Brief Definitive Report

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_HJ606 (10), V_HJ558 (60), V_HS107 (4), V_HQ52 (15), V_H7183 (12), V_HX24 (2), D, J_H, $C\mu$. 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). AT11-2 is an Abelson virus (A-MuLV)–transformed B precursor cell line originating from BALB/c mice and capable of differentiating from Ig^- to μ^+ cells via functional recombination of V_H segments to preexisting DJ_H complexes (1, 2). AT11-2 can further class-switch from μ^+ to $\gamma 2b^+$ or $\gamma 3^+$ cells by the deletion mechanism of intervening C_H genes (10). Recently we have reported that AT11-2 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 AT11-2, 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 $C\kappa$ probe, or to a 5′ D probe as previously described (1, 11).

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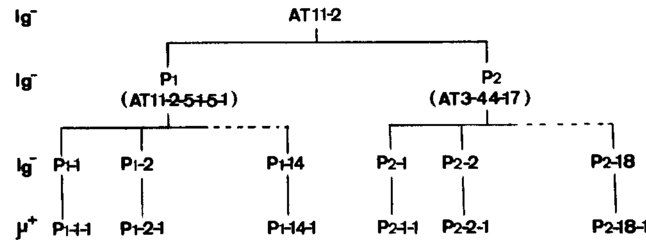

FIGURE 1. Generation of intracytoplasmic μ^+ subclones from Ig^- parent clones with a DJ_H/DJ_H configuration on both chromosomes by functional V_H to DJ_H rearrangements during culture. Two Ig^- subclones P_1 (AT11-2-5-1-5-1) and P_2 (AT3-44-17) were cloned from Ig^- AT11-2. P_1 was recloned and 14 Ig^- subclones (P_1–14) were isolated. Because each Ig^- subclone contained 0.1–0.2% of μ^+ cells, only one μ^+ subclone per each Ig^- subclone was isolated by the cloning and a total of 14 μ^+ subclones (P_1-1-1 to P_1-14-1) were independently isolated. P_2 was also recloned and 18 Ig^- subclones (P_2–18) were isolated. Because the Ig^- subclones also contained 0.1–0.2% of μ^+ cells, only one μ^+ subclone per one Ig^- subclone was isolated and a total of 18 μ^+ 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, 5× SSC, Denhardt's solution, 20 mM sodium phosphate (pH 6.5), 10% SDS, and 100 μg/ml denatured salmon sperm DNA. The filters were washed three times in 2× SSC and 0.1% SDS at room temperature and twice in 0.1× SSC and 0.1% SDS at 50°C. DNA fragments containing functional V_HDJ_H rearrangements were used as the probes: V_H36-60, a 2.5 kb Eco RI–Eco RI fragment from MOPC315; V_HJ606, a 8.0 kb Eco RI–Eco RI fragment from J606; V_HJ558, a 2.0 kb Bam HI–Bam HI fragment from ARS; V_HS107, a 6.7 kb Eco RI–Eco RI fragment from TEPC15; V_HQ52, a 2.8 kb Hind III–Hind III fragment from MOPC141; V_H7183, 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_HDJ_H rearrangement, which was isolated from the μ^+ subclone P_1-7-1 (original name, AT11-2-5-1-5-51-1) 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 μ^+ cells that were generated from in vitro functional V_H to DJ_H recombinations, we isolated only one μ^+ subclone per each Ig^- subclone by the cloning in 0.33% soft agarose medium (see Fig. 1). In total, 32 μ^+ subclones were isolated which were generated from independent functional V_H to DJ_H recombinational events. All μ^+ subclones synthesized only μ chains, but not heavy chains of other isotypes nor κ or λ chains, when tested by immunofluorescence. They had κ genes in germline configuration on both chromosomes (data not shown).

The configuration of immunoglobulin heavy chain genes was examined in all μ^+ 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^-

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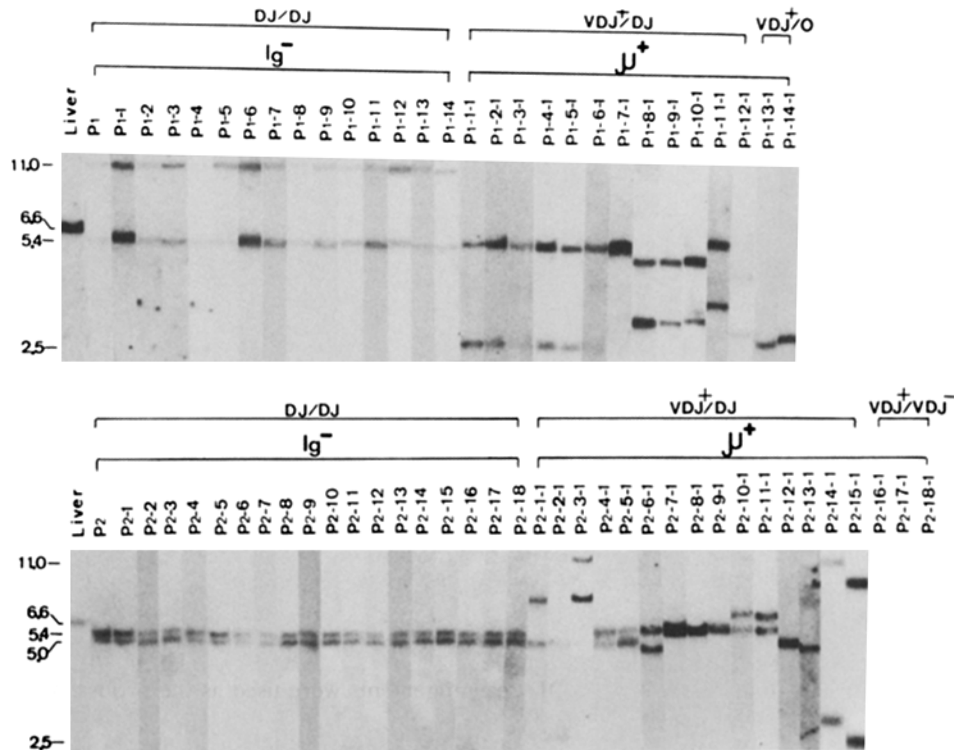

FIGURE 2. Analysis of heavy chain gene rearrangements of μ^+ subclones. DNAs were digested with Eco RI and hybridized to a J_H probe. When a μ^+ subclone retained or lost 5′ D flanking sequences, it was determined to be VDJ^+/DJ or VDJ^+/VDJ^-, respectively, as previously described (11). VDJ^+/O means the deletion of the J_H gene on one chromosome. A mixture of 5′ D_{FL}, 5′ D_{SP} and 5′ D_{Q52} flanking sequences was used as the 5′ D probe. Some μ^+ subclones (e.g., P_2-3-1) 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 P_1 and P_2. This might suggest secondary DJ_H complex formation as previously described (11).

subclones (P_1-1–14) revealed 11.0 and 5.4 kb Eco RI fragments, and another Ig^- parent clone P_2 and its Ig^- subclones (P_2–18) showed 5.4 and 5.0 kb Eco RI fragments. On the other hand, all μ^+ subclones (P_1-1-1 to P_1-14-1 and P_2-1-1 to P_2-18-1) revealed one or two further rearranged bands different from those of the parent clones. Note that 32 μ^+ subclones were generated from completely independent V_H to DJ_H rearrangements (see Fig. 1). Six μ^+ subclones from P_1-1-1 to P_1-6-1 showed the same rearranged pattern.

To determine which V_H segments were used in the rearrangements, RNAs were prepared from μ^+ subclones and analyzed by a Northern blotting procedure using probes specific for the V_H families, V_H36-60, V_HJ606, V_HJ558, V_HS107, V_HQ52, and V_H7183 (4, 5) (Fig. 3). Surprisingly, of 32 μ^+ subclones that were generated by independent V_H to DJ_H rearrangements, 31 (P_1-1-1 to P_1-13-1, and P_2-1-1 to P_2-18-1) used V_H segments of the V_HQ52 family. The remaining one μ^+ subclone (P_1-1-14-1) used a V_H segment of the V_H7183 family. No V_H to DJ_H rearrangements used the V_H36-60, V_HJ606, V_HJ558, or V_HS107 families. These

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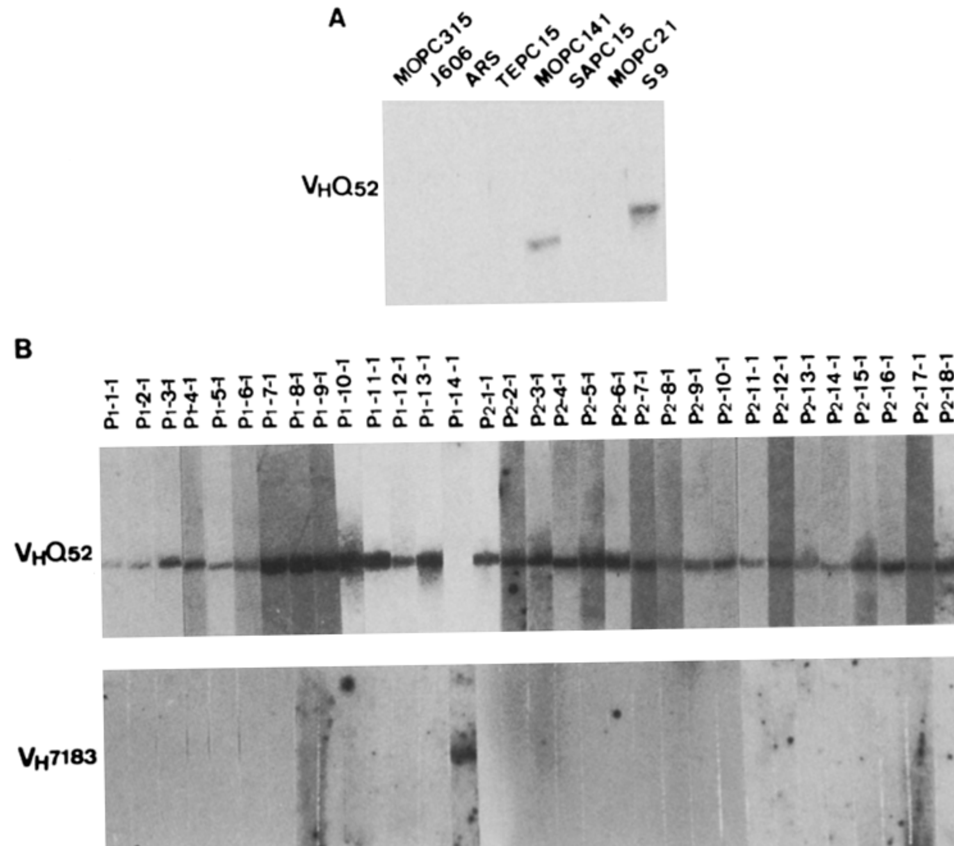

FIGURE 3. V_H expression of the V_HQ52 family in μ^+ subclones. *A*, specificity of the probes used for Northern blotting analysis. DNA probes were electrophoresed, blotted to nitrocellulose filters and hybridized to the 0.72 kb nick-translated Hind III–Sac I DNA fragment specific for the variable region of MOPC141 prepared by the incision from the 2.8 kb Hind III–Hind III fragment of MOPC141. *B*, Northern blot analysis. RNAs were prepared and analyzed as described in Materials and Methods.

results suggest that V_H segments of the V_HQ52 family were almost exclusively used, as this B-lineage precursor line gave rise to pre-B cell progeny.

The functional V_HDJ_H rearrangement of μ^+ P_1-7-1 (original name, AT11-2-5-1-5-51-1) was cloned and named S9 as previously described (2). Because S9 shares 85, 50, and 51% V_H coding region sequence homology with MOPC141 (V_HQ52 family), MOPC21 (V_H7183 family), and V_H81X (V_H7183 family [6]), respectively, S9 (V_H segment of P_1-7-1) is a member of the V_HQ52 family. This is consistent with the results of Northern blotting analysis.

Discussion

Yancopoulos et al. reported the preferential use of V_H segments of the V_H7183 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_HDJ_H rearrangements examined, and the preferential use of this V_H family was correlated with its proximity to

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the J_H 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 V_HQ52 family were preferentially or selectively used. The analysis of the V_H expression by pre-B and B hybridomas of fetal and neonatal mice indicated that the V_H repertoire of fetal B-lineage cells is largely restricted to the V_H7183 family and that subsequent recruitment of additional V_H families occurred during neonatal development (7). 78% of fetal liver–derived pre-B hybridomas used the V_H7183 family whereas no neonatal liver–derived pre-B hybridomas used the family. The determination of the V_H 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 V_H7183 family resulted in higher expression of more J_H-distal V_H families in the mature B cells of the adult with a concomitant decrease in the representation of the more J_H-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 V_HQ52 family but not the V_H7183 family. Thus, our results strongly indicated 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. Recently, the biased use of V_H segments of the V_HQ52 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′ V_H families between the two strains. They demonstrated that in the BALB/c strain, the V_H7183 family was the most J_H proximal whereas in the NIH/Swiss strain, at least a portion of the V_HQ52 family, which was preferentially used, occurred 3′ to the bulk of the V_H7183 family. We have demonstrated selective use of the V_HQ52 family in a BALB/c-derived clone.

Furthermore, it seems that selective use of the V_HQ52 family is independent of the types of preexisting DJ_H complexes because a $D_{SP2.8}$-J_{H3} complex in the P_1 clone (2) and $D_{FL16.1}$-J_{H3} and $D_{FL16.1}$-J_{H4} complexes in the P_2 clone (11) could also join selectively to the V_HQ52 family.

Summary

AT11-2, an Abelson virus–transformed cell line has DJ_H complexes on both chromosomes and is able to form functional variable region genes by the joins of V_H genes to the DJ_H complexes during culture. Therefore we examined which V_H gene family was used in functional V_H to DJ_H recombinations in AT11-2. Surprisingly, of 32 independent functional V_H to DJ_H recombinational events in AT11-2, 31 events used the V_H segments of the V_HQ52 family, and the remaining one used the V_H segment of the V_H7183 family. Thus, we describe here the first B precursor cell line that almost selectively uses the V_HQ52 family in functional V_H to DJ_H rearrangements. The selective use of the V_HQ52 family in this B precursor cell line strongly indicates nonrandom use of V_H gene families, and the existence of a stage at which the V_HQ52 family is preferentially used during

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the normal development of early pre-B cells and has important implications for understanding the ontogeny of V_H repertoire development. Furthermore, this cell line should prove extremely valuable in further studies of this kind.

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