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Concomitant T-cell receptor α and δ gene rearrangements in individual T-cell precursors

(development/murine/lineage/thymus/ontogeny)

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ABSTRACT **A debate has recently surfaced concerning the degree of precommitment attained by αβ and γδ T-cell precursors prior to T-cell receptor (TCR) gene rearrangement. It has been suggested that precursors may be precommitted to rearrange either α or δ genes, but not both, thus giving rise to αβ- and γδ-producing T cells, respectively. Alternatively, the precursors may be flexible with regard to potential TCR gene rearrangements. To address this controversy, the gene rearrangements among a group of T-cell hybridomas from fetal, newborn, and early postnatal mouse thymi were examined. Six probes spanning the δ and α loci were used in Southern blot analyses to characterize the rearrangements which occurred on homologous chromosomes in each cell. Although homologous chromosomes often rearranged in synchrony within the α locus, a number of hybridomas were found which had retained a δ rearrangement on one chromosome and an α rearrangement on the second. Results show that a precommitment by T cells to rearrange δ or α genes in a mutually exclusive manner is not an absolute feature of mouse thymocyte development.**

The major T-cell receptors (TCRs) in mice and humans are each composed of two protein chains (α and β or γ and δ) linked by disulfide bonds (for review, see refs. 1–4). α, β, γ, and δ chains are composed of either three or four gene products: variable (V), diversity (D), joining (J), and constant (C). While β and γ genes are situated on distinct chromosomes (6 and 13, respectively, in the mouse), δ genes are positioned between the 50–100 Vα genes and 50–100 Jα genes on mouse chromosome 14. The fact that αβ and γδ TCRs are expressed in a mutually exclusive manner among T-cell subsets has warranted explanation and has yielded two contrasting hypotheses (refs. 5–12; see ref. 13 for review).

In the first case, it has been suggested that precursor T cells are committed to either αβ or γδ TCR expression prior to the full gene rearrangement process. The cells are therefore said to rearrange genes either in α or δ loci, but not both (5, 6). de Villartay *et al.* (8) have proposed that Jα pseudogene rearrangement may be instrumental in the commitment of cells to the αβ lineage.

The second explanation suggests that rearrangements may occur at α and δ loci within single developing cells, that lineage commitment is affected by the rearrangement event, and that cells which have rearranged genes in the δ locus nonproductively may be candidates for subsequent rearrangement and expression of the α genes (10–12).

To resolve the controversy, we have isolated single cells from normal mice by hybridoma production and analyzed the α and δ gene contexts on homologous chromosomes. Hybridoma production has been proven to be an effective technology with which cells may be isolated at various stages of thymocyte development and normal gene rearrangements may be assessed (14–19). Results show that during multiple stages of thymocyte ontogeny α and δ gene rearrangements appear concomitantly in single cells. Clearly, a precommitment to rearrange α or δ genes, but not both, is not an absolute feature of T-cell development.

MATERIALS AND METHODS

Mice. Female C57BL/6, male DBA/2, and (B6 × DBA/2)F_1 (BDF1) mice were purchased from The Jackson Laboratory. C57BL/6 and DBA/2 mice were mated overnight. Males were removed the following morning, which was designated day 0 for successful matings.

Fusions. Hybridomas were prepared as described previously (20). In brief, cells from freshly excised thymic tissue were suspended, fused with the BW5147 (BW/α-β-) thymoma line (21), and plated in HAT medium (Iscove's medium, 10% fetal calf serum, 4 mM glutamine, penicillin and streptomycin at 100 units/ml each, 100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine). Following a 1- to 2-week incubation period, viable cells were expanded and cloned by limiting dilution in 96-well microtiter plates in the presence of irradiated [2200 rad; 1 rad = 0.01 Gy] splenic feeder cells. Cell populations were then expanded for the preparation of DNA.

Southern Blot Analysis of Hybridoma DNA. Hybridoma DNA was isolated as described previously (22), digested with restriction enzymes, fractionated on agarose gels, transferred to nitrocellulose filters, and hybridized with ^{32}P-labeled probes at 65°C overnight. The cloned probes used for hybridizations are illustrated in Fig. 1. Probes were as follows: probe 1, a genomic 600-base-pair (bp) *Hin*dIII–*Hin*dIII fragment 5′ to Dδ1; probe 2, a genomic 850-bp *Hin*dIII–*Eco*RI fragment 6 kilobases (kb) 5′ to Jδ1; probe 3, Jδ1-specific genomic *Pvu* II–*Mbo* I 400-bp fragment; probe 4, a Cδ-specific probe, 2.5-kb *Hpa* I–*Kpn* I fragment from cosmid clone 51.1w7 (23); probe 5, probe 14 of ref. 23; and probe 6, 700-bp *Sac* I–*Hin*dIII fragment isolated from the phage clone HYα1 (a gift from Y. Uematsu, Basel Institute for Immunology, Basel).

RESULTS

A panel of thymic hybridomas was obtained by fusing pooled BDF1 mouse thymocytes with the BW5147 thymoma line. Thymic stages at the time of fusion (and the number of cloned hybridomas analyzed) were as follows: fetal day 16 (1), fetal day 17 (2), fetal day 18 (5), newborn (10), and postnatal day 1 (11). DNAs obtained from each of the hybridomas were first analyzed for BDF1-derived α gene rearrangements by Southern blots. Among the 29 cells, 14 showed no apparent

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Abbreviations: TCR, T-cell receptor; V, variable; D, diversity; J, joining; C, constant; BDF1 (C57B1/6 × DBA/2)F_1.

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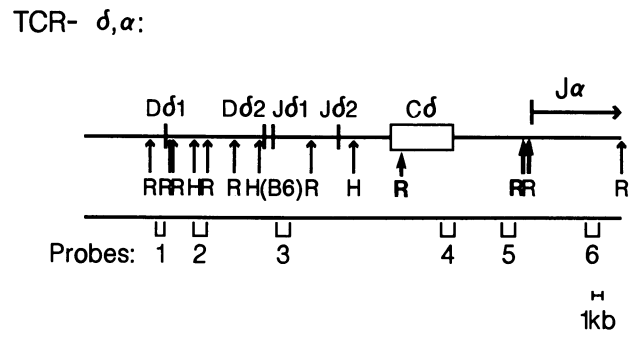

FIG. 1. TCR δ and α loci and probes. Portions of the δ and α loci are shown. The probes utilized in Southern blot analyses are designated at the bottom. See *Materials and Methods* for probe description. Relevant enzyme restriction sites are R, *Eco*RI, and H, *Hin*dIII. The polymorphic restriction site specific to C57BL/6 is designated B6.

rearrangements in the α region on either chromosome, 9 showed rearrangement within the α locus on each chromosome, and 6 showed rearrangement within the α locus on only one of the two homologous chromosomes (31).

The existence of the latter cells presented an opportunity to determine whether developing cells could rearrange δ and α genes simultaneously. If commitments were made to rearrange only α or δ genes, but not both, the second chromosome in these cells should show no rearrangement at δ. If no commitment was made, rearrangements within the δ locus could be present.

The six probes illustrated in Fig. 1 were used for the examination of δ and α loci in the hybridoma set. Representative Southern blot samples are shown in Fig. 2. Results from the complete analysis are summarized in Table 1. All bands on Southern blots derived from the BDF1 fusion partner, as the BW5147 line had no genes in the δ and 5′ Jα regions. Fig. 2*A* shows representative hybridoma and liver DNA samples digested with *Eco*RI and hybridized with the 5′ Dδ1 probe (probe 1, illustrated in Fig. 1). As seen among these DNAs, 5′ Dδ1 sequences were often deleted (as in 8/4/16/G9 and 6/16/T/F10.1), while in some instances (e.g., 7/18/18/G4) the germ-line gene was retained. Fig. 2*B* shows the hybridization of the 5′ Dδ2 probe (probe 2) with *Hin*dIII-digested DNA. In this case, the C57BL/6 and DBA/2 germ-line genes could be distinguished by the band size, being 5.8 and 15 kb, respectively. In the sample DNAs shown in Fig. 2*B*, patterns matched those of Fig. 2*A* in that only one of the hybridomas (7/18/18/G4) retained the germ-line sequence. In this case, the germ-line band could be shown to represent the DBA/2-derived gene. Fig. 2*C* exhibits the hybridization of the Jδ1 probe (probe 3) with *Eco*RI-digested DNA. Jδ1 genes were rearranged in hybridomas 8/4/16/G9 and 6/16/T/F10.1. Fig. 2*D* shows the hybridization of each *Eco*RI-digested DNA with a probe located between δ and α loci (probe 5). Each hybridoma shown in this figure maintained the germ-line sequence recognized by probe 5. Hybridizations with probe 4 also showed germ-line sequences after hybridization to *Eco*RI-digested DNA (data not shown).

BDF1 liver DNA was next digested with the *Sca* I enzyme, yielding two bands on Southern blots hybridized with probe 4. This revealed the polymorphism between C57BL/6- and DBA/2-derived gene sequences. Digestion of the sample hybridoma DNAs with *Sca* I and hybridization with probe 4 showed that one BDF1-derived chromosome from each of the sample cells retained the Cδ germ-line sequence, while the second chromosome had deleted the Cδ sequence (data not shown).

Finally, in Fig. 2*E* is shown hybridization of *Eco*RI-digested DNAs with the Jα probe, probe 6. As was previously noted (31), each hybridoma showed two bands (germ-line and rearranged) consistent with a Jα (normal or pseudogene) rearrangement on one of the BDF1-derived chromosomes in each cell.

All Southern blot data are shown in Table 1 along with interpretations of blot results. In three of the six hybridomas known to contain an α rearrangement on only one chromosome, the homologous chromosome bore a rearrangement within the δ locus (hybridomas 8/4/16/G9, 6/14/T/D4, and 6/16/T/F10.1). All rearrangement patterns were different as determined by Southern blot analyses with multiple enzymes (data not shown) and all patterns were consistent with Jδ1 joining on one chromosome and Jα (normal or pseudogene) joining on the second. Whether the Jδ and Jα joins in these hybridomas could render functional TCR protein products was not determined. Each of these three hybridomas derived from a separate fusion and different stage of development. A preliminary analysis of hybridomas prepared by fusions of adult thymocytes to the BW parent cell revealed several further cases of δ and α gene rearrangements in single cells (unpublished results). Again, at least 20% of all hybridomas which bore an α gene rearrangement bore a δ gene rearrangement on the homologous chromosome.

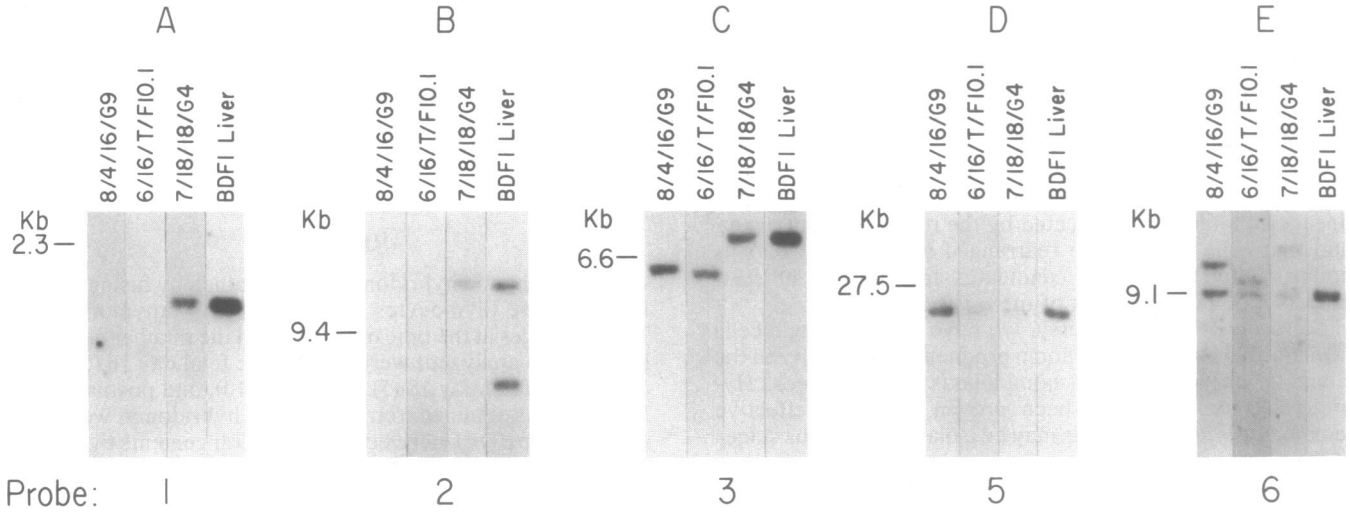

FIG. 2. TCR gene rearrangements among thymic hybridomas. Sample hybridoma and liver DNAs are shown after Southern blot analyses with each of the probes designated in Fig. 1.

Table 1. TCR α and δ gene rearrangements within early thymus hybridomas

Hybridoma	Age*	Chromosome	Band patterns with gene probes†						Gene context‡
			1	2	3	4	5	6	
8/4/16/G9	F-16	1	–	–	R	G	G	G	δ rearrangement
		2	–	–	–	–	–	R	α rearrangement
7/18/18/G4	F-18	1	G	G	G	G	G	G	Germ-line
		2	–	–	–	–	–	R	α rearrangement
6/14/T/C1	Neo	1	–	–	–	–	–	G	?
		2	–	–	–	–	–	R	α rearrangement
6/14/T/D4	Neo	1	–	–	R	G	G	G	δ rearrangement
		2	–	–	–	–	–	R	α rearrangement
6/14/T/E9	Neo	1	G	G	G	G	G	G	Germ-line
		2	–	–	–	–	–	R	α rearrangement
6/16/T/F10.1	Post-1	1	–	–	R	G	G	G	δ rearrangement
		2	–	–	–	–	–	R	α rearrangement

*Thymocyte stage at the time of hybridoma formation is designated F-16 and F-18 for fetal days 16 and 18, respectively; Neo for neonate; and Post-1 for postnatal day 1.

†Band patterns contributed by the two BDF1-derived chromosomes are designated G, R, and – for germ-line, rearranged, and deleted, respectively.

‡Predicted gene context on BDF1-derived homologous chromosomes in each of the hybridoma samples.

In the case of hybridoma 6/14/T/C1, D, J, and Cδ genes were lost on both chromosomes. Both chromosomes bore a rearrangement positioned between probes 5 and 6. Two hybridomas, 7/18/18/G4 and 6/14/T/E9, maintained the δ complex in germ-line configuration on one chromosome.

DISCUSSION

The analyses described here reveal the potential among developing cells to rearrange both α and δ genes during T-cell development. Clearly, rearrangements within α and δ loci in single cells need not occur in a mutually exclusive manner.

It was once argued that cell fusion to the BW line might promote gene rearrangements at TCR loci and that hybridoma technology could therefore not provide an effective system for T-cell analysis. Multiple studies have since illustrated that TCR gene rearrangements in fused cell populations are stable and accurately represent the cells from which they derive. When T-cell-depleted bone marrow cells are fused to the BW line, the bone-marrow-derived chromosomes do not acquire TCR gene rearrangements (22). The same is true for fetal liver cells (15). When immature T-cell populations are fused to BW, their rearrangements do not progress (15). Due to their stability, hybridomas have provided a great deal of information concerning TCR gene rearrangements and function in developing and mature T-cell populations (e.g., see refs. 14–19, 24).

Past analyses have yielded conflicting results regarding the ability of T-cell precursors to rearrange both δ and α gene sequences. Experiments performed by Winoto and Baltimore (5) involved the examination of thymic DNA circles from 4- to 5-week-old mice. These authors suggested that the circles which derived from α rearrangements had maintained δ genes in the germ-line context. They concluded that a precommitment of cells to the $\alpha\beta$ lineage prevented rearrangement in the δ region (5, 6).

In a separate study Takeshita *et al.* (12) examined sequences from thymic and splenic circular DNAs, also thought to derive from α gene rearrangements. In these sequences, δ gene rearrangements were indicated. These authors concluded that consecutive rearrangements at δ and α loci may represent a common feature of T-cell development.

An explanation for these apparently contrasting results may emerge by consideration of the rigid order maintained in the δ and α gene rearrangement events. Distinct patterns of rearrangement have been clearly illustrated within both δ and α loci during the progression of ontogeny. As an example of this, rearrangements have been shown to appear predominantly in the 5′ region of the Jα locus among early thymocytes, but in the middle or 3′ region of the locus in mature peripheral T cells (25, 31). Nonrandom joining has also been revealed by the finding that homologous chromosomes usually rearrange within similar regions of the Jα locus (25, 29, 31).

The patterned rearrangements are likely prompted by multiple factors. An example of the effect environmental factors may have on gene rearrangement patterns is provided by studies of the immunoglobulin isotype switch rearrangement during B-cell development. It has been demonstrated in several laboratories that the switch rearrangements within B-cell cultures may be manipulated by altering the interleukin content of culture media prior to the rearrangement event (26–28). Culture conditions may similarly affect TCR α gene rearrangements in T-cell differentiation cultures (29).

Additional factors which may skew rearrangements within the δ and α loci include cell stage, gene proximity, and enhancer/repressor elements (6, 30). Due to the multiple, complex influences that promote certain patterns of gene rearrangement and that prompt homologous chromosomes to rearrange similarly, many cells which undergo α gene rearrangement may never rearrange δ genes. Likewise, many cells which undergo δ gene rearrangements may never rearrange α genes. These effects, however, give no hint as to the gene rearrangement potential of the precursor cell.

At present there is no evidence that a normal T cell which has rearranged δ genes is prohibited from rearranging genes within the α locus and ultimately producing $\alpha\beta$ TCR protein products. Therefore, the precursors from which $\alpha\beta$ and $\gamma\delta$ producers derive likely constitute overlapping rather than distinct T-cell populations. By clonally marking precursor cells in normal mice and examining their gene rearrangement patterns throughout development, ultimate restrictions on developmental gene patterns, or the lack thereof, may finally be elucidated.

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1. Winoto, A., Mjolsness, S. & Hood, L. (1985) *Nature (London)* **316**, 832–836.

2. Arden, B., Klotz, J. L., Siu, G. & Hood, L. E. (1985) *Nature (London)* **316,** 783–787.
3. Hayday, A. C., Diamond, D. J., Tanigawa, G., Heilig, J. S., Folsom, V., Saito, H. & Tonegawa, S. (1985) *Nature (London)* **316,** 828–832.
4. Wilson, R. K., Lai, E., Concannon, P., Barth, R. K. & Hood, L. E. (1988) *Immunol. Rev.* **101,** 149–172.
5. Winoto, A. & Baltimore, D. (1989) *Nature (London)* **338,** 430–432.
6. Winoto, A. & Baltimore, D. (1989) *Cell* **59,** 649–655.
7. Bonneville, M., Ishida, I., Mombaerts, P., Katsuki, M., Verbeek, S., Berns, A. & Tonegawa, S. (1989) *Nature (London)* **342,** 931–934.
8. de Villartay, J.-P., Hockett, R. D., Coran, D., Korsmeyer, S. J. & Cohen, D. I. (1988) *Nature (London)* **335,** 170–174.
9. Ferrick, D. A., Ohashi, P. S., Wallace, V., Schilham, M. & Mak, T. W. (1989) *Immunol. Today* **10,** 403–407.
10. Pardoll, D. M., Fowlkes, B. J., Bluestone, J. A., Kruisbeek, A., Maloy, W. L., Coligan, J. E. & Schwartz, R. H. (1987) *Nature (London)* **326,** 79–81.
11. Allison, J. P. & Lanier, L. L. (1987) *Immunol. Today* **8,** 293–296.
12. Takeshita, S., Toda, M. & Yamagishi, H. (1989) *EMBO. J.* **8,** 3261–3270.
13. Raulet, D. H. (1989) *Annu. Rev. Immunol.* **7,** 175–207.
14. Born, W., Rathbun, G., Tucker, P., Marrack, P. & Kappler, J. (1986) *Science* **234,** 479–482.
15. Born, W., Yagüe, J., Palmer, E., Kappler, J. & Marrack, P. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2925–2929.
16. Chien, Y.-h., Iwashima, M., Wettstein, D. A., Kaplan, K. B., Elliott, J. F., Born, W. & Davis, M. M. (1987) *Nature (London)* **330,** 722–727.
17. Iwashima, M., Green, A., Davis, M. M. & Chien, Y.-h. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8161–8165.
18. Korman, A. J., Maruyama, J. & Raulet, D. H. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 267–271.
19. Ito, K., Bonneville, M., Takagaki, Y., Nakanishi, N., Kanagawa, O., Krecko, E. G. & Tonegawa, S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 631–635.
20. Hurwitz, J. L., Heber-Katz, E., Hackett, C. J. & Gerhard, W. (1984) *J. Immunol.* **133,** 3371–3377.
21. White, J., Blackman, M., Bill, J., Kappler, J., Marrack, P., Gold, D. P. & Born, W. (1989) *J. Immunol.* **143,** 1822–1825.
22. Hurwitz, J. L., Samaridis, J. & Pelkonen, J. (1988) *Cell* **52,** 821–829.
23. Malissen, M., Trucy, J., Letourneur, F., Rebaï, N., Dunn, D. E., Fitch, F. W., Hood, L. & Malissen, B. (1988) *Cell* **55,** 49–59.
24. O'Brien, R. L., Happ, M. P., Dallas, A., Palmer, E., Kubo, R. & Born, W. K. (1989) *Cell* **57,** 667–674.
25. Hurwitz, J. L., Samaridis, J. & Pelkonen, J. (1989) *J. Immunol.* **142,** 2533–2539.
26. Lutzker, S., Rothman, P., Pollack, R., Coffman, R. & Alt, F. W. (1988) *Cell* **53,** 177–184.
27. Coffman, R., Shrader, B., Carty, J., Mosmann, T. & Bond, M. (1987) *J. Immunol.* **139,** 3685–3690.
28. Berton, M. T., Uhr, J. W. & Vitetta, E. S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 2829–2833.
29. Thompson, S. D., Pelkonen, J., Rytkönen, M., Samaridis, J. & Hurwitz, J. L. (1990) *J. Immunol.* **144,** 2829–2834.
30. Bories, J. C., Loiseau, P., d'Auriol, L., Gontier, C., Bensussan, A., Degos, L. & Sigaux, F. (1990) *J. Exp. Med.* **171,** 75–83.
31. Thompson, S. D., Pelkonen, J. & Hurwitz, J. L. (1990) *J. Immunol.*, in press.