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Involvement of the *D* Segment (DQ_{52}) Nearest to the J_H Region in Immunoglobulin Gene Rearrangements of Lymphoid-Cell Precursors

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A. Introduction

The specific antigen recognition molecules expressed by B and T lymphocytes, the immunoglobulins (Ig) and the T-cell receptor for antigen (TCR) are coded for by genes which are assembled in an ordered series of somatic DNA recombination events during lymphocyte differentiation. In B lymphocytes the heavy chain gene of the Ig molecule (IgH) is the first to be assembled and this occurs by two successive DNA rearrangements in which first a diversity segment (*D*) and then a variable gene segment (*V*) are joined, usually by a process of intrachromosomal deletion, to a joining segment (*J*) to form a complete variable region sequence (for a review, see Alt et al. 1986). A similar sequence of DNA recombinations leads to the assembly of variable region sequences from *V*, *D* and *J* gene segments in the *TCR* genes (for a recent review, see Kronenberg et al. 1986).

The initial gene rearrangement events are not entirely lineage restricted. About half of mouse T-cell lines, hybridomas and thymomas have IgH rearrangements (Forster et al. 1980; Cory et al. 1980; Kurosawa et al. 1981; Zuniga et al. 1982). *IgH* gene rearrangements in T lineage cells and *TCR* (β, γ) gene rearrangements in B lineage cells have been reported in 10%–30% of human lymphoid neoplasms (Pelicci et al. 1985; Rabbitts et al. 1985; Tawa et al. 1985; Greaves et al. 1986). These "cross-lineage" rearrangements appear to be nonfunctional and incomplete with no involvement of *V* regions.

In order to better understand the nature of these DNA alterations and their possible significance for lymphoid lineage commitment we have analysed the *IgH* gene rearrangements occurring in a group of human T-cell leukaemias and T-cell lines with precursor phenotypes and a similar group of B lineage leukaemias. These studies indicate that immature T cells frequently undergo an unusual type of DJ rearrangement of the *IgH* gene and that this rearrangement is only seen in B cells with the most primitive immunophenotypes. A detailed account of these studies is reported elsewhere (Mizutani et al. 1986).

B. Methods

Leukaemic blood cells from untreated patients were isolated and immunophenotyped with a panel of monoclonal antibodies as described previously (Greaves et al. 1982; Furley et al. 1986).

DNA isolation and restriction enzyme analysis was carried out essentially as previously described (Ford et al. 1983), except that the DNA probes were radiolabelled by the random primer method (Feinberg and Vogelstein 1984).

The *J* gene probes used are shown in Fig. 1. Probe D was excised from λ CH 28-6 (Ravetch et al. 1981; a kind gift from P. Leder) and subcloned in a plasmid vector. Probes A, B, C and E were excised from fragment D. Probe C was subcloned and re-excised before use. Probe F derives from

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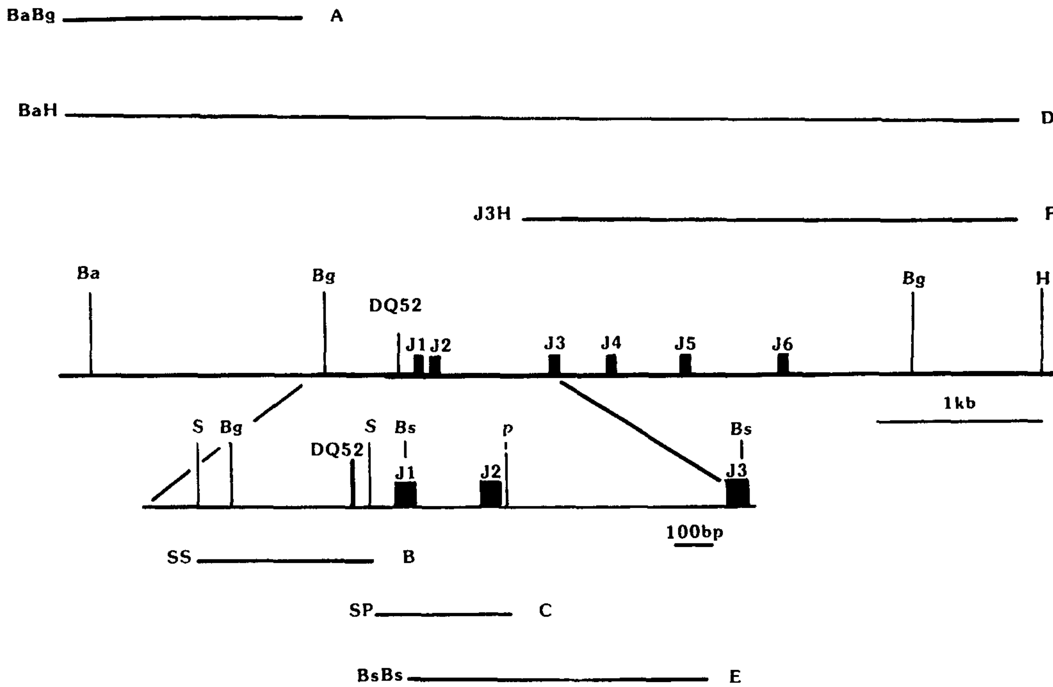

Fig. 1. Map of the human J_H gene region showing the restriction enzyme sites used to generate the DNA probes. *Ba, Bam*HI; *Bg, Bgl*II; *Bs, Bst*EII; *H, Hin*dIII; *S, Sma*I; *P, Pst*I. The restriction sites used to excise the probes are shown beside each probe. The 5′ end of fragment F terminates at J_3 in a *Bam*HI site created during the cloning of the genomic DNA (Rabbitts et al. 1981). This map is based on the published map and sequence of the *J* gene region (Ravetch et al. 1981). The restriction enzyme sites for *Eco*RI and one of the *Bam*HI and *Hin*dIII sites used in the gene rearrangement studies lie outside the region shown

C76R51 (Rabbitts et al. 1981; a kind gift from Dr. T. Rabbitts).

In order to analyse the *J* gene rearrangements, we isolated a series of DNA fragments from the *J* gene region as shown in Fig. 1. These DNA probes allowed us to identify gene rearrangement and to test whether it was accompanied by deletion of sequences 5′ to and within the *J* gene region as would be expected if rearrangement had occurred by intrachromosomal deletion. The *J* gene rearrangements were analysed with these probes using Southern transfers of DNA from the leukaemias digested with restriction enzymes which cut outside the *J* gene region.

I. Immature T Cell Leukaemias

In order to identify *IgH* J_H gene rearrangements in T-cell leukaemia DNAs, we first used the complete *J* region probe, D in Fig. 1. Since rearrangement usually involves the loss of DNA 5′ to the *J* regions, we next probed the Southern transfers with fragment A (see Fig. 1). DNA from nine T-cell leukaemias and 21 B-cell lineage leukaemias was tested in this way. Figure 2 shows the results of this procedure. The T-ALL DNA shown (Fig. 2I) has clearly retained sequences 5′ to J. DNA from five other T-cell leukaemic cells gave a similar result (Table 1). The rearranged allele retaining fragment A (cf. Fig. 1) shows a small decrease in size of 1–2 kb from the unrearranged fragment size irrespective of the restriction enzyme used. This suggested that these rearrangements resulted from small deletions within or close to the *J* gene segments. This was confirmed using probes C and E (see Fig. 1) which reveal that sequences between J_1 and J_3 have been deleted. These rearrangements are therefore consistent with DQ_{52} joining to J_3 or *J* segments 3′ to J_3. Two T-ALLs did not retain 5′ J in their rearranged μ gene and one (JM) had an unusual rearrangement probably involving DQ_{52}, but with no detectable deletion at all.

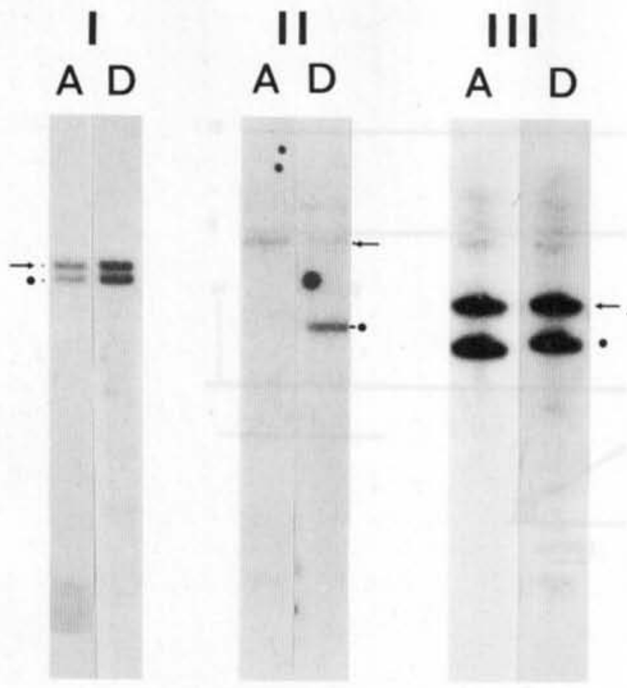

Fig. 2 I–III. J_H gene rearrangement and retention of 5′ to *J* sequences in some lymphoid precursor leukaemias. **I.** DNA from a T-ALL digested with *Bam*HI. **II.** DNA from common (B cell precursor) ALL digested with *Eco*RI. **III.** DNA from null (early B cell precursor) All digested with *Hin*dIII. *A*. Detected with probe A (see Fig. 1). *D*. Detected with probe D (see Fig. 1). Note retention of rearranged band/5′ *J* in samples **I** and **III**, but not **II**. →, germ line position, o, rearranged allele

No retention of the 5′ J fragment was observed in DNA from ten common ALL B-cell precursor leukaemias (Fig. 2 II); however, DNA from three out of ten null ALL which have a more immature B-precursor phenotype appear to have undergone the same rearrangement involving DQ_{52} as seen in T cells (Fig. 2 III; Table 1).

The occurrence of very similar recombination signal sequences – the conserved heptamer and nanomer sequences (for a review, see Alt et al. 1986) in the *V*, *D* and *J* gene segments in both the *Ig* genes and the *TCR* genes suggests that both sets of genes make use of common enzymes during rearrangement. This has further been established by the ability of AMuLV transformed pre-B cell lines to rearrange an introduced *TCR β* gene (Yancopoulos et al. 1986). The endogenous *TCR β* gene in the AMuLV transformed line was not rearranged, suggesting that control of gene rearrangement in lymphocyte development is mediated by accessibility in cells where the recombinases are active. The occurrence of bigenotypic (*IgH*/*TCR*) rearrangements in immature T cells as reported here and elsewhere indicates that the *J* gene segment is accessible to recombinases of some committed T cells undergoing *TCR* gene rearrangement. If accessibility in primitive lymphoid precursors was, initially at least, restricted to a limited window, then this might favour local gene rearrangements involving the *D* gene which lies close to the J_H gene segments, i.e. DQ_{52}. However, our data and that of others indicates the involvement in some T cells of more distant *D* regions.

It is not known what role if any DQ_{52} plays in the normal B-cell repertoire. On its own it cannot code for any of the mouse (Sakano et al. 1981) or human third hypervariable regions which have been reported on. It is found rearranged in the mouse myeloma QUPC52 (Sakano et al. 1981) and may also be present at the 3′ end of the third hy-

Table 1. Summary of *J* region rearrangements in the T and B lineage leukaemic cells

Leukaemias	Numbers tested	Numbers showing retention of 5′ region (DQ_{52}) in at least one allele
T Lineage (T-ALL)	9[a]	6[b]
B Lineage[c]		
Null ALL[d]	10	3
Common ALL	10	0
Mature B	1	0

[a] Selected from a larger group of T-ALL investigated; 15 others tested had no IgH rearrangements (Furley et al. 1986; Greaves et al. 1986). Of the nine, three were cell lines, the remainder were diagnostic samples from untreated patients.

[b] One T-ALL (the cell line JM) had undergone a different type of nondeletional recombination involving insertion or inversion of DNA near the *J* locus, but the breakpoint appears to be at or close to DQ_{52} (see Mizutani et al. 1986, for details).

[c] All diagnostic samples except one common ALL cell line (Nalm-6) and one mature B cell line (B85).

[d] Reactive with the pan B monoclonal antibody B4 (CD19; Nadler et al. 1984), but not expressing the common ALL (gp100/CD10) associated antigen (Greaves et al. 1981).

pervariable region in the expressed allele of a human CLL (Ravetch et al. 1981); in this latter case it may be part of a *VDDJ* rearrangement.

It is possible that DQ_{52} to *J* joins occur in normal B cells as a relatively common early or primary rearrangement event (reflecting limited accessibility) to be masked by later *D* to *J* or *D* to *DJ* rearrangements. This accords with the observation that the only three B lineage leukaemias (out of 21 tested) found to have J rearrangement with no deletion of the 5′ *J* region were in the subgroup with the most immature B lineage phenotypes (null ALL, see Table I). An analysis of *J* gene rearrangement in AMuLV transformed B-cell precursors also revealed a few examples of events which could be due to DQ_{52} to *J* rearrangement (Alt et al. 1981).

Further studies involving DNAase I sensitivity and hypersensitive site analysis are in progress to further investigate the possibility that the DQ_{52}-*J* region of the *IgH* gene might become preferentially accessible as a consequence of early events underlying commitment of the lymphoid lineages.

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