SELECTIVE USE OF THE V_HQ52 FAMILY IN FUNCTIONAL V_H TO DJ_H REARRANGEMENTS IN A B PRECURSOR CELL LINE

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Functional transcription of immunoglobulin heavy chain genes in B lymphocyte lineage cells follows two sequential rearrangement events: D to J_H joins occur first on both chromosomes, followed by V_H and DJ_H rearrangement (1-3). The second of these events involved selection of one variable region segment from among seven major families, which were determined on the basis of nucleotide sequence homology (4, 5). Seven families have been mapped on chromosome 12 of BALB/c mice in the following order: V_H36-60 (5), V_H606 (10), V_H558 (60), V_HS107 (4), V_HQ52 (15), V_H7183 (12), V_HX24 (2), D, J_H, C_H. The numbers in parentheses indicate the estimated number of V_H segments within the family. There are so far some indications that the choice of V_H segment genes in V_H to DJ_H rearrangements may be not random (6-9). AT-11-2 is an Abelson virus (A-MuLV)-transformed B precursor cell line originating from BALB/c mice and capable of differentiating from Ig to \( \alpha' \) cells via functional recombination of V_H segments to preexisting DJ_H complexes (1, 2). AT-11-2 can further class-switch from \( \alpha' \) to \( \gamma_{2b}^{+} \) or \( \gamma_{3}^{+} \) cells by the deletion mechanism of intervening C_H genes (10). Recently we have reported that AT-11-2 is able to create secondary DJ_H complexes by the replacement of the preexisting DJ_H complexes (11).

In the present study, we examine V_H gene families of the V_H segments that were used in functional V_H to DJ_H recombinations in AT-11-2, and describe nonrandom use of V_H gene families and the existence of a stage at which the V_HQ52 family is preferentially used during the normal development of early pre-B cells.

Materials and Methods

**Cells and Immunofluorescence.** Cell line, cell cloning, and immunofluorescence were previously described (1, 10).

**Southern Blot Analysis.** Southern blot analysis was performed as described (1). DNAs were digested with the indicated restriction enzyme, electrophoresed in 0.5% agarose gels, blotted onto nitrocellulose filters, and hybridized to a J_H probe, to a C_H probe, or to a 5' D probe as previously described (1, 11).
FIGURE 1. Generation of intracytoplasmic \( \mu^+ \) subclones from Ig\(^-\) parent clones with a DJ\(_m\)/DJ\(_m\) configuration on both chromosomes by functional V\( \rightarrow \)DJ, H rearrangements during culture. Two Ig\(^-\) subclones P\(_1\) (AT11-2-5-1-5-1) and P\(_2\) (AT3-44-17) were cloned from Ig\(^-\) AT11-2. P\(_1\) was recloned and 14 Ig\(^-\) subclones (P\(_1\)-1-14) were isolated. Because each Ig\(^-\) subclone contained 0.1–0.2% of \( \mu^+ \) cells, only one \( \mu^+ \) subclone per each Ig\(^-\) subclone was isolated by the cloning and a total of 14 \( \mu^+ \) subclones (P\(_1\)-1-1 to P\(_1\)-14-1) were independently isolated. P\(_2\) was also recloned and 18 Ig\(^-\) subclones (P\(_2\)-1-18) were isolated. Because the Ig\(^-\) subclones also contained 0.1–0.2% of \( \mu^+ \) cells, only one \( \mu^+ \) subclone per one Ig\(^-\) subclone was isolated and a total of 18 \( \mu^+ \) subclones (P\(_2\)-1-1 to P\(_2\)-18-1) were isolated.

**Northern Blot Analysis.** Total RNAs were prepared from cells by a guanidium/CsCl method. RNAs were electrophoresed through 1% of agarose gels after denaturation with glyoxal and dimethylsulfoxide, and transferred to nitrocellulose filters. The filters were incubated at 42°C for 16 h with the probes in reaction mixture containing 50% formamide, 5X SSC, Denhardt’s solution, 20 mM sodium phosphate (pH 6.5), 10% SDS, and 100 \( \mu \)g/ml denatured salmon sperm DNA. The filters were washed three times in 2X SSC and 0.1% SDS at room temperature and twice in 0.1X SSC and 0.1% SDS at 50°C. DNA fragments containing functional V\( \rightarrow \)DJ\(_m\) rearrangements were used as the probes: V\( \rightarrow \)DJ\(_m\) 36-60, a 2.5 kb Eco RI-Eco RI fragment from MOPC315; V\( \rightarrow \)DJ\(_m\) 606, a 8.0 kb Eco RI-Eco RI fragment from J606; V\( \rightarrow \)DJ\(_m\) 558, a 2.0 kb Bam HI-Bam HI fragment from ARS; V\( \rightarrow \)DJ\(_m\) 107, a 6.7 kb Eco RI-Eco RI fragment from TEPC15; V\( \rightarrow \)DJ\(_m\) 52, a 2.8 kb Hind III-Hind III fragment from MOPC141; V\( \rightarrow \)DJ\(_m\) 7183, a 3.4 kb Eco RI-Eco RI fragment from SAPC15 and a 6.7 kb Hind III-Hind III fragment from MOPC21. S9 is the 5.4 kb cloned DNA containing the functional V\( \rightarrow \)DJ\(_m\) rearrangement, which was isolated from the \( \mu^+ \) subclone P\(_1\)-7-1 (original name, AT11-2-5-1-5-1-1) as previously described (2).

**Results**

We isolated 14 Ig\(^-\) subclones from the Ig\(^-\) P\(_1\) clone containing D\(_{SP2.8}\)/J\(_{H3}\) and D\(_{FL16.1}\)/J\(_{H3}\) complexes being carried by 11.0 kb and 5.4 kb Eco RI fragments, respectively, and 18 Ig\(^-\) subclones from the Ig\(^-\) P\(_2\) clone containing D\(_{FL16.1}\)/J\(_{H3}\) and D\(_{FL16.1}\)/J\(_{H4}\) complex being carried by 5.4 kb and 5.0 kb Eco RI fragments, respectively (Fig. 1). P\(_2\) was generated by the replacement of the preexisting D\(_{SP2.8}\)/J\(_{H3}\) complex by the secondarily formed D\(_{FL16.1}\)/J\(_{H4}\) complex (11). Because each Ig\(^-\) subclone also contained 0.1–0.2% of intracytoplasmic \( \mu^+ \) cells that were generated from in vitro functional V\(_{\mu}\) to DJ\(_{H}\) recombinations, we isolated only one \( \mu^+ \) subclone per each Ig\(^-\) subclone by the cloning in 0.3% soft agarose medium (see Fig. 1). In total, 32 \( \mu^+ \) subclones were isolated which were generated from independent functional V\(_{\mu}\) to DJ\(_{H}\) recombinational events. All \( \mu^+ \) subclones synthesized only \( \mu \) chains, but not heavy chains of other isotypes nor \( \kappa \) or \( \lambda \) chains, when tested by immunofluorescence. They had \( \kappa \) genes in germline configuration on both chromosomes (data not shown).

The configuration of immunoglobulin heavy chain genes was examined in all \( \mu^+ \) subclones by a Southern blotting procedure. DNAs were digested with Eco RI and hybridized to a J\(_{H}\) probe (Fig. 2). The Ig\(^-\) parent clone P\(_1\) and its Ig\(^-\)
FIGURE 2. Analysis of heavy chain gene rearrangements of $\mu^+$ subclones. DNAs were digested with Eco RI and hybridized to a $J_\mu$ probe. When a $\mu^+$ subclone retained or lost 5' D flanking sequences, it was determined to be VDJ*/DJ or VDJ*/VDJ, respectively, as previously described (11). VDJ*/O means the deletion of the $J_\delta$ gene on one chromosome. A mixture of 5' DFC, 5' Dsp and 5' DQ32 flanking sequences was used as the 5' D probe. Some $\mu^+$ subclones (e.g., P2-3-1) were determined to be VDJ*/DJ because of the retention of the fragments detected by the 5' D flanking sequence probe, although they had two rearranged bands quite different from parent clones P1 and P2. This might suggest secondary DJx complex formation as previously described (11).

subclones (P1-1-14) revealed 11.0 and 5.4 kb Eco RI fragments, and another Ig parent clone P2 and its Ig- subclones (P2-18) showed 5.4 and 5.0 kb Eco RI fragments. On the other hand, all $\mu^+$ subclones (P2-1-1 to P2-14-1 and P2-1-1 to P2-18-1) revealed one or two further rearranged bands different from those of the parent clones. Note that 32 $\mu^+$ subclones were generated from completely independent $V_\mu$ to DJ rearrangements (see Fig. 1). Six $\mu^+$ subclones from P1-1-1 to P1-6-1 showed the same rearranged pattern.

To determine which $V_\mu$ segments were used in the rearrangements, RNAs were prepared from $\mu^+$ subclones and analyzed by a Northern blotting procedure using probes specific for the $V_\mu$ families, $V_\mu$36-60, $V_\mu$J606, $V_\mu$J558, $V_\mu$S107, $V_\mu$Q52, and $V_\mu$7183 (4, 5) (Fig. 3). Surprisingly, of 32 $\mu^+$ subclones that were generated by independent $V_\mu$ to DJ rearrangements, 31 (P1-1-1 to P1-13-1, and P2-1-1 to P2-18-1) used $V_\mu$ segments of the $V_\mu$Q52 family. The remaining one $\mu^+$ subclone (P1-1-14-1) used a $V_\mu$ segment of the $V_\mu$7183 family. No $V_\mu$ to DJ rearrangements used the $V_\mu$36-60, $V_\mu$J606, $V_\mu$J558, or $V_\mu$S107 families. These
results suggest that \( V_\mu \) segments of the \( V_{\mu Q52} \) family were almost exclusively used, as this B-lineage precursor line gave rise to pre-B cell progeny.

The functional \( V_\mu DJ_\mu \) rearrangement of \( \mu^+ P_{1-7-1} \) (original name, AT11-2-5-1-5-1-5-1) was cloned and named S9 as previously described (2). Because S9 shares 85, 50, and 51\% \( V_\mu \) coding region sequence homology with MOPC141 (\( V_{\mu Q52} \) family), MOPC21 (\( V_{\mu 7183} \) family), and \( V_{\mu 81X} \) (\( V_{\mu 7183} \) family [6]), respectively, S9 (\( V_\mu \) segment of \( P_{1-7-1} \)) is a member of the \( V_{\mu Q52} \) family. This is consistent with the results of Northern blotting analysis.

Discussion

Yancopoulos et al. reported the preferential use of \( V_\mu \) segments of the \( V_{\mu 7183} \) family in A-MuLV-transformed cell lines originated from BALB/c strain mice (6). This occurred in at least 19 (58\%) out of 33 \( V_\mu DJ_\mu \) rearrangements examined, and the preferential use of this \( V_\mu \) family was correlated with its proximity to
the Jₜₜ locus. The reason for the differences between their results and our findings is not clear. However, we analyzed spleen cell–derived lines established by the injection of A-MuLV into neonatal BALB/c mice, whereas they examined fetal liver- and bone marrow–derived lines established by the viral infection in vitro. It may be that our B precursor clone was frozen at a stage of B cell development at which members of the VₜₜQ52 family were preferentially or selectively used. The analysis of the Vₜₜ expression by pre-B and B hybridomas of fetal and neonatal mice indicated that the Vₜₜ repertoire of fetal B-lineage cells is largely restricted to the Vₜₜ7183 family and that subsequent recruitment of additional Vₜₜ families occurred during neonatal development (7). 78% of fetal liver–derived pre-B hybridomas used the Vₜₜ7183 family whereas no neonatal liver–derived pre-B hybridomas used the family. The determination of the Vₜₜ expression in the fetal liver (developing B cells) and adult spleen (mature B cells) also indicated that the initial pattern of preferential use of the Vₜₜ7183 family resulted in higher expression of more Jₜₜ-distal Vₜₜ families in the mature B cells of the adult with a concomitant decrease in the representation of the more Jₜₜ-proximal families (9). Because our clone was established by the culture of the transformed spleen cells after viral injection into neonatal BALB/c mice (12), it may be derived from early pre-B cells in neonatal spleen. Therefore, it might be plausible that early pre-B cells in neonatal spleen preferentially use the VₜₜQ52 family but not the Vₜₜ7183 family. Thus, our results strongly indicated nonrandom use of Vₜₜ gene families and the existence of a stage at which the VₜₜQ52 family is preferentially used during the normal development of early pre-B cells. Recently, the biased use of Vₜₜ segments of the VₜₜQ52 family in an NIH/Swiss–derived 300-19 line but not in BALB/c-derived lines was described (8). They related the differences to organization of the more 3’ Vₜₜ families between the two strains. They demonstrated that in the BALB/c strain, the Vₜₜ7183 family was the most Jₜₜ proximal whereas in the NIH/Swiss strain, at least a portion of the VₜₜQ52 family, which was preferentially used, occurred 3’ to the bulk of the Vₜₜ7183 family. We have demonstrated selective use of the VₜₜQ52 family in a BALB/c-derived clone.

Furthermore, it seems that selective use of the VₜₜQ52 family is independent of the types of preexisting DJₜₜ complexes because a DₜₜSP2.8-JₜₜHS complex in the P₁ clone (2) and DₜₜFL16.1-JₜₜHS and DₜₜFL16.1-JₜₜH₄ complexes in the P₂ clone (11) could also join selectively to the VₜₜQ52 family.

Summary

AT11-2, an Abelson virus–transformed cell line has DJₜₜ complexes on both chromosomes and is able to form functional variable region genes by the joins of Vₜₜ genes to the DJₜₜ complexes during culture. Therefore we examined which Vₜₜ gene family was used in functional Vₜₜ to DJₜₜ recombinations in AT11-2. Surprisingly, of 32 independent functional Vₜₜ to DJₜₜ recombinational events in AT11-2, 31 events used the Vₜₜ segments of the VₜₜQ52 family, and the remaining one used the Vₜₜ segment of the Vₜₜ7183 family. Thus, we describe here the first B precursor cell line that almost selectively uses the VₜₜQ52 family in functional Vₜₜ to DJₜₜ rearrangements. The selective use of the VₜₜQ52 family in this B precursor cell line strongly indicates nonrandom use of Vₜₜ gene families, and the existence of a stage at which the VₜₜQ52 family is preferentially used during
the normal development of early pre-B cells and has important implications for understanding the ontogeny of V<sub>N</sub> repertoire development. Furthermore, this cell line should prove extremely valuable in further studies of this kind.

We thank Profs. Susumu Tonegawa (Massachusetts Institute of Technology, Cambridge, MA) and Yoshikazu Kurosawa (Fujita Gakuen Health University) for providing the V<sub>N</sub> probes. We also thank Prof. Paul W. Kincade (Oklahoma Medical Research Foundation) for his helpful discussion and criticism.

Received for publication 4 March 1987 and in revised form 14 May 1987.

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