Brief Definitive Report

γ GENE REARRANGEMENT AND EXPRESSION IN AUTOREACTIVE HELPER T CELLS

BY MAURICE ZAUDERER,* AIKICHI IWAMOTO,‡ AND TAK W. MAK†

From the *Cancer Center and Department of Microbiology, University of Rochester, Rochester, New York 14642; and ‡The Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada M4X 1K9

Three sets of genes are known to undergo T cell–specific rearrangements. The genes that encode the α (1–3) and β (4, 5) subunits of the T cell receptor and a third gene, γ (6, 7), are assembled from gene segments similar to those that comprise the V and C regions of immunoglobulin chains. Although the function of the γ gene product is unknown, mRNA transcripts of this gene have been detected in greatest abundance in Thy-1+, Lyt-2−, L3T4− thymocytes and peripheral T cells (8). Among mature T cells, it has been reported (9) that γ gene transcripts were detected in all cytotoxic but only 1 of 11 helper T cell clones or hybridomas examined. This accords with the low level of γ mRNA detected in adult lymph node T cells (8). It is particularly striking that a single predominant γ DNA rearrangement is observed in many T cells (7, 9). This contrasts with the diversity of β (10) and α (11, 12) gene rearrangements, and appears to reflect expression of the same germline V and C region γ genes in different cytotoxic T cell clones. These results have suggested that the γ gene product has a role in early T cell differentiation and perhaps in some mature T cell subset associated function.

Autoreactive T cell clones with Ia-specific receptors have been isolated (13), and their nonspecific helper functions have been described (13, 14). Unlike antigen-specific, Ia-restricted T cells, these T cell clones are activated by MHC-syngeneic stimulators in the absence of foreign antigens. MHC recognition during T cell differentiation is known to influence the functional repertoire of mature T cells. It is possible that some self-MHC–reactive T cells in the periphery may represent extrathymic migration of T cell precursors at an early stage of differentiation. Since γ gene expression may be a marker for an early stage of differentiation, we screened RNA from several autoreactive T cell clones for γ gene transcripts. We report here that γ gene rearrangements were observed in all helper T cell clones examined and that high levels of γ RNA expression were detected in four of five L3T4+, Ia-specific autoreactive T cell clones.

Materials and Methods

T Cell Clones. Antigen-specific and autoreactive T cell clones were selected directly from KLH-primed lymph nodes by limiting-dilution culture in the presence of 10% Con A–stimulated rat spleen supernatant (Con A–SN) as previously described (13, 14). Two autoreactive clones, KNA1 and KNA2, were selected from a single pool of normal BALB.K lymph node cells. All other clones derived from independent KLH-primed
donors. Clonal specificity was determined from the requirements for induction of IL-2 secretion. Autoreactive T cell clones CLA1, FGB1, and CLD1 are I-A<sup>d</sup>-specific; KNA1 and KNA2 are I-A<sup>e</sup>-specific. Among KLH-specific clones, CKC4 is of BALB/c origin, and KKC5, KKB2, and KKC1 are BALB.K derived.

**Southern Blots.** DNA was extracted from ~10<sup>7</sup> cells in 0.5% SDS. Samples were incubated overnight at 37°C with 100 μg/ml proteinase K followed by extraction with phenol and chloroform. DNA was precipitated in 0.1 M NaCl with two volumes of ethanol for 30 min at room temperature. The precipitate was transferred (lifted) and readily dissolved in 2 mM Tris, 0.2 mM EDTA. Ethanol precipitation from 0.1 M NaCl solution was repeated three times. 10 μg of each DNA sample was digested at 37°C with 40 U Eco RI for 16 h followed by an additional 10 U for 3 h. DNA fragments were separated by electrophoresis on 0.8% agarose gel and transferred to nitrocellulose. Hybridization with P<sup>32</sup>-labeled nick-translated probes (10<sup>6</sup> cpm/ml) was for 18 h at 65°C. Filters were washed at 65°C in 3x SSC, 0.1% SDS and then in 1x SSC, 0.1% SDS.

**Northern Blots.** RNA was extracted in guanidinium thiocyanate (Fluka Chemical Co., Hauppauge, NY) followed by centrifugation through CsCl. 20 μg total RNA was denatured by reaction with glyoxal in DMSO. RNA was fractionated by electrophoresis in 1% agarose gel and transferred to nitrocellulose filters. After baking 2 h at 80°C, filters were washed once in boiling 20 mM Tris, pH 8.0, to remove any remaining glyoxal. Hybridization conditions were as described above for Southern blots.

**Results**

Hayday et al. (7) reported that a preferential rearrangement of one of three homologous genes for V<sub>V</sub> and one of three genes for C<sub>V</sub> give rise to an ~16 kb Eco RI fragment in all cytotoxic T cell lines examined. To determine whether a similar rearrangement occurs in L3T4<sup>+</sup> T cell clones, we analyzed Eco RI digests of DNA from five autoreactive and four KLH-specific cloned helper T cell lines. V<sub>V</sub> and C<sub>V</sub> region probes were subcloned from a γ cDNA isolated from a cytotoxic T cell line (A. Iwamoto, manuscript submitted for publication). This cDNA uses the same V and J-C gene segments (15) as the γ cDNA clone first described by Saito et al. (6). As previously described (7), the V<sub>V</sub> probe hybridizes to germline 10.8 kb and 5.7 kb fragments, and the C<sub>V</sub> probe hybridizes to germline 13.4 kb, 10.5 kb, and 7.5 kb fragments in an Eco RI digest of BALB/c liver DNA (Fig. 1). Hybridization to a 2.3 kb J region fragment was also detected but is not shown in this figure. Among differentiated T cells, a 16 kb rearrangement is detected with either the V or C region probes in DNA digests of seven of the eight helper T cell lines analyzed (Fig. 1). In DNA of the KLH-specific CKC4 clone, the 16 kb rearrangement is absent and an ~14 kb rearrangement is detected. An ~21 kb rearrangement is absent and not with this V<sub>V</sub> region probe in all clones except CLA1. Retention of the germline γ V<sub>10.8</sub>, C<sub>10.5</sub>, and C<sub>13.4</sub> restriction fragments is variable among the different T cell lines. The 16 kb and 21 kb DNA rearrangements in these cloned helper T cell lines are similar or identical to γ gene rearrangements previously described (7, 9) for cytotoxic T cell clones.

Although γ gene rearrangements have been detected in diverse T cells (9), γ RNA transcripts have been detected primarily in immature or cytotoxic T cells (8, 9). γ gene expression in helper T cells was assessed by Northern analyses of RNA extracted from autoreactive and KLH-specific cloned T cell lines. A γ cDNA encompassing both V and C region segments was used as a hybridization probe for homologous V<sub>V</sub> and C<sub>V</sub> sequences. As shown in Fig. 2, high levels of
FIGURE 1. Southern analysis of Eco RI digests of DNA from diverse autoreactive and KLH-specific T cell lines. A γ cDNA was isolated from a cytotoxic T cell line (15). An Ava I site at the V-J boundary was exploited to prepare hybridization probes for V and J-C gene segments. Blots of two separate gels were first hybridized to the J-C, probe (lower panels). Filters were then washed and hybridized to the V, probe (upper panels). Migration of λ Hind III markers on the two gels is indicated.

FIGURE 2. Northern analysis of γ RNA transcripts. A γ cDNA clone isolated from the 5/10-13 cytotoxic T cell line (15) was used as a hybridization probe. This cDNA encompasses both γ V and C region segments. Shown are: A20.2J, a B cell lymphoma; J774, a macrophage cell line; FBG1 and CLD1, BALB/c autoreactive cloned T cell lines; KKC5 and KKB2, BALB.K KLH-specific T cell clones; and CKC4, a BALB/c KLH-specific T cell clone.

an apparently full-length 1.5 kb γ transcript were detected in RNA of two I-Aα-specific autoreactive T cell clones, FGB1 and CLD1, and one KLH-specific clone, KKB2. Low but detectable hybridization to 1.5 kb RNA was observed for two additional KLH-specific clones, KKC5 and CKC4. There is no hybridization detected to RNA of a B cell lymphoma, A20.2J, or a macrophage cell line, J774, in either this or longer exposures. The results of a second experiment (Fig. 3a) show high-level γ gene expression in two additional autoreactive T cell clones, KNA1 and KNA2, but not in a fifth autoreactive clone, CLA1. To clarify the
FIGURE 3. Northern analysis of (a) γ and (b) α RNA transcripts in cloned autoreactive T cell lines. The same filter was hybridized to P32-labeled, nick-translated inserts of first p5/10-13γ and then p8(0.8), a C region subclone of a murine α cDNA (Dr. Dennis Loh, Washington University, St. Louis, MO). After hybridization to the γ probe, the filter was washed three times in boiling 20 mM Tris, pH 8.0. Removal of the labeled probe was confirmed by autoradiography before hybridization with the α probe. KNA1 and KNA2 are BALB.K, and FGB1 and CLA1 and BALB/c autoreactive cloned T cell lines.

significance of variation in the level of γ mRNA detected in different lines, the same filter was washed and hybridized to a Cα probe (Fig. 3b). Although low in γ gene expression, the CLA1 clone expresses relatively high levels of α mRNA.

Discussion

γ gene rearrangements similar to those described for cytotoxic T cell lines are found in all L3T4+, autoreactive, or KLH-specific cloned helper T cell lines we have examined. High levels of γ RNA transcripts were, in addition, detected in four out of five L3T4+, class II MHC–specific, autoreactive T cell clones, and in at least one of three KLH-specific, class II MHC–restricted clones. Relatively low but significant levels of γ RNA expression were detected in the remaining clones. This contrasts with previously reported (9) expression of γ RNA in only 1 of 11 antigen-specific helper T cell lines.

γ RNA transcripts have been reported (8) to be most abundant in immature thymocytes. At this stage of T cell maturation, precursors may be selected for direct recognition of MHC-encoded molecules expressed in the thymus. It has been suggested, therefore, that the γ gene product plays a role in positive selection of MHC-reactive precursors (8, 16). If the γ RNA transcripts detected in autoreactive T cell clones are indeed functional, then this observation would extend the association between γ gene expression and reactivity to self-MHC. This may, however, reflect the origin of many autoreactive T cells in γ-expressing precursors, rather than the specific function of the γ gene product. Moreover, expression of at least low levels of γ RNA in all three KLH-specific clones analyzed here suggests that the frequency of γ gene expression in these L3T4+ helper T cells may be higher than previously anticipated (9) from analysis of T cell hybridomas and T cell clones selected under somewhat different conditions.

Summary

γ gene rearrangements similar to those described for cytotoxic T cell lines are found in L3T4+, autoreactive, or KLH-specific cloned helper T cell lines. High levels of γ RNA transcripts were, in addition, detected in four out of five L3T4+,
class II MHC–specific, autoreactive T cell clones, and in at least one of three KLH–specific, class II MHC–restricted clones. This contrasts with previously reported (9) expression of γ RNA in only 1 of 11 antigen-specific helper T cell lines.

Received for publication 11 December 1985 and in revised form 10 February 1986.

References
1. Chien, Y., D. M. Becker, T. Lindsten, M. Okamura, D. I. Cohen, and M. M. Davis. 1984. A third type of murine T-cell receptor gene. Nature (Lond.). 312:31.
2. Saito, H., D. M. Kranz, Y. Takagaki, A. C. Hayday, H. N. Eisen, and S. Tonegawa. 1984. A third rearranged and expressed gene in a clone of cytotoxic T lymphocytes. Nature (Lond.). 312:36.
3. Sim, G. K., J. Yague, J. Nelson, P. Marrack, E. Palmer, A. Augustin, and J. Kappler. 1984. Primary structure of human T-cell receptor α-chain. Nature (Lond.). 312:771.
4. Yanagi, Y., Y. Yoshikai, K. Leggett, S. Clark, J. Aleksander, and T. W. Mak. 1984. A human T cell–specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. Nature (Lond.). 308:145.
5. Hedrick, S., D. Cohen, E. Nielsen, and M. Davis. 1984. Isolation of cDNA clones encoding T cell–specific membrane-associated proteins. Nature (Lond.). 308:149.
6. Saito, H., D. M. Kranz, Y. Takagaki, A. C. Hayday, H. N. Eisen, and S. Tonegawa. 1984. Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. Nature (Lond.). 309:757.
7. Hayday, A. D., H. Saito, S. D. Gillies, D. M. Kranz, G. Tanigawa, H. N. Eisen, and S. Tonegawa. 1985. Structure, organization, and somatic rearrangement of T cell gamma genes. Cell. 40:259.
8. Raulet, D. H., R. D. Garman, H. Saito, and S. Tonegawa. 1985. Developmental regulation of T-cell receptor gene expression. Nature (Lond.). 314:103.
9. Kranz, D. M., H. Saito, M. Heller, Y. Takagaki, W. Haas, H. N. Eisen, and S. Tonegawa. 1985. Limited diversity of the rearranged T-cell γ gene. Nature (Lond.). 313:752.
10. Patten, P., T. Yokota, J. Rothbard, Y. Chien, K. Arai, and M. M. Davis. 1984. Structure, expression and divergence of T-cell receptor β-chain variable regions. Nature (Lond.). 312:40.
11. Arden, B., J. L. Klotz, G. Sui, and L. E. Hood. 1985. Mouse T-cell antigen receptor genes: Diversity and structure of genes of the α family. Nature (Lond.). 316:783.
12. Becker, D. M., P. Patten, Y. Chien, T. Yokota, Z. Eshhar, M. Giedlin, N. R. J. Gascoigne, C. Goodnow, R. Wolf, K. Arai, and M. M. Davis. 1985. Variability and repertoire size of T-cell receptor Vα gene segments. Nature (Lond.). 317:430.
13. Zauderer, M., H. Campbell, D. R. Johnson, and M. Seman. 1984. Helper functions of antigen-induced specific and autoreactive T cell colonies. J. Mol. Cell. Immunol. 1:65.
14. Imperiale, M. J., D. A. Faherty, J. F. Sproviero, and M. Zauderer. 1982. Functionally distinct helper T cells enriched under different culture conditions cooperate with different B cells. J. Immunol. 129:1843.
15. Iwamoto, A., F. Rupp, P. Ohashi, C. Walker, H. Pircher, R. Joho, H. Hengartner, and T. W. Mak. 1986. Sequence and expression of new constant and variable region genes. J. Exp. Med. 163:1203.
16. Pernis, B., and R. Axel. 1985. A one and a half receptor model for MHC-restricted antigen recognition by T lymphocytes. Cell. 41:13.