DETECTION OF T CELL RECEPTOR α CHAIN LOCUS REARRANGEMENTS IN HUMAN ANTIGEN-SPECIFIC T LYMPHOCYTE CLONES USING A DNA PROBE SPECIFIC FOR CHROMOSOME REGION 14q11

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A central feature of antigen recognition by T lymphocytes is that it occurs only when the antigen is associated on the cell surface with a molecule encoded by the MHC, (2-4). The antigen-MHC recognition unit of the human T cell (T3-Ti) has been defined as a clonotypic, disulfide-linked heterodimer (Ti) composed of one α and one β chain associated with three monomorphic but distinct 20-25 kD T3 molecules (5, 6). The genes encoding functional α and β chains are generated by recombination of at least three (variable [V], joining [J], and constant [C]) DNA regions for the α chain (7), and four (V, diversity [D], J, and C DNA regions for the β chain (8). The human locus of the β chain has been mapped to band q35 of the chromosome 7 (9), whereas that of the α chain was assigned to chromosome 14 (band 14q11) (10, 11).

We have recently reported the isolation of a human DNA clone from the 14q11 chromosome band (1). We took advantage of a t(8;q14) (q24;ql 1) translocation in the KE-37R human leukemic T cell line. Since the breakpoint on chromosome 8q+ was close to the 3' end of the c-myc oncogene, a c-myc representative probe was used to screen a genomic library of the KE-37R cell line and to isolate a junction fragment containing the c-myc 3' end and a 14q11-specific DNA segment which was subcloned. This subclone was designated K40, and used as a probe, it revealed the presence of rearranged restriction patterns with leukemia cell DNA from several patients with T cell malignancies, whereas germline patterns were observed in DNA from normal tissue and non-T cell malignancies (1). These data suggested that K-40 may be a part of the T cell receptor (TCR)-α chain locus. To further address this question, we have used human T cell clones derived from in vitro allostimulated lymphocyte populations or PHA-stimulated lymphocytes. In this report we show that DNA rearrangements in T-cell clones are detected by hybridization (a) not only with a TCR-α chain cDNA clone, but also (b) with the K40 probe. Taken together, the present data and previous reports (1) show that K40 is a part of the TCR-α chain locus,

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and will be valuable for studying rearrangements of TCR-α gene family in T cells.

Materials and Methods

**Human T Cell Clones.** The human T cell clones BJ1, BJ28, and BJ37 were derived from the allostimulated IL-2-dependent T cell line BJL1. Cloning was performed by micromanipulation, as described elsewhere (12).

SA-K10, SA-1, and SA-6 clones derived from PBL of a healthy donor incubated with rIFN-γ (Biogen, Cambridge, MA) for 48 h before cloning and recloning, as previously described (13). Briefly, cloning was performed by limiting dilution in Terasaki plates at 0.3 cell/well in the presence of 5% IL-2-conditioned medium, autologous irradiated feeder cells, and PHA (Wellcome, Beckenham, United Kingdom). PHA was used as a mitogenic stimulus because of its ability to provide high cloning efficiency and growth of T cells regardless of antigenic specificity. Several subclones were expanded by feeding every 2–3 d with IL-2. Restimulation was performed every 2 wk in the presence of PHA, IL-2, and autologous feeder layers. All the experiments were carried out at least 8 d after replacing the PHA-containing media to avoid any interference of the mitogen in the results of the tests.

Determination of phenotype, function, and specificity were described previously (14).

**DNA Probes and Southern Blot Hybridization.** The pGA5 clone originated from a TCR-α cDNA clone isolated from Jurkat cells (15), and was supplied by Dr. E. Palmer.

The TCR-β clone (pDO-β1 clone) was obtained from Dr. Strominger. A Bgl II 0.4 kb fragment was used as a probe for the Cβ sequences.

The K40 clone has been described elsewhere (1), and is presented in Fig. 2. For hybridization, a mixture of two probes corresponding to a 1.3 kb Xba fragment (K40-B) and a 1.5 kb Hind III fragment (K40-C) (see Fig. 2) was used.

Results and Discussion

Individual T cell clones were isolated either by limiting dilution (13) or micromanipulation (12). The BJ T cell clones derived from an in vitro allostimulated IL-2-dependent T cell line, BJL, whereas the SA T cell clones were obtained by cloning cells directly from PBMC treated with rIFN-γ and rIL-2 (Bourge, Degos, and Bensussan, manuscript submitted for publication). The phenotype and function of these IL-2-dependent cell populations are listed in Table I.

To ascertain their clonality, DNA from each individual clone was first hybridized with a probe representative for the C region of the TCR-β chain gene. An Eco RI restriction pattern of the germline DNA showed the presence of two 11.5 kb and 4.0 kb fragments corresponding, respectively, to Cβ1 and Cβ2 regions. All the clones displayed rearranged patterns (Fig. 1). With the exception of clone SAK10, deletion of the 11.5 kb band was consistently observed in all the clones, in agreement with recent work (17) showing that this event is a marker for the T cell lineage. Digestion with Hind III allowed a clearcut distinction between those cases where Eco RI rearranged bands had a similar size.

Another pattern of TCR-β rearrangement was observed in clone SA-K10 (Fig. 1). Although present, the Eco RI 11.5 kb fragment exhibited a fainter intensity, and new bands were visible, suggesting that only one Cβ1 allele was rearranged in this clone. Such a situation has been already described (18). Hind III patterns also indicated the presence of rearranged TCR-β regions. Taken together, these results were consistent with clonality of these T cells.
| Clone | Phenotype | Function | TCR-β gene patterns (Cp region) | TCR-α gene patterns | K40-B + K40-C patterns |
|-------|-----------|----------|-------------------------------|---------------------|------------------------|
|       |           |          | Eco RI | Hind III | Eco RI | Hind III | Eco RI | Hind III |
| BJL*  | T3+,T4+, T8-,T11* | Cytotoxic MHC class I or II-specific | C1 (smear) C2 | GL | GL, 1.5 kb | GL | GL** |
| B cells |          |          | C1 (11.5 kb) C2 (4.0 kb) | 7.0 kb | GL* (23, 8.0, and 4.4 kb) | GL (8.5, 3.5, and 2.2 kb) | GL** (8.5, 5.0 kb) | GL (5.0, 1.5 kb) |
| BJ-17 | T3+,T4+, T8-,T11* | Proliferative class II-specific | C1, C2 10 kb | GL, 7.2, 5.0 kb | GL | GL | GL |
| BJ-28 | T3+,T4+, T8-,T11* | Cytotoxic class II-specific | C1, C2 12 kb | GL, 15.0 kb | Deleteion of 8 kb | GL | GL |
| BJ-37 | T3+,T4+, T8-,T11* | Cytotoxic class II-specific | C1, C2 10.5 kb, 8.0 kb | GL, 7.2, 17, and 5.0 kb | GL, 9 kb | GL, 7.2 kb | GL, 8 kb | GL, 5.0, 7.2, and 17 kb |
| SA-1  | T3+,T4+, T8-,T11* | Suppressor of proliferation; restricted | C1, C2 9 kb | GL, 5.0 kb | Deleteion of 8.0 kb | Deletion of 1.5 kb | GL |
| SA-6  | T3+,T4+, T8-,T11* | Suppressor of proliferation; restricted | C1, C2 | Deletion of 8.2 kb | GL | GL | Deletions of 3.0, 8.5 kb | Deletions of 1.5 kb; partial deletion of 5.0 kb |
| SA-K10 | T3+,T4+, T8-,T11* | NK activity | C1, C2, 5.0 kb, 12 kb | GL, 5.0 kb | Deleteion of 8.0 kb | Deletion of 8.5 kb | Deletions of 3.0, 8.5 kb | Deletion of 1.5 kb |

* Allostimulated polyclonal population.
† Autologous B lymphoblastoid cell line.
‡ This germline pattern corresponds to the most frequent restriction fragment length polymorphism.
§ Deletion of C1 or C2.
GL indicates the presence of major germline bands (Eco RI: 23, 8, and 4.4 kb; Hind III: 8.5, 3.5, and 2.2 kb).
** Germline bands (Eco RI: 8.5, and 5.0 kb; Hind III: 5.0, and 1.5 kb).

Next, the Southern blot hybridization was repeated using a TCR-α probe (pGA5 clone, Fig. 2A). Alterations of the germline pattern were detected in cytotoxic MHC class II-specific BJ-28 and BJ-37 T cell clones, T3+ SA-K10 T cell clone with natural killer–like activity, and restricted suppressor T cell clone SA-1. SA-1 and SA-K10 T cell clones showed deletion of a 8.0 kb Eco RI band corresponding to the Vα region (15). SA-1 and SA-K10, derived from the same individual and differing by phenotype and function, did not generate identical Hind III patterns (Table I). The findings obtained with the clone SA-1 confirmed that restricted suppressor T3+ T cells can be rearranged in TCR-α and TCR-β genes (6).

Hybridization of the same blots with the K40 probe revealed rearranged patterns in the T cell clones BJ-37, SA-6, and SAK-10. Strikingly, the two latter clones showed a complete absence of the 8.5 and 3.0 kb Eco RI germline fragments, indicating that all this region had been deleted. DNA rearrangements in SA-6 could not be detected with the TCR-α cDNA probe. The clone BJ-37 exhibited rearranged fragments.

The data show that the K40 probe, when used as a TCR-α probe, has the
FIGURE 1. TCR-\(\gamma\) gene rearrangements in individual T cell clone DNA. (A), Eco RI-digested DNA; (B), Hind III-digested DNA. Lanes a-h refer respectively to BJL-1 (a), BJ-17 (b), BJ-28 (c), BJ-37 (d), SA-6 (e), SA-1 (f), SA-10 (g), and B lymphoblastoid line (h).

FIGURE 2. TCR-\(\alpha\) gene rearrangements (left) and K40 rearrangements (right) detected in Eco RI-digested DNA (A) and Hind III-digested DNA (B) in T cell clones presented in Table I. The bottom of the figure depicts a schematic representation of the K40 clone originally isolated from KE-57R cells bearing a t (8;14) translocation involving the 14q11 band (1). Probes K40 B and C used in the present work consisted of the Xba 1.3 kb (B) and Hind III fragments (C). DNAs are ordered as in Fig. 1.
capacity to detect alterations in T cells displaying functional activity. Because K40 is located in band 14q11, we can conclude that it is a part of the TCR-α chain locus that is also mapped to this band. Furthermore, the fact that a TCR-α cDNA probe (pGA5) and our K40 probe do not detect the same rearrangements in individual clones suggests that the genomic segments recognized by each probe do not encompass the same region of the α chain locus. Indeed, it was previously shown (1) that K40 and pGA5 sequences do not crosshybridize and do not recognize any common genomic DNA fragments. The question then arises as to whether they might recognize closely located regions. In that respect, recent works (19, 20) have shown that t(11;14) (p13;q11) translocations characterizing certain types of acute T cell leukemias split the TCR-α chain locus between the Vα region on one hand, and Jα and Cα regions on the other. It would be worthwhile to determine whether K40 sequences segregate with Vα in leukemic cells presenting such a translocation. DNA sequencing of K40 presently in progress should also provide useful information by comparing it with DNA sequences of the TCR-α locus.

Up to now, only leukemic human T cells have been shown (7) to be affected by a variety of TCR-α chain rearrangements. As expected, our results indicate that normal T cell clones displaying cytotoxic as well as suppressor functions also have rearrangements in their TCR-α chain genes. Further investigation will be required to correlate cell functions with rearrangements.

Finally, since the K40 probe detected different patterns of rearrangement from those identified by the TCR-α chain probe, it constitutes an efficient tool to extend the study of T cell receptor DNA rearrangements in T lymphocytes, since the full extent of this region of the genome is still unknown.

Summary

Human cloned antigen-specific T lymphocytes were used to characterize DNA rearrangements using a cDNA probe from the T cell receptor (TCR)-α chain gene. Rearranged patterns were detected in some T cell clones, confirming that normal mature T cells are rearranged in TCR-α locus. Similarly, rearranged DNA patterns were found in T cell clones from the same panel, using a DNA probe (clone K-40, [1]) isolated from chromosome 14 (14q11), where the TCR-α locus has been mapped. These results suggest that this genomic DNA clone is located within the TCR-α chain locus.

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