Heterogeneity of N insertion capacity in fetal hematopoietic stem cells

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Abstract

TCR gene rearrangement is strictly regulated during mouse ontogeny. The V-(D)-J junctions of αβ and γδ TCR transcripts expressed in the adult thymus are more highly diverse than those in the fetal thymus. We previously showed that adult hematopoietic stem cells (HSC) have a higher capacity to insert N nucleotides into Vγ4 TCR transcripts than fetal HSC and that the level of N nucleotide insertion is determined, at least in part, at the level of HSC. To analyze this developmental change of HSC at the single cell level, we investigated N nucleotide insertions in three TCR transcripts (Vγ1, Vγ2 and Vγ8) derived from limiting numbers of fetal liver HSC by fetal thymic organ culture. Eight day-14 fetal liver HSC clones showed various levels of N nucleotide insertions in Vγ4 transcripts (0-78%). On the other hand, the level of N insertions was similarly regulated in Vγ4, Vγ2 and Vγ8 TCR transcripts in a clone-specific way. These results suggested that the level of N insertion is programmed at the level of single HSC and that fetal liver contains a heterogeneous population of HSC in terms of N insertion capacity. After 3 weeks culture with a stromal cell line, fetal HSC showed higher levels of N insertion capacity than before culture. This result and the presence of HSC with intermediate N insertion capacity support the hypothesis that the developmental potential of individual HSC gradually changes from fetal to adult type in one stem cell lineage.

Introduction

Hematopoietic stem cells (HSC) play an essential role in hematopoiesis. They have two major characteristics: the capacity for self-renewal and the capability to differentiate into all kinds of hematopoietic cells. However, the process by which this occurs is not yet well understood. HSC have been highly enriched from adult bone marrow and fetal liver (1-4). By the combination of Thy-1 antigen expression at low levels (Thy-1⁺) and the absence or low expression of lineage markers, B220, Gr-1, Mac-1, TER-119, CD4, CD8 and CD5 (Lin⁻) and the expression of Sca-1 antigen (Sca-1⁺), Thy-1⁺ Lin⁻ Sca-1⁺ cells from adult bone marrow and fetal liver were demonstrated to contain highly enriched HSC (5-7).

To understand the mechanisms of self-renewal and differentiation of HSC, it could be informative to compare fetal HSC with their adult counterparts. The sites at which hematopoiesis occurs change during mouse ontogeny (8). The first place is the yolk sac where primitive erythropoiesis occurs during day 7 to day 12 of embryonic life. The aorta–gonad–mesonephros region is also shown to support hematopoiesis from day 9 to day 11 (9,10). Hematopoiesis takes place in the fetal liver after day 11, presumably when HSC migrate into the organ. The fetal liver remains the major hematopoietic site during mid and late gestational stages. After day 15, hematopoiesis begins in fetal spleen and bone marrow, and the bone marrow becomes the major site throughout animal life after birth. In some experiments, transplantation of yolk sac cells into the yolk sac cavity revealed their potential to become adult bone marrow colony-forming units and thymocytes (11,12). However, the precise developmental pathway of hematopoietic stem cell lineages is not yet well understood.

TCR gene rearrangement is strictly regulated during mouse ontogeny and there are several successive waves...
A developmental switch in the stem cell lineage

Fig. 1. Limiting dilution analysis of hanging drop organ culture with fetal HSC. Thy-1<sup>10</sup>Lin<sup>−/−</sup>Sca-1<sup>+</sup> cells (3, 5, 10, 30 or 100 cells) from day 14 fetal liver were cultured with 2'-deoxyguanosine-treated fetal thymic lobes in a hanging drop for 2 days. The lobes were then cultured on Nucleopore filters for 14 days and the repopulation of the thymic lobes were checked.

Methods

Limiting dilution analysis of fetal thymic organ culture

Isolation of Thy-1<sup>10</sup>Lin<sup>−/−</sup>Sca-1<sup>+</sup> (c-kit<sup>+</sup>) cells from adult bone marrow and fetal liver was done as described (6,23). Fetal thymic lobes were dissected from BALB/c mouse fetuses at day 15 of gestation and treated with 2'-deoxyguanosine as described (6). Limiting numbers of the sorted HSC were cultured with the thymic lobes in hanging drops and then transferred onto Nucleopore filters as described (24,25).

Table 1. Heterogeneity of HSC population of day 14 fetal liver

<table>
<thead>
<tr>
<th>Stem cell clone</th>
<th>TCR transcripts with N nucleotide insertion (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Type&lt;sup&gt;b&lt;/sup&gt;</th>
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<tbody>
<tr>
<td></td>
<td>V&lt;sub&gt;γ&lt;/sub&gt;4</td>
<td>V&lt;sub&gt;δ&lt;/sub&gt;3</td>
</tr>
<tr>
<td>FL&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0.0</td>
<td>28</td>
</tr>
<tr>
<td>B</td>
<td>3.7</td>
<td>55</td>
</tr>
<tr>
<td>C</td>
<td>4.3</td>
<td>ND</td>
</tr>
<tr>
<td>D</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>E</td>
<td>35</td>
<td>45</td>
</tr>
<tr>
<td>F</td>
<td>47</td>
<td>ND</td>
</tr>
<tr>
<td>G</td>
<td>57</td>
<td>ND</td>
</tr>
<tr>
<td>H</td>
<td>78</td>
<td>85</td>
</tr>
<tr>
<td>FL average</td>
<td>30</td>
<td>47</td>
</tr>
<tr>
<td>BM&lt;sup&gt;c&lt;/sup&gt; oligoclonal</td>
<td>83</td>
<td>65</td>
</tr>
</tbody>
</table>

<sup>a</sup>Between 20 and 30 V(D)J junctional sequences were determined per each sample. The percentage of transcripts with N nucleotide insertion is shown.

<sup>b</sup>The type of N insertion level was relatively designated as low (0-25 %), intermediate (26-60%) and high (>60%) in the V<sub>γ</sub>4 gene.

<sup>c</sup>FL, Fetal liver; BM, adult bone marrow.
Fig. 2. V_{Y4}-J_{y1} junctional sequences of TCR transcripts derived from single HSC. Thymocytes were derived from single HSC of day 14 fetal liver by organ culture and 20–30 DNA sequences were determined in V_{Y4}-X1 junctions. Clones B, E and H (Table 1) are representively shown. The DNA sequences are aligned with published germline sequences (13,19). N nucleotides and P nucleotides are defined as described in Methods. The frequency with which the sequence was found among DNA clones is listed in the last column. Asterisks indicate the canonical sequence and short homotogy sequences (more than two nucleotides) between two segments of germline sequences are underlined (19,20).

A developmental switch in the stem cell lineage

Thymocytes were released from organ-cultured lobes by dissection with fine forceps. From the Poisson formula, the probability \( P(r) \) of precisely \( r \) cells in a lobe, where \( m \) is the mean number of cells per lobe, is \( P(r) = (m^r/e^m)/r! \). Therefore, the probability that the repopulated lobe is derived from two or more HSC is calculated as \( 1 - (1 - e^{-m})P(1) \) (where \( P(1) \) is the proportion of negative lobes).

Culture of HSC on a stromal cell line

A bone marrow-derived stromal cell line, AC-11, was cultured in RPMI 1640 medium supplemented with 5% FCS, 50 \( \mu \)M 2-mercaptoethanol, 1 mM sodium pyruvate, 2 mM glutamine, 100 U/ml penicillin and 100 \( \mu \)g/ml streptomycin at 37°C under 7% CO\(_2\) in air (26). For passage of culture, the confluent stromal cell layer was washed three times with HBSS supple-
mented with 10 mM HEPES (pH 7.4) and incubated in HBSS without Ca2+ and Mg2+ containing 500 μg/ml collagenase-dispase (Boehringer, Mannheim, Germany) for 30 min at 37°C. The cells were detached by vigorous pipetting and washed with the medium. Resuspended cells were seeded in six-well plates at a density of 1×104 cells/ml. Thy-10 Lin− Sca-1+ (c-kit+) cells from adult bone marrow and fetal liver were cultured at a density of 100 cells/well under the same conditions described above for 2-12 weeks. After culture, the cells were recovered by gentle pipetting and filtered through nylon screens. A colony-forming unit in spleen (CFU-S) assay of the cultured cells was done as described (27). Progeny of 10-50 Thy-10 Lin− Sca-1+ cells from fetal liver was cultured with a fetal thymic lobe. Five to ten repopulated lobes were pooled and thymocytes were recovered.

RT-PCR and sequencing of V(D)J junctional sequences
Poly(A)+ mRNA was isolated as described previously (6). cDNA was synthesized from the poly(A)+ mRNA using 25 μg/ml oligo(dT)12-18 (Pharmacia, Uppsala, Sweden), 6 μJ/ml of ribonuclease inhibitor (Takara, Kyoto, Japan) and 10 μJ/ml of Moloney mouse leukemia virus reverse transcriptase (SuperScript; Gibco/BRL, Gaithersburg, MD) at 37°C for 1 h. cDNA was amplified by PCR in a reaction buffer consisting of 50 mM KCl, 10 mM Tris (pH 8.3 at room temperature), 1.5 mM MgCl2, 3 ng/ml of each primer of appropriate pair, 0.2 mM each deoxynucleotide triphosphate, 50 μM tetracycline hydrochloride and 0.025 μJ/ml of Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT). Each cycle consisted of denaturation (1 min at 94°C), primer annealing (1 min at 55°C), extension (1 min at 72°C) and ramp time (1 min) between each two segments. The reaction was carried out in a programmable heat block (Thermal Cycler 480; Perkin-Elmer Cetus) for 40 cycles. Amplified cDNA was digested with EcoRI and HindIII, and fractionated by electrophoresis in 3% NuSieve agarose gel (FMC Bioproducts, Rockland, ME). The PCR fragment was purified with glass beads (Mermaid Kit; BIO101, La Jolla, CA) and cloned into plasmid. Plasmid DNA from positive colonies was sequenced with a DyeDeoxy Terminator Cycle Sequencing Kit by a 373A DNA Sequencer (Applied Biosystems, Foster City, CA).

N nucleotides and P nucleotides were defined as reported previously (19). N and P nucleotides were scored according to the following criteria: (i) the stretch of N nucleotides was taken as narrow as possible, (ii) P nucleotides (template addition) were considered as up to five bases (19,28-30), (iii) in Vβ sequences, fusion of Dβ1-Dβ2 was considered when both segments consist of three or more nucleotides, (iv) in this case, N nucleotides between Dβ1 and Dβ2 were not taken into account.

PCR primers
For V4Δ-J11 transcripts: V4, 5′-CCGAATTCTAGTCTCCACCAT-3′; J11, 5′-GCAAGCTTAGTCTCTTCTT-3′ (22). For V2Δ-J11 transcripts: V2, 5′-AAGGAATTCAGAATGAGGC-3′ (13, 19). For Vβ8-Cβ transcripts: Vβ8, 5′-AGACCAAGGACAAGAGACAATCCCTC-3′; Cβ, 5′-AAGGAATTCGCTTCTGGAGTC-3′ (31). EcoRI or HindIII restriction sites for subcloning into plasmid are underlined.

Results
Limited dilution analysis of hanging drop-organ culture of day 14 fetal liver HSC
To test the capacity of N nucleotide insertion of single fetal HSC, limited dilution analysis was carried out with fetal liver HSC by hanging drop organ culture (Fig. 1). Limiting numbers of Thy-10 Lin− Sca-1+ cells, isolated from day 14 fetal liver, were cultured with 2′-deoxyxanosine-treated fetal thymic lobes in a hanging drop for 1-2 days. The lobes were then transferred onto Nucleopore filters and cultured for a further 2 weeks. Repopulation of fetal thymic lobes with HSC was judged by presence or absence of thymocytes after dissecting with fine forceps.

In the first experiment, 5, 10, 30 or 100 Thy-10 Lin− Sca-1+ cells from day 14 fetal liver were cultured with fetal thymic lobes. Twenty-five percent of lobes (5 lobes/20 lobes) cultured with five cells were repopulated; 30% (3/10), 80% (8/10) and 100% (9/9) of lobes were repopulated after culture with 10, 30 and 100 cells respectively. Therefore, in this experiment the precursor frequency was 1 in 20 cells (Fig. 1). In the second experiment, thymic lobes were repopulated with three or six Thy-10 Lin− Sca-1+ cells per lobe; 6.7% (1/15) of lobes cultured with three cells per lobe were repopulated and 30% (3/10) of lobes cultured with six cells per lobe were also repopulated. In this experiment, the precursor frequency was also 1 in 20 cells. The efficiency of repopulation in these experiments was as high as that observed in the intrathymic injection experiment described previously (6). Because the repopulation frequency was low (6.7-30%), most of the lobes repopulated with three, five or six cells were considered to be the progeny of single HSC. The five lobes repopulated with five cells in the first experiment and one and three lobes repopulated with three and six cells respectively in the second experiment (nine lobes in total) were further analyzed.

HSC in day 14 fetal liver are heterogeneous in terms of N insertion capacity
To check the N insertion capacity of individual HSC, PCR amplification of cDNA was carried out. Poly(A)+ RNA from nine thymic lobes repopulated with three to six cells was reverse transcribed and the cDNA was amplified by PCR with V4 and J11 primers. One lobe repopulated with three cells was not amplified, but the remaining eight lobes were successfully amplified. The PCR products were cloned into plasmids and 20-30 DNA sequences were determined. The percentage of V4Δ-J11 transcripts with N nucleotide insertion is shown in Table 1 and the sequences of three representative clones are shown in Fig. 2. The percentage of N insertions of eight fetal HSC clones ranged from 0 to 78%. Four clones, A, B, C and D, showed low levels (0-14%) of N insertion, while three clones, E, F and G, showed intermediate levels (35-57%). Only one clone, H, showed a relatively high level (78%) of N insertion.

The average percentage of N insertion in eight fetal HSC clones was 30%. This level is comparable to that of day 14 whole fetal liver cells (21%, after correction with P nucleotide) reported previously (22). As a control using adult HSC, 30 Thy-10 Lin− Sca-1+ cells from adult bone marrow were cultured with fetal thymic lobes. Thymocyte development
was observed in 80% of lobes (8/10), indicating that the repopulated lobes are derived from oligoclonal precursors (data not shown). The percentage of N insertion in V4-J1 transcripts was 83% (Table 1). This value is also comparable to that of whole adult bone marrow cells (77%, after correction for P nucleotides) reported previously (22). Therefore, fetal liver and adult bone marrow HSC always gave rise to cells with low and high N insertion frequencies, respectively, in the different experiments. These results indicate that the difference of N insertion level among repopulated lobes is not due to unevenness of individual thymic lobes, but rather due to the difference in individual HSC. Furthermore, they also suggested that most of the HSC in adult bone marrow have high N insertion capacity. Because the level of N insertion of clone H (78%) was the same as that of adult HSC, this clone can be considered as an adult type HSC in terms of N insertion capacity. In general, the HSC population of day 14 fetal liver consists of stem cells with various levels of N insertion capacity, many with low, some with intermediate and few with high levels. All of these data suggest that day 14 fetal liver contains a heterogeneous population of HSC consisting of cells with different levels of N insertion capacity.

Table 2. V(D)J junctional sequences of TCR transcripts with N nucleotide insertion within out-of-frame sequences

<table>
<thead>
<tr>
<th>Stem cell clone</th>
<th>TCR transcripts with N nucleotide insertion (%)</th>
<th>V4A</th>
<th>V22</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>10</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>40</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>28</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>50</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>82</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>BMP oligoclonal</td>
<td>100</td>
<td>85</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. V(D)J junctional sequences of TCR transcripts derived from cultured HSCa

<table>
<thead>
<tr>
<th>Stem cells</th>
<th>in vitro culture (days)</th>
<th>Transcripts with N nucleotides (%)</th>
<th>V4A</th>
<th>Vp8</th>
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</thead>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLb</td>
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<td>42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>40</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>61</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>83</td>
<td>83</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

aName of FL clones is same as in Table 1
bFL, fetal liver
bBM, adult bone marrow

The level of N insertion in different TCR is programmed at the level of individual HSC

To check whether N insertion frequency is determined by the status of the clonal progenitor and would similarly be determined in the post-fetal program TCR as well as the fetal program TCR, V2-J1, and Vp8-Jg junctional sequences of five out of eight fetal liver HSC clones were determined by PCR (Table 1 and Fig. 3). The percentage of transcripts with N insertion turned out to be generally higher in V4 and Vp8 genes than in the V4 gene. Because both the V-D and D-J joints are counted, it is reasonable that N insertion tends to be scored at a higher level in Vp8 transcripts. Interestingly, the level of N insertion in different V genes showed a similar tendency when compared within the individual stem cell clones. For example, clone A, which showed no N insertion in V4 transcripts, revealed relatively low levels of N insertion in V2 (28%) and Vp8 (32%) transcripts when compared among each V gene. On the other hand, clone H showed the highest level of N insertion in all the three TCR transcripts. One exception is clone B. This clone showed a high level (55%) in V4 transcripts, while the level in V4 and Vp8 transcripts is relatively low. The average of five fetal HSC clones was 47% in V2 and 54% in Vp8 transcripts. On the other hand, oligoclonal adult HSC showed higher levels of N insertion in V2 (65%) and Vp8 (93%) transcripts. These data suggest that N nucleotide insertion in different TCR species is regulated in a clone-specific way.

It can be argued that these results are influenced by selection at the cellular level or the frequency of canonical junctional sequences in V4 transcripts. The canonical sequence of V4 transcripts is included in in-frame rearrangement. Therefore, we re-calculated the percentage of transcripts with N insertion within out-of-frame sequences to exclude the influence of these factors. Out-of-frame sequences were 10-50% of V4 and V2 transcripts, while almost all Vp8 transcripts were in-frame. The percentage of sequences with N insertions ranged from 0 to 82% within out-of-frame V4 transcripts in eight fetal clones (Table 2). As far as V4 and V2 transcripts were concerned, clones of low N insertion type showed low percentages and clones of high N insertion type showed high percentages within out-of-frame transcripts, similar to the results calculated from whole sequences shown in Table 1. These data suggest that the conclusion above was not biased by cellular selection on the basis of surface expressed TCR.

It has been demonstrated that N nucleotide insertion is caused by terminal deoxynucleotidyl transferase (TdT) (32–34). To test whether the difference of N nucleotide insertion capacity was caused by the difference in the level of TdT expression, TdT transcripts were detected by quantitative PCR from organ-cultured thymocytes derived from single HSC. Thymocyte cDNA was amplified by PCR with TdT primers from fetal liver HSC clones, C and G, and from oligoclonal adult bone marrow HSC, which were the only samples available. The amount of TdT expression was normalized by hypoxanthine phosphoribosyl transferase cDNA levels. In general, the thymic clones derived from fetal HSC manifested relatively lower levels of TdT expression than adult HSC (data not shown). These results suggest the possibility that programming of TdT expression is predetermined at the level of individual HSC.
The junctions between Dpi and Dp2 are shown as slants in the Dp column.

segments used are also shown and the numbers of nucleotides deleted from the Jp segments are listed in the column designated as del.

are shown in the similar way as Rg. 2. The V-O-J junctional sequences are aligned with published germline sequences (31, 40).

Fig. 3. Vβ8–Dβ7–Jβ8 junctional sequences of TCR transcripts derived from single HSC. Between 20 and 30 sequences from clones B, E and H are shown in the similar way as Fig. 2. The V–D–J junctional sequences are aligned with published germline sequences (31, 40). The Jβ segments used are also shown and the numbers of nucleotides deleted from the Jβ segments are listed in the column designated as del. Short homology sequences are underlined and Jβ gene segments which include short homology sequences at the terminal are also underlined. The junctions between Dβ1 and Dβ2 are shown as slants in the Dβ column.
In V₄ transcripts, there was a tendency that the clones with low N insertion capacity showed a few kinds of transcripts and the clones with high N insertion capacity showed many kinds of transcripts (Fig. 2). It has been suggested that V(D)J recombination is often directed by short sequence homology (35,36) and the frequency of recombination directed by short homology influences the variability of transcripts. Homology-directed recombination is supposed to cause the canonical sequences of V₃ and V₄ TCR which are seen more often in fetal than in adult thymus. Furthermore, it was reported that the expression of TdT results in the low frequency of homology-directed recombinations in vitro (37) and in vivo (38). Therefore, we investigated the frequency of recombinations mediated by short sequence homology among the transcripts without N insertion. Short homology was defined as those sequences in which there are two or more common nucleotides between adjacent junctional regions. There was no correlation between the frequency of homology-directed recombination and the frequency of N insertion (data not shown). These results suggested that the difference in the variability between eight fetal HSC clones was due to N insertions rather than short homology-directed recombination.

The N insertion capacity of fetal HSC increases after coculture with the stromal cell line

Because HSC with various levels of N insertion capacity were found in day 14 fetal liver, it is possible that at least some HSC in fetal liver and adult bone marrow may be related to each other as one cell lineage. To check whether HSC with low N insertion capacity can change to HSC with intermediate or high N insertion capacity, we performed in vitro culture of day 14 fetal liver HSC with a bone marrow-derived stromal cell line, AC11 (26). First, Thy-1⁺ Lin⁻ Sca-1⁺ cells from day 14 fetal liver and adult bone marrow were cultured on a bone marrow-derived stromal cell line, AC11, for 2–12 weeks CFU-S activity of the cultured cells was analyzed. The ratio of the CFU-S activity after the culture to that before the culture was plotted.

CFU-S activity of adult bone marrow HSC quickly decreased after 2 weeks of culture, that of fetal liver HSC increased during 2–3 weeks and was maintained until 8 weeks. These results suggest that AC11 may be able to support fetal HSC activity in vitro for several weeks.

After culture with AC11 for 1–3 weeks, day 14 fetal liver HSC were organ-cultured for 2 weeks, and N nucleotide insertions in V₄ and V₈ transcripts were analyzed (Table 3). The frequencies of transcripts with N nucleotides before culture were 25 and 42% in V₄ and V₈ transcripts respectively. These values were comparable to the average levels of eight fetal HSC clones (Table 1). The level of N insertion in V₄ transcripts increased to 40 and 61% after 1 and 3 weeks of culture respectively. The level in V₈ transcripts also increased to 73–84% after the culture. Although the levels after the culture were not as high as those of adult bone marrow HSC, the N insertion capacity obviously increased by culture with AC11. In addition, the variability of V₄ transcripts also increased after the culture. These data suggest the possibility that fetal HSC with low N insertion capacity may change into adult HSC with high N insertion capacity as one cell lineage during the course of development.
Discussion

In the limiting dilution analysis the probability of single cell reconstitution can be calculated from the frequency of thymic lobe repopulation (Fig. 1). We chose five lobes cultured with five cells per lobe from the first experiment. The frequency of negative lobes was 75%. In this case, the probability that any repopulated lobe was reconstituted from only one cell is statistically calculated as 86.3%. Next, we analyzed three lobes cultured with six cells per lobe in the second experiment. In this experiment the frequency of negative lobes was 70%. In this case the probability of single cell reconstitution is 83.2%. From these two experiments, we can expect that only 1.2 out of eight lobes were repopulated with two or more HSC. Therefore, it is suggested that most of the eight clones were derived from single HSC.

It has been reported that the repopulation with fetal liver cells resulted in the high levels of N insertions in Vp17 transcripts by fetal thymic organ culture (39). It was also suggested that the change of TdT expression in thymocytes depends on a change of the thymic microenvironment, rather than a change of HSC potential. From these points, there can be an argument that the various levels of N insertion capacity observed in fetal HSC clones were due to unevenness of thymic lobes used in the experiments. This argument is not likely in our case for the following reasons. First, the average percentage of N insertion in Vp4 transcripts of eight fetal HSC clones (30%) was comparable to that of total fetal liver cells (21%) (22) and sorted fetal liver HSC (25%) (Table 3) in different experiments at different time. Secondly, the N insertion capacity of adult bone marrow HSC was always very high in three different TCR transcripts in different experiments (Table 1) (22). Finally, fetal liver HSC gave a higher level of N insertion after culture with a stromal cell line (Table 3). Collectively, these considerations support the interpretation that the level of N insertion correlates not with the state of the thymic microenvironment but with the capacity of stem cells.

In this study, we showed the property of fetal liver HSC at the single cell level by limiting dilution analysis of fetal thymic organ culture. It was shown that day 14 fetal liver HSC were heterogeneous in terms of N insertion capacity. When we consider the developmental switch in N insertion capacity from fetal to adult HSC, a question occurs to us whether the observed changes resulted from a change in the stem cell progeny of fetal HSC or whether they resulted from two waves of HSC not related to each other in a cell lineage. Because the average percentage of N insertions of eight fetal liver HSC clones was comparable to that of total fetal liver cells and polyclonal fetal liver HSC, it seemed to be that these eight clones represented well the HSC in day 14 fetal liver. The presence of HSC with intermediate levels of N insertion capacity in day 14 fetal liver suggested that fetal and adult HSC populations might be related to each other in cell lineages. In the experiment of in vitro culture with a stromal cell line, the N insertion capacity of day 14 fetal liver HSC increased after the culture. Although this result can be influenced by cellular selection during the culture or by a particular environment such as a bone marrow-derived stromal cell line, there is a possibility that the property of fetal HSC can change within a defined cell lineage.

These results lead us to propose a model on developmental switch of N insertion capacity in HSC (Fig. 5). In this model, a fetal type of HSC with low N insertion capacity changes through a HSC with intermediate capacity into an adult type of HSC with high N insertion capacity during the course of mouse development (Fig. 5A). This developmental switch of HSC in terms of N insertion capacity may be applicable not only in T cell development but also in B cell development. This developmental change at the single cell level involves not only the change of individual HSC in the developmental stage but also the change of the stem cell population in the mouse ontogeny. The stem cell population in fetal liver, consisting mainly of fetal type of cells, may gradually change to the stem cell population in adult bone marrow, consisting mostly of adult type cells (Fig. 5B). Our previous study showed that the HSC population begins to lose the capacity to generate Vp3 T cells around day 16 of gestation and completely loses it ~1 week after birth (6). Therefore, it is possible that the transition of the HSC population occurs during these gestational stages. If we had investigated day 12 or late fetal liver HSC, this model might be clearly confirmed. This change at the single cell level can be caused by various factors. For example, cell division number or aging ('biological clock') can be listed as internal factors and the hematopoietic microenvironments can be considered as external factors.

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Abbreviations

CFU-S colony-forming unit in spleen
HSC hematopoietic stem cells
TdT terminal deoxynucleotidyl transferase

References

A development switch in the stem cell lineage


