Circular DNA Resulting from Recombination between V-(D)-J Joining Signals and Switch Repetitive Sequences in Mouse Thymocytes

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Summary

During the course of analyzing circular DNA in mouse thymocytes, novel recombinants were identified with immunoglobulin heavy chain joining gene and switch region probes. These circles represent excision products of recombination between the heptamer-nonamer motif for V-(D)-J joining and a repetitive sequence for class switching. The molecular mechanisms that generate “hybrid circles” are discussed.

Somatic DNA recombination plays a key role in activating and diversifying the Ig and TCR genes during lymphocyte development. For the V-(D)-J type of joining, recombination signal sequences (RSS's) are found adjacent to each germline V, D, or J segment, consisting of a highly conserved heptamer, CACTGTG, and nonamer, GGTGTTTGT, separated by a spacer of constant length (1). Normally, recombination occurs between one RSS containing a 12-bp spacer and a second RSS containing a 23-bp spacer; this is the so-called 12/23-bp spacer rule.

Another type of rearrangement, known as class switch recombination, occurs only in the Ig H chain genes. This recombination is responsible for changing the isotype of Ig H chains, by replacing an upstream set of C gene exons with another downstream set. Switch recombination takes place between a pair of sites, one in the intron between the JH and Cμ gene, the other in a region upstream from one of the other C genes (2). These recombination sites are variable and lie in the switch (S) regions. The S regions lack conserved recombination signal sequences, such as the heptamer-nonamer motifs or V-(D)-J joining, but are rich in repetitive sequences. Although little is known about the enzymatic machinery, it is generally believed that two distinct “recombinases” mediate V-(D)-J joining and class switch recombination.

To study the mechanisms for V-(D)-J joining and class switch recombination, we and others have previously characterized extrachromosomal circular DNA in lymphocytes. Thymocyte circular DNA contains the excision products derived from the V-(D)-J joining of TCR genes (3, 4). More recently, it was shown that switch-activated B cells contain circular DNA derived from the switch recombination between two distinct S regions (5–8). In general, the characterization of circular DNA in lymphocytes has shown that both V-(D)-J joining and the Ig class switch are accompanied by intramolecular DNA deletion, which results in covalently closed excision products.

During the course of analyzing circular DNA in thymocytes, we noticed that clones positive with Ig Jα region probes could be isolated from the circular DNA library. Previous work on the JN genes from T cell lines showed that IgH D-to-J joining often occurs in T lineage cells (9). Curiously, we have found that most of the rearranged clones isolated with Jα region probes did not contain the normal signal joint of two fused RSS's, but instead contained structures resulting from recombination between a RSS of D or J segments and a site in the switch repetitive region. In this report, we characterize the unusual circular molecules by restriction enzyme mapping and DNA sequencing.

Materials and Methods

Preparation of Circular DNA. Thymus glands from 3-wk-old BALB/c mice were used for the preparation of thymocyte circular DNA as previously described (3). The circular DNA material was treated with ATP-dependent DNAase of Micrococcus luteus (U.S. Biochemical Corp., Cleveland, OH) to eliminate residual chromosomal DNA. A phage library was made with AgtWES.

Flow Cytometric Analysis. For FACS® staining analysis (Becton Dickinson & Co., Mountain View, CA), cells from disrupted thymic tissue were passed through Nitex, and washed three times in HBSS with 2% FCS, 0.1% azide. Cells (10⁶) were incubated at 4°C for 1 h with saturating amounts of FITC-conjugated anti-Thy-1.2 mAb (30-H12; Becton Dickinson & Co.) or FITC-conjugated anti-B220 mAb (RA3-6B2; courtesy of N. Glaichenhaus, American Type Culture Collection, Rockville, MD). After washing, cells were analyzed on a FACS® 440 (Becton Dickinson & Co.).

DNA Probes. DNA probes used for screening of the circular DNA clones are as follows: (a) DFL16.1, 0.8-kb BamHI-BamHI; (b) 3'-D128.8, 340-bp PstI-PstI; (c) 5'-Jα, 1.6-kb EcoRI-XbaI;
DNA Sequencing and Other DNA Methods. Nucleotide sequences were determined by the chain-termination method with dideoxynucleotides (10). DNA clones were analyzed by standard procedures.

Results and Discussion

The mouse thymocyte circular DNA library was screened with a 4.0-kb XbaI-XbaI probe containing DQ52 and the entire JH region. With this probe, >200 JH-positive clones were isolated from the library of 350,000 recombinant phage. The frequency of JH-positive clones was comparable with that previously observed for screenings with TCR Jα probes (3). This indicates that JH excision events are not rare in thymocytes from 3-wk-old mice. 38 of these clones were single plaque purified and analyzed further with several JH region probes. The hybridization data showed that 31 of these clones represent excision products in which both DQ52 and the JH segments remain in the germline configuration. Seven other JH-positive clones contained rearrangement in the 5′ region. They were positive with JH1-a and 3′JH probes, but negative with the 5′Jα probe. The library was also screened with the 5′-Spα probe, a 309-bp EcoRI-XbaI fragment. 23 5′-Spα-positive clones were single plaque purified and analyzed further with various IgH probes. These hybridization experiments showed that most of the Spα-positive clones also contain JH1-a, and JH sequences. Some of these clones were further analyzed by restriction mapping. They include clones ICD116, ICD137, ICD144, ICD162, ICD171, ICD172, and ICD165.

By comparing restriction enzyme cleavage maps, the recombination sites on the clones were predicted. In Fig. 1, DNA regions excised into the circular DNA are shown underneath the germline map of the Jα-Cμ region. Clones were charac-

Figure 1. Circular DNA molecules resulting from recombination between RSS and switch sequences. Seven clones were analyzed by restriction enzyme mapping. By comparing the maps of recombinants with those of the germline regions, recombination sites were predicted. DNA regions excised into the circles are shown. In the map, switch (Sp) regions are stippled. Coding sequences (DQβ, L'S, and Cα exons) are indicated by vertical bars. Triangles represent recombination signal sequences (RSS's) for V(D)J joining. Restriction sites are shown: EcoRl (E), BamHI (B), XbaI (X), and ScaI (S).

Figure 2. Nucleotide sequences of seven clones of hybrid circles. Clones ICD116, ICD137, ICD144, ICD162, ICD171, ICD172, and ICD165 were analyzed. Sequences around the breakpoint were compared with their corresponding germline sequences. Precise recombination sites (indicated by vertical lines) were determined by comparing the sequences of recombinant clones with those of germline counterparts. For the clone ICD165, the best fitted Spα consensus sequence (MUSIGCD09) was used as the germline sequence. Heptamers and nonamers of the RSS's are boxed. DQα and Jα coding sequences are bold underlined. The Spα trinucleotide sequence GTG, found at the breakpoint, is underlined. Colors between two sequences indetical residues.
Jogous to the excision product of so-called pseudonormal RSS is not retained at the recombination breakpoint, but the middle of the overall SA region (Fig. 1). In ICD165, the mapping predicted that the recombination site lies in the Sp. region and at J.,2. In Fig. 2 b, the recombinant sequence is compared with germline JH coding sequence. Therefore, the clone ICD165 is analogous to the excision product of so-called pseudonormal recombination (3, 11), in which rearranged coding sequence is retained on the circular DNA, and the signal joint is formed on the chromosome (Fig. 3 b).

In this report, we have characterized novel circular DNAs that represent excision products of recombination between the RSS and a switch repetitive sequence (Fig. 3). These “hybrid circles” were discovered during the course of analyzing thymocyte circular DNA. Since the FACS analysis demonstrated that the thymocyte sample contained <0.3% B220-positive cells, it is unlikely that these Ig circular DNAs were isolated from B lineage cells. In addition to the unusual recombination reported here, D-to-J joining of IgH is rather common in T cells. Why the Ig genes are rearranged in T cells is an unresolved question. However, it is assumed that a common recombinase is responsible for both Ig and TCR gene rearrangements, and that the Ig J region is activated for recombination at early stages of T cell development. It is somewhat curious that Ig Jμ region is involved in the rearrangement in T lineage cells. More puzzling here is why the RSS can join with the Jμ sequence. To account for the origin of the Ig “hybrid circles”, one possibility is that some basic components are shared by the V-(D)-J class switch recombinases. Another possibility is that the GTG in the switch repetitive sequence is recognized by the V-(D)-J recombinase, as it was part of an RSS heptamer. As shown in Fig. 2 a, the trinucleotide GTG was found in the Jμ sequence at the breakpoints in most of the clones. In the heptamer, the trinucleotide adjacent to the recombinase site is essential (12, 13), and appears to serve by itself as a joining signal, at least in the Vn gene replacement (14, 15).

In the Ig H chain genes, the Jn-Sμ region often serves as a target for aberrant DNA rearrangement. It has been postulated that the V-(D)-J or switch recombinase is responsible for aberrant rearrangements in some lymphoid tumors (16). The “hybrid circles” described in this report appear to be formed, at least in part, by the action of a V-(D)-J recombinase, but in a manner that is aberrant with respect to the 12/23-bp spacer rule. We have analyzed T cell lines and T cell hybridomas for the Sμ rearrangement on the chromosome. Among 24 samples analyzed, rearrangement was found in one pre-T cell line, KKA (17), which is Thy-1+ and CD3- . The Sμ region was not rearranged in the other T cell samples tested, most of which represent mature stages of development (our unpublished observation). It is possible that the hybrid circle formation may be limited to certain stages of T cell maturation in the thymus. Deregulation of the recombinase machinery may occur in dying T cells, leading to the formation of hybrid circles. Further studies with separated thymocyte populations will elucidate the biological significance of hybrid circle formation in thymocytes. It is of interest to study whether this type of recombination also occurs in B cells. However, if it occurs during B cell ontogeny, such cells would be either blocked in their ability to form functional VDJ structures or blocked in their ability to undergo class switch at later stages. In any case, the discovery of hybrid circles is of intrinsic interest for the understanding of the recombination mechanisms of antigen receptor genes.

Figure 3. Schematic diagrams of hybrid circle formation. Two types of circles are generated between the RSS and Sμ sequences, depending upon relative orientations of RSS to Sμ sequence. In pathway a, the 3'-RSS of the Dμ recombines with Sμ sequence, and both are retained on the circle, while the Dμ-coding sequence remains on the chromosome. In pathway b, the 5'-RSS of Jn recombines with Sμ, but remains on the chromosome. The Jn-coding sequence, recombined with Sμ sequence, is retained on the circle. Coding sequences for D, J, and Cμ exons are shown as filled bars. Switch regions are stippled. Triangles represent RSS's.
We thank A. Otsuka, M. E. Koshland, and R. J. Aguilera for critical reading of the manuscript, and Y. Hashimoto and A. Winoto for furnishing T cell DNA samples. We also thank P. Duplay and N. Glaichenhaus for help with the FACS®, and C. M. Samson, W. Chung, and W. A. Weinberg for technical assistance.

This work was supported by grants from the National Institutes of Health (AI-18790) and the American Cancer Society (IM-366).

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Received for publication 13 November 1990 and in revised form 13 December 1990.

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