for use of JH proximal DQ52 and DH proximal VH and JH gene segments. To test the extent to which these differences reflect limited perinatal TdT activity versus differences in the fetal/adult environment, we used the Hardy scheme to sort fractions B–F B lineage cells from TdT-deficient BALB/c adult BM. Vh7183-containing VDJC transcripts from these cells were amplified, cloned, sequenced, and compared with transcripts from wild-type perinatal liver and adult BM. The pattern of VhDjH usage in TdT-deficient BM largely matched that of TdT-sufficient adult cells. What minor transcripts from these cells were amplified, cloned, sequenced, and compared with transcripts from wild-type perinatal liver and adult BM. The pattern of VhDjH usage in TdT-deficient BM largely matched that of TdT-sufficient adult cells. What minor differences were detected in the pro-B cell stage tended to diminish with B cell maturation, suggesting strong environmental or Ag-driven pressure to achieve a specific range of VhDjH usage regardless of the extent of N nucleotide addition. However, although the patterns of VhDjH usage in the TdT-deficient B lineage cells paralleled that of wild-type adult cells, the length distribution, global amino acid composition, and charge distribution of the CDR-H3 repertoire proved to be a close, although not exact, homologue of the CDR-H3 repertoire first expressed by late pre-B cells in the TdT-insufficient perinatal liver. Thus, although differing in Vh content, TdT-deficient mice appear to represent a good, although not perfect, model for testing the role of perinatal CDR-H3 limitations on late B cell development and Ab responses. The Journal of Immunology, 2010, 185: 6075–6084.

For Ig, the B cell Ag receptor, diversity is the property of the variable (V) domains of the H and L chains, which are manufactured and then sequentially tested and selected during B cell development (1–5). Diversity is asymmetrically distributed within each V domain (6, 7). In the primary sequence, three intervals of hypervariability, termed CDRs, are separated from each other by four relatively conserved framework regions. CDR-3 of the Ig H chain (CDR-H3), which is encoded by the 3′ end of the VH, the 5′ end of the JH, and the entire D, is the direct product of VDJ joining and can be supplemented by non-germline encoded nucleotides (N nucleotides) introduced randomly at the sites of joining by TdT. Its location at the center of the Ag binding site means that CDR-H3 often plays a critical role in Ab specificity (6–8). The inclusion of N nucleotides in CDR-H3 allows B cells to escape potential germline constraints on the sequence of their Ag receptor repertoires (1, 2, 9–11).

In previous studies, we have shown that the essential outlines of the adult TdT-sufficient CDR-H3 repertoire, including patterns of gene segment use, amino acid composition, charge, predicted base and loop structure, and length, are established early in B cell development, prior to the expression of H chain protein (12). B lineage cells sequentially express a pre-B cell Ag receptor, rearrange an L chain gene, express surface IgM, and, as they are released into the periphery, begin to coexpress surface IgD. During this developmental process, the CDR-H3 repertoire is sequentially focused to fit into what appears to be a preferred range in terms of the distributions of length, amino acid composition, and average hydrophobicity. This developmental process is heavily influenced by the amino acid composition of the reading frame of the included D (12).

The composition of the CDR-H3 repertoire varies during ontogeny. For example, the perinatal liver CDR-H3 repertoire, which has its own distinct pattern of VDJ gene segment usage and lacks N nucleotides, differs significantly from the CDR-H3 repertoire expressed in adult bone marrow (BM) (13–15). It has been proposed that the differences in these repertoires contribute heavily to the differences between the neonatal and the adult response to Ags, that is, the antigenic hierarchy (16) that underlies both vaccination schedules and the altered susceptibility of young children to infection.

By comparing the CDR-H3 repertoire of Vh7183-containing transcripts from TdT-deficient mice with that of physiologically TdT-insufficient perinatal liver and with that of TdT-sufficient adult BM, we sought to test the extent to which differences between the perinatal repertoire and the adult repertoire reflect the effects of N nucleotide inclusion. Although the patterns of VDJ

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Abbreviations used in this paper: BM, bone marrow; CDR-H3, CDR-3 of the Ig H chain; E, extended; IMGT, international ImMunoGeneTics information system; K+, extrakinked; K–, kinked; KO, knockout; V, variable; WT, wild-type.

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usage in early pre-B cell progenitors from the BM of TdT-deficient mice differed from those observed in the physiologically TdT-insufficient perinatal liver, we found that the CDR-H3 repertoire expressed by the mature BM TdT-deficient B cells is a similar, but not exact, homologue of the CDR-H3 repertoire expressed by mature B cells in the TdT-insufficient perinatal liver.

Materials and Methods

Mice

TdT-deficient animals generated by Gillfillan and colleagues (11) on a mixed 129/C57BL/6 background were the kind gift of Dr. John Kearney of the University of Alabama at Birmingham. The animals were back-crossed for 10 generations onto BALB/cj (stock no. 000651; The Jackson Laboratory, Bar Harbor, ME) and bred in the University of Alabama at Birmingham vivarium. The mice were maintained in a specific pathogen-free barrier facility. All experiments with live mice were approved by and performed in compliance with the University of Alabama at Birmingham Institutional Animal Care and Use Committee regulations.

Flow cytometric analysis and fluorescence-activated cell sorting

Flow cytometric analysis and FACS from mononuclear cells from the liver were performed as previously described for mononuclear cells from the BM of 8-wk-old BALB/c mice (12, 17, 18). A MoFlo instrument (Cytometry, Ft. Collins, CO) was used for cell sorting. Developing B lineage cells in the liver were identified on the basis of the surface expression of CD19, CD43, IgM, BP-1, and/or IgD.

RNA preparation, RT-PCR, and sequencing

Total RNA isolation, VH7183-specific VDJCμ RT-PCR amplification, cloning, sequencing, and sequence analysis were performed as previously described (12, 17, 18). The sequences reported in this work have been placed in the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/) under the accession numbers HM154548–HM154936. A listing of the 470 unique, in-frame VH7183DJCμ sequences used for analysis in this work is provided in Supplemental Table I.

Structural analysis

We used the “H3 rules,” as published by Shirai et al. (19, 20), to predict structural features of the CDR-H3 base and loop, as previously described (21). Briefly, the structure of the CDR-H3 base (termed kinked, extra-kinked, or extended) can be predicted in sequences that contain a minimum of five amino acid residues, including international ImMunoGeneTics information system (IMGT) positions 105–118 (Kabat positions 93–103). In ~25–30% of the sequences with a kinked or extra kinked CDR-H3 base, the H3 rules can predict whether an intact hydrogen bond ladder may be formed within the loop of the CDR-H3 region or whether the hydrogen bond ladder is likely to be broken. For example, proline residues tend to inhibit formation of a stable hydrogen bond ladder; the presence of a V H-formation system (IMGT) positions 105–118 (Kabat positions 93–103). In ~25–30% of the sequences with a kinked or extra kinked CDR-H3 base, the H3 rules can predict whether an intact hydrogen bond ladder may be formed within the loop of the CDR-H3 region or whether the hydrogen bond ladder is likely to be broken. For example, proline residues tend to inhibit formation of a stable hydrogen bond ladder; the presence of a V H-
of DH/JH distal block 18-8 in the perinatal liver consistently lagged behind that of DH/JH proximal block 6-3 (15). The pattern of the DH distal VH gene block (block 18-8) in the TdT-deficient mice proved identical to that of adult WT mice and thus divergent from that of the perinatal TdT-insufficient mice (Fig. 2). The pattern of the DH proximal VH gene block 6-3 proved more torturous, sharing identity with adult BM in fraction B and diverging from both adult BM and perinatal liver in fraction D. Interestingly, however, the three groups of mice converged to the same pattern in early pre-B cell fraction C and then in immature B cell fraction E and mature B cell fraction F. (Fig. 2).

Increased identification of DH DSP family members in CDR-H3s from TdT-deficient B cells

In previous deconstructions of VH7183DJCm sequences from adult TdT-sufficient WT BM, we were unable to identify the donor DH in up to one-sixth of the CDR-H3 sequences (12, 17, 18, 23). By contrast, we were able to identify the donor DH in 99% of the transcripts we obtained from TdT-deficient fraction F (Fig. 3). This was associated with a striking increase in the donor assignment of the various members of the DSP gene segment family. The contribution of the other DH families, DFL, DQ, and DST, was statistically indistinguishable from that in adult TdT-sufficient WT BM.

Although there was a statistical trend for decreased use of JH1 and JH3 among the CDR-H3 sequences from the TdT-deficient mice, with a potential compensatory increase in the use of JH4, none of the differences achieved statistical significance (Fig. 3). In previous deconstructions of VH7183DJCm sequences from adult TdT-sufficient WT BM, we were unable to identify the donor DH in up to one-sixth of the CDR-H3 sequences (12, 17, 18, 23). By contrast, we were able to identify the donor DH in 99% of the transcripts we obtained from TdT-deficient fraction F (Fig. 3). This was associated with a striking increase in the donor assignment of the various members of the DSP gene segment family. The contribution of the other DH families, DFL, DQ, and DST, was statistically indistinguishable from that in adult TdT-sufficient WT BM.

Increased use of reading frame 1 in CDR-H3s from TdT-deficient B cells

We observed a statistical trend (p = 0.09) for increased use of reading frame 1 among those sequences from TdT-deficient adult BM that used members of the DFL or DSP families, with 78% using RF1 in fraction B versus 89% in fraction F (Fig. 3). The use of RF1 in the TdT-deficient BM repertoire was greater than that of adult BM and less than that of perinatal liver in fractions B, C, and D. The converse was true for RF3. Whereas this pattern of an increased use of RF1 and decreased use of RF3 was maintained
between TdT-sufficient and TdT-deficient adult BM in fraction F, the differences in reading frame usage between TdT-deficient adult BM and TdT-insufficient neonatal liver essentially resolved, creating a very similar pattern. Unlike RF1 and RF3, the use of RF2 in the TdT-deficient adult BM matched that of TdT-insufficient perinatal liver at all five stages of BM B cell development, with both using RF2 much less frequently than that in TdT-sufficient adult BM.

The frequency of CDR-H3s containing D–J and V–D overlaps increased with development in TdT-deficient adult BM

One mechanism known to influence D_H reading frame usage is the tendency for D→J rearrangement to occur at sites of sequence microhomology (13, 28). Rearrangement at these regions of microhomology has the effect of restricting the diversity of the repertoire by enriching for shared terminal amino acid sequence. The extent of this microhomology varies, with J_H1 and J_H2 gene segments sharing up to six nucleotides of homology with the 3′ termini of 12 of the 13 D_H gene segments and J_H4 sharing five nucleotides of homology, all in reading frame 1. J_H3, in contrast, exhibits only one nucleotide of terminal homology in more than one D_H reading frame.

To test whether the increase in the use of RF1 reflected an increased incidence of rearrangement at the site of D–J microhomology in the absence of N nucleotides, we evaluated the frequency of rearrangements involving D–J microhomology (Fig. 4). Among sequences obtained from TdT-sufficient adult BM, rearrangements occurring at sites of D–J microhomology represented only 2–5% of the transcripts. In contrast, among the sequences from TdT-deficient adult BM, ~40% from fraction B had evidence of D–J homology rearrangements. The prevalence of these types of rearrangements exhibited a steady increase with development, representing ~80% of fraction F sequences. Among those sequences using members of the DSP or DFL families that lacked evidence of rearrangement at sites of D–J microhomology, the prevalence of RF1 was 70% of the total regardless of developmental stage. Among those sequences containing evidence of...
rearrangement at sites of D–J microhomology, use of RF1 reached up to 100% in several fractions (data not shown). The non-RF1 exceptions in this latter category tended to use JH3 and thus contained only one shared nucleotide.

The frequency of V→D overlap was also greater among the sequences from TdT-deficient adult BM. The prevalence of such sequences matched that observed in physiologically TdT-insufficient perinatal liver for fractions B–E (Fig. 4). However, in fraction F, the prevalence of sequences sharing D–J or V–D overlaps proved lower in TdT-insufficient perinatal liver than in TdT-deficient adult BM. The divergence in the prevalence of the D–J overlap bore no relation to the use of individual JH gene segments.

The distribution of CDR-H3 lengths was focused with development

In TdT-sufficient adult BM, the average CDR-H3 length increases with B cell development. This increase was also observed in TdT-deficient BM (10.0 ± 0.3 amino acids, fraction B, versus 10.4 ± 0.2 amino acids, fraction F; respectively) (Fig. 5A), although this slight increase did not achieve statistical significance (p = 0.37). However, the two codons difference in the average CDR-H3 length in TdT-deficient fraction F CDR-H3s versus that of TdT-sufficient adult BM fraction F (12.5 ± 0.2) was highly significant (p < 0.0001).

Whereas the average length of adult BM CDR-H3 is significantly longer than that of perinatal liver at all stages in WT mice (15), the CDR-H3s from TdT-deficient adult BM fraction B were almost one codon longer than the CDR-H3s from TdT-insufficient perinatal liver fraction B (p = 0.05). The average length of the sequences from the TdT-deficient mice approached that of TdT-insufficient perinatal liver in fraction C and then converged in fraction D. Equivalence was then maintained from fraction E through fraction F (Fig. 5A). A major reason for the lower average length of CDR-H3 in both TdT-deficient adult BM and TdT-insufficient perinatal liver compared with that in TdT-sufficient adult BM is the complete absence of CDR-H3s containing more than 16 codons (Fig. 6) (15).

In TdT-sufficient BM, the distribution of CDR-H3 lengths also becomes more focused with development (12). This primarily reflects a progressive decrease in the representation of shorter CDR-H3s. This same pattern was also observed in TdT-deficient BM (Fig. 6), where a progressive decrease in the representation of sequences containing CDR-H3s of fewer than eight codons is readily apparent.

When the lengths of CDR-H3s from TdT-deficient BM and TdT-insufficient perinatal liver were compared with each other, it became clear that the divergence in average length largely reflected the decreased number of very short CDR-H3s in the samples from adult BM. However, as the developing B cells passed through their sequential checkpoints, this divergence declined to the point that the CDR-H3 length distribution in fraction F was nearly identical between the two sets of samples (the single exception was an overrepresentation of CDR-H3s that use nine codons in the TdT-deficient adult cells) (Fig. 6, right panel).

The amino acid composition of CDR-H3 was greatly restricted in the absence of TdT and matches that of perinatal liver

To assess the effect of N addition on CDR-H3 amino acid content, we compared the use of individual amino acids in the CDR-H3 loop, which comprises aa 95 to 100 by the Kabat nomenclature (6). In Fig. 7, the amino acids are arranged by relative hydrophobicity, as assessed by the Kyte–Doolittle scale (29) as normalized by Eisenberg (30).

Compared with the TdT-sufficient adult BM repertoire, the TdT-deficient adult BM repertoire was significantly enriched for use of tyrosine, histidine, and alanine. Use of aspartic acid and asparagine was also variably increased. Conversely, the TdT-deficient adult BM repertoire was depleted of arginine, threonine, and leucine and virtually devoid of lysine, glutamine, glutamic acid, proline, methionine, cysteine, phenylalanine, valine, and isoleucine. There was relatively little change in this pattern with development.

The amino acid composition of the CDR-H3 repertoire in TdT-deficient adult BM proved strikingly similar to the TdT-insufficient perinatal liver repertoire. The few differences that achieved statistical significance were primarily detected in fraction B. Indeed, for B cells in fraction F, there were no statistically significant differences in global amino acid usage between the two repertoires.

The perinatal repertoire was enriched for use of DQ52 (15), which encodes tryptophan and glycine in RF1, threonine, leucine, and glycine in RF2, and asparagine, aspartic acid, and glycine in RF3. Close inspection revealed a statistical trend for an increase in the use of threonine, aspartic acid, asparagine, and tryptophan in the perinatal versus the adult N-less repertoires. However, the numbers of sequences were insufficient to achieve statistical significance. What differences were present appeared to be minimized in fraction F (Fig. 7).

Average CDR-H3 hydrophobicity declined with development

The calculation of average CDR-H3 hydrophobicity provides a measure of the relative assortment of amino acids by a particular physical property: water solubility. In TdT-sufficient adult BM, the average hydrophobicity of CDR-H3 becomes more, then less hydrophobic in the transition from fraction B to C to D. It then stabilizes in a slightly hydrophilic range (−0.18 ± 0.2; Fig. 5B) for fractions E and F. The average hydrophobicity of CDR-H3s produced by TdT-deficient BM B lineage cells began at the same point in fraction B (−0.15 ± 0.05) but then progressively declined from fraction B to C to D, becoming increasingly charged. It then

![FIGURE 5. Average CDR-H3 length and average CDR-H3 loop hydrophobicity as a function of B cell development in TdT-deficient adult BM compared with that in the adult BM and perinatal liver of WT mice. Average CDR-H3 length (A) and average CDR-H3 loop hydrophobicity (B) encoded by the VH7183DJCμ transcripts from TdT-deficient adult BM, WT TdT-sufficient adult BM, and WT TdT-insufficient perinatal liver. The SEM is shown.](http://www.jimmunol.org/DownloadedFrom/)
stabilized through fraction F, achieving an average hydrophobicity of $-0.24 \pm 0.03$. The difference between the TdT WT and knockout (KO) adult repertoires just missed achieving statistical significance ($p = 0.06$).

The average hydrophobicity of the CDR-H3 repertoire produced by TdT-insufficient perinatal liver B lineage cells starts more heavily charged than that of either TdT-sufficient or TdT-deficient adult BM. There is again a set of transitions from fraction B to C to D, but in the opposite direction from that for TdT-sufficient adult BM. The charge differential in fraction C achieved statistical significance compared with that of both TdT-sufficient and TdT-deficient adult BM ($p < 0.001$). And then, in a pattern that matched that observed for the progression in the average length of CDR-H3 (Fig. 5A), there was convergence among cells that have progressed to fraction D. At the end of this selection process, the average hydrophobicity for both TdT-deficient adult BM and TdT-insufficient neonatal liver fraction F cells proved the same (Fig. 5B).

In TdT-sufficient adult BM, the distribution of average hydrophobicity in the CDR-H3 repertoire achieved a tighter focus as the cells progressed through successive developmental checkpoints (12). This focusing was associated with a progressive loss of highly charged and highly hydrophobic CDR-H3s. This same general process was observed in TdT-deficient adult BM, with one major exception. At all stages of development, there was a relative paucity of highly hydrophobic CDR-H3s (Fig. 8, left panel). Still, the paucity of both highly hydrophobic and highly charged CDR-H3s was most apparent in fraction F, with evidence by inspection of the same trimming and focusing pattern observed in TdT-sufficient BM (12).

As with the differences in average length, distribution of lengths, and average charge, the greatest divergence between the distribution of charge in CDR-H3s from TdT-deficient BM and those from TdT-insufficient perinatal liver occurred in fractions B and C. The distribution pattern nearly normalized in fraction D and then showed mild patterns of divergence in fractions E and F, none of which achieved statistical significance (Fig. 8).

**FIGURE 6.** Distribution of CDR-H3 length of V$_{H}$7183DJC$_{\mu}$ transcripts as a function of B cell development in TdT-deficient adult BM: divergence between TdT-deficient versus WT adult BM and perinatal liver. *Left,* Distribution of CDR-H3 lengths of the sequenced population of unique, in-frame transcripts from TdT-deficient adult BM Hardy fractions B–F. *Center,* Divergence in the distribution of CDR-H3 lengths in TdT-deficient versus WT TdT-sufficient adult BM. *Right,* Divergence in the distribution of CDR-H3 lengths in TdT-deficient adult BM versus WT TdT insufficient perinatal liver. To facilitate visualization of the change in variance of the distribution, the vertical lines mark the preferred range of lengths in the BM fraction F. Arrows point to features of particular interest; see text for detail.
phenylalanine, aspartic acid, and tyrosine contributed by the J_H at Kabat positions 100K–102. To assess the effect of N addition on CDR-H3, we calculated the properties of the CDR-H3 base and CDR-H3 loops according to the H3 rules of Shirai et al. (19) (Fig. 9). In the CDR-H3s from both TdT-deficient adult BM and TdT-insufficient perinatal liver, all of the sequences with a predictable loop structure contained intact hydrogen bond ladders, whereas in the sequences from WT adult BM, more than half of the CDR-H3 loops were deformed (Fig. 9, right column).

Although N nucleotides rarely contribute to the CDR-H3 base, extended CDR-H3 bases were significantly more frequent among BM fraction B sequences from TdT KO mice than from that of WT mice (p = 0.007) (Fig. 9, left column). No significant differences were observed in this regard among fraction C–F sequences.

**Discussion**

The two most prominent differences between the perinatal liver and the adult BM H chain repertoire in BALB/c mice are a divergence in V_H, D_H, and J_H gene segment use and the paucity or abundance of N nucleotides (13–15). We sought to test what role, if any, the paucity of N addition during the perinatal period might play in the control of V_H gene segment use among the TdT-deficient and TdT-sufficient adult BM. The mechanisms for these differences are unclear, but one potential possibility is that TdT influences the interaction between the RAG rearrangement complex and the rearranging gene segments, perhaps due to steric hindrance. However, as the developing B cells passed through sequential checkpoints of development and became more and more dependent on the Ag-binding properties of the V domain, we found that the use of these gene segments in the TdT-deficient state converged and then matched their use in the normal TdT-sufficient state.

For the rest of the V_H, D_H, and J_H gene segments, there were few, if any, statistically significant differences between TdT-deficient and TdT-sufficient adult BM other than increased recognition of the use of DSP gene segment sequences. Indeed, it is possible that most “unassignable” CDR-H3s in the TdT-sufficient adult repertoire are derived from DSP-containing DJ rearrangements. What differences were present at early stages also tended to diminish with maturation of the B cells.

Between TdT-deficient adult BM and physiologically TdT-insufficient perinatal liver, the divergence between V_H, D_H, and J_H gene segment sequence and reading frame preference proved greatest in fraction B and the least in fraction F. However, the signature differences in D_H and J_H gene use that mark the perinatal repertoire (i.e., preferential use of DQ52 and J_H2) were not recapitulated in the absence of TdT and N nucleotides. Thus, the activation and rearrangement of D_H and J_H do not appear to be as sensitive as V_H81X and V_H7183.10 to the presence or absence of

**FIGURE 7.** Distribution of amino acids in the CDR-H3 loops of the V_H7183DJC4 transcripts during B cell development in TdT-deficient adult BM: divergence between TdT-deficient versus WT adult BM and perinatal liver. Top, Distribution of amino acid use is shown as the percentage of CDR-H3 loop sequences as a function of B cell development in TdT-deficient adult BM. Center, Divergence in the distribution of individual amino acid use in the CDR-H3 loop between TdT-deficient versus WT TdT-sufficient adult BM. Right, Divergence in the distribution of individual amino acid use in the CDR-H3 loop between TdT-deficient adult BM versus WT TdT-insufficient perinatal liver. The amino acids are arranged by relative hydrophobicity, as assessed by a normalized Kyte–Doolittle scale (29, 30). All comparisons were made using χ² or Fisher’s exact test as appropriate. Significant differences among each fraction in the different mice are indicated by asterisks. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001.
TdT. Also, these differences appeared to be less amenable to the Ag receptor-influenced selective pressures that act to converge V_{H} use among the mature B cell fraction. This suggests that increased use of DQ52 and J_{H}2 reflects either the effects of the fetal environment or differences in gene activation that are unique to the fetal B lineage cell. Determination of the contribution of these two possibilities likely will require transplantation studies.

The H3 rules of Shirai et al. (19) exclusively predicted intact hydrogen bond ladders for the CDR-H3 loops of adult TdT-deficient and perinatal liver Abs, whereas the adult TdT-sufficient repertoire contained more than 50% deformed hairpins. Thus compared with adult WT, the Ag binding grooves of TdT-deficient and perinatal TdT-insufficient mice are deeper due to shorter CDR-H3 loops and much less diverse due to the predominance of intact hydrogen bond ladders. It can be assumed that these structural properties may contribute to the functional features of the fetal Ab repertoire, which is characterized by low affinity, polyreactivity, and mild autoreactivity.

The greatest divergence in amino acid composition, charge, and length between TdT-deficient adult BM B lineage cells and TdT-sufficient adult BM or TdT-insufficient perinatal liver occurred in fractions B and C, with B greater than C. These fractions are the most likely to reflect differences in the stromal environment or in endogenous factors related to the derivation of the cells themselves. The perinatal liver, for example, clearly presents a different range of adjacent external cells and soluble molecules, and the progenitor cells that give rise to the B-1 lineage that predominates in the fetus are supported by thymic stromal lymphopoietin (31), whereas the progenitor cells that give rise to the B-2 lineage that predominates in the adult depend strictly on IL-7 (32). However, as the cells transition from fraction C to fraction D, a developmental checkpoint that depends on the successful association between the nascent H chain and surrogate L chain, the composition of the CDR-H3 repertoire in the TdT-deficient BM became very similar to the composition of the CDR-H3 repertoire in the TdT-deficient perinatal liver. Using Occam’s razor, this would suggest that the surrogate L chain is acting to select a specific range of CDR-H3 lengths and charge irrespective of external environment or cell derivation. In particular, the surrogate L chain appears to discriminate against very short or highly hydrophobic CDR-H3s.

FIGURE 8. Distribution of CDR-H3 loop charge of V_{H}7183DJC_{a} transcripts as a function of B cell development in TdT-KO adult BM: divergence between TdT-deficient versus WT adult BM and perinatal liver. Left, Distribution of CDR-H3 loop hydrophobicity of the sequenced population of unique, in-frame transcripts from TdT-KO adult BM Hardy fractions B–F. Center, Divergence in the distribution of CDR-H3 loop hydrophobicity in TdT-deficient versus WT TdT-sufficient adult BM. Right, Divergence in the distribution of CDR-H3 loop hydrophobicity in TdT-deficient adult BM versus WT TdT-insufficient perinatal liver. The normalized Kyte–Doolittle hydrophobicity scale (30) has been used to calculate average hydrophobicity. Although this scale ranges from −1.3 to +1.7, only the range from −1.0 (charged) to +1.0 (hydrophobic) is shown. To facilitate visualization of the change in variance of the distribution, the vertical lines mark the preferred range of average hydrophobicity in the BM fraction F.
The two most apparent differences between the H chain repertoire expressed in TdT-insufficient perinatal liver and the repertoire expressed in TdT-deficient adult BM reflect differences in VDJ usage. First, adult cells can access a full range of V_{H} families, and second, fetal B cell progenitors enrich for the use of DQ52 and for DH proximal J_{H}2. Studies of the anatomy of hot spots in protein interfaces (33) have shown that arginine, tyrosine, and tryptophan contribute disproportionately to binding energies. In this light, our data would suggest that with regard to CDR-H3, the use of tyrosine and arginine is similar, and the primary difference between TdT-insufficient perinatal liver and TdT-deficient adult BM reflects the loss of DQ52-encoded tryptophan in CDR-H3. This latter population appears to be small, but could still be significant in certain cases.

In the final analysis, whereas we found small, but subtle, differences in the composition of the CDR-H3 repertoire between TdT-deficient adult BM and TdT-insufficient perinatal liver, the repertoire expressed in TdT-deficient adult BM reflect differences in VDJ usage. First, adult cells can access a full range of V_{H} families, and second, fetal B cell progenitors enrich for the use of DQ52 and for D_{H} proximal J_{H}2. Studies of the anatomy of hot spots in protein interfaces (33) have shown that arginine, tyrosine, and tryptophan contribute disproportionately to binding energies. In this light, our data would suggest that with regard to CDR-H3, the use of tyrosine and arginine is similar, and the primary difference between TdT-insufficient perinatal liver and TdT-deficient adult BM reflects the loss of DQ52-encoded tryptophan in CDR-H3. This latter population appears to be small, but could still be significant in certain cases.

FIGURE 9. Distribution of the predicted structures of the base and the loop of CDR-H3 from V_{H}7183DJC_{\mu} transcripts as a function of B cell development in TdT-KO adult BM: divergence between TdT-deficient versus WT adult BM and perinatal liver. Left column, Frequency of kinked (K\textsuperscript{-}), extrakinked (K\textsuperscript{+}), and extended (E) CDR-H3 bases. Right column, Frequency of broken and intact hydrogen bond ladders within the CDR-H3 loop for those H chains that contain kinked or extrakinked bases. Top panels, Transcripts from TdT\textsuperscript{−/−} adult BM Hardy fractions B–F. Middle panels, Divergence in the transcripts from TdT\textsuperscript{−/−} versus WT adult BM. Bottom panels, Divergence in the transcripts from TdT-KO adult BM versus WT perinatal liver.

The two most apparent differences between the H chain repertoire expressed in TdT-insufficient perinatal liver and the repertoire expressed in TdT-deficient adult BM reflect differences in VDJ usage. First, adult cells can access a full range of V_{H} families, and second, fetal B cell progenitors enrich for the use of DQ52 and for D_{H} proximal J_{H}2. Studies of the anatomy of hot spots in protein interfaces (33) have shown that arginine, tyrosine, and tryptophan contribute disproportionately to binding energies. In this light, our data would suggest that with regard to CDR-H3, the use of tyrosine and arginine is similar, and the primary difference between TdT-insufficient perinatal liver and TdT-deficient adult BM reflects the loss of DQ52-encoded tryptophan in CDR-H3. This latter population appears to be small, but could still be significant in certain cases.

In the final analysis, whereas we found small, but subtle, differences in the composition of the CDR-H3 repertoire between TdT-deficient adult BM and TdT-insufficient perinatal liver in fraction F, the overall view is one of extensive similarity on a global level between the CDR-H3s of TdT-deficient adult BM and of TdT-insufficient perinatal liver. Because the fetus is relatively protected from external environmental Ags, these observations would suggest that study of immune responses in TdT-deficient animals is likely to allow us to continue to gain some insight into the biologic significance of CDR-H3 control on the Ag hierarchy. Studies that have already been completed include an examination of the effect of N addition on immunity to infectious agents or polysaccharide Ags. Heterosubtypic immunity to influenza A virus infection is impaired in TdT\textsuperscript{−/−} mice (35), and Mahmoud and Kearney have shown that TdT activity is required for the generation of optimal anti-DEX Ab response and the dominance of the J558 idiotype in adult BALB/c mice (36). These studies would suggest that restrictions in the addition of N nucleotides could function as a mechanism for the increased susceptibility of the young to some infectious diseases. Conversely, a number of studies have shown that autoimmune disease is inhibited by the absence of N nucleotides (37–41). In particular, the decrease in the use of arginine may diminish the likelihood of generating pathogenic anti-DNA Abs (42). Gene expression patterns in the embryo and fetus differ significantly from those of the adult. Thus, it is possible that control of the CDR-H3 repertoire with the resultant limitations in the diversity of the Igs expressed by the developing child functions to buffer the effects of a changing universe of self-Ags, preventing the development of autoimmunity in the very young. Still, the ex-
tensive similarities in CDR-H3 content between the mature B cell repertoire in TdT-deficient adult BM and TdT-insufficient perinatal liver suggest that TdT-deficient mice represent a good, although not perfect, model for testing the role of the limited perinatal CDR-H3 repertoire on Ab responses to Ags. Such studies are currently being pursued in our laboratory.

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Disclosures

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References

11. Gilfillan, S., A. Dierich, M. Lemeur, C. Benoist, and D. Mathis. 1993. Mice devoid of TdT represent a unique, although not perfect, model for testing the role of the limited perinatal CDR-H3 repertoire on Ab responses to Ags. Such studies are currently being pursued in our laboratory.

Development of the N-Less CDR-H3 repertoire