

FUNCTIONAL ISOTYPES ARE NOT ENCODED
BY THE CONSTANT REGION GENES OF THE β
SUBUNIT OF THE T CELL RECEPTOR FOR
ANTIGEN/MAJOR HISTOCOMPATIBILITY COMPLEX

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Studies in both human and murine systems have identified the antigen receptors on T lymphocytes as belonging to a novel class of 80–90 kD mol wt disulfide-linked heterodimers that express clonotypic epitopes (1–4). In man, these molecules, termed Ti, are each comprised of one 49–51 kD mol wt α and one 43 kD β subunit that in turn are noncovalently associated with the invariant 20 and 25 kD T3 molecules. Recently, N-terminal amino acid sequencing and molecular cloning techniques have identified the Ti β gene and demonstrated that it has a distant but definite homology to immunoglobulin light chains (5–7).

Given the extraordinary functional heterogeneity of the T lymphocytes, it was of interest to determine whether different C region genes of the Ti β subunit are used preferentially by one or another T cell population and hence define a unique T cell isotype. To address this question, we used interleukin 2 (IL-2)-dependent clonal T cell populations with known function and a cDNA probe specific for the two constant region genes of the Ti β subunit, TiC β ₁ and TiC β ₂.¹

Materials and Methods

Human T Cell Clones. The phenotype, function, and specificity of these IL-2-dependent cell populations are listed in Table I. Note that all of the clones were derived from a single donor (8–12).

Isolation of a cDNA Clone Encoding Ti β REX. cDNA libraries were constructed in λ_{gt10} from the previously described human T lineage tumor REX (5). cDNA was synthesized according to a procedure described by Land et al. (13). The cDNA library was amplified on *E. coli* C600 hflA and cloned inserts were size fractionated on a sucrose gradient. The 1–2 kilobase (kb) fraction was then recloned in λ_{gt10} and plated on *E. coli* C600 hflA. Synthetic oligonucleotide probes corresponding to the N-terminal amino acid sequences of the Ti β chain were labeled to high specific activity with polynucleotide kinase and γ -ATP (New England Nuclear, Boston, MA) and used to screen plaque transfers (Benton and Davis) (14). Hybridization was at 50°C and 6 \times SSPE overnight. Positives were plated and rescreened twice. Plaque-purified recombinant phages (λ β REX) were amplified, the

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DNA extracted, and inserts subcloned in plasmid pBR 322 (p β REX). Probes specific for the constant region gene of Ti β REX (C β REX) were generated after digesting p β REX with EcoRI and BglII (New England Biolabs, Beverly, MA) and purifying the 800-base-long fragment on a preparative agarose gel. The fragment was eluted and labeled with ^{32}P by nick translation (13).

Transfer Hybridizations. DNA and RNA were prepared by standard techniques (13). DNA was digested with EcoRI and size fractionated on agarose gels, transferred to nitrocellulose, and hybridized to ^{32}P -labeled Ti β probes. RNA was treated with formaldehyde and run on agarose gels containing formaldehyde. Before transfer to nitrocellulose, gels were soaked in 20 \times standard sodium citrate (SSC) for 30 min. After transfer, nitrocellulose membranes were hybridized to radioactively labeled C β REX probes (6 \times SSPE, 50% formamide; 42°C, 2 \times 10⁶ cpm/ml).

Results and Discussion

To ascertain whether different functional T cell populations selectively used one or another of the two genes, termed TiC β ₁ and TiC β ₂, encoding the constant domain of the Ti β subunit (15),¹ a series of experiments was performed using 12 T cell clones of differing functions and specificities derived from a single donor. As shown in Table I, six regulatory T cell populations and six cytotoxic T cell populations were examined. DNA was extracted from individual clones, digested to completion with EcoRI, size fractionated by agarose gel electrophoresis, and DNA molecules transferred to a nitrocellulose membrane. EcoRI was used as the restriction enzyme since earlier studies¹ indicated that it provided the greatest size separation of the two Ti β C region genes and permitted rearrangements to be readily visualized. Subsequently, the nick-translated C β REX probe was hybridized to a nitrocellulose membrane under standard conditions.

Fig. 1 shows the results of Southern blot analysis of the EcoRI digest of these clonal DNAs when hybridized with the Ti β C region cDNA probe. The germline configuration of these genes is shown in lane 13 for the B lymphoblastoid line Laz 509. Two bands can be readily identified at 10.2 and 4.2 kb and represent TiC β ₁ and TiC β ₂, respectively. The C β REX cDNA probe identifies both bands with similar intensity in the radioautograph, suggesting that it hybridized equally well to the two genes and that the same number of copies of each are present in the genome.

Southern blot analysis of the various clonal T cell DNAs (Fig. 1, lanes 1–12) yields several patterns that are distinct from the germline configuration in the autologous B lymphoblastoid line Laz 509. In all cases, there is an alteration of the 10.2 kb TiC β ₁ gene. For example, the inducer clones RW16C and AC3 (lanes 1 and 12), three T4⁺ or T8⁺ cytotoxic T cell populations (lanes 3, 4, and 10), and the noncytotoxic suppressor T cell clone T8AC6 (lane 6) show a third rearranged band on Southern blot analysis with concomitant reduction by ~50% of the intensity of the germline TiC β ₁ gene. Note that the diffuseness of the pattern of the TiC β ₁ gene rearrangements in CT8EL is probably secondary to multiple distinct TiC β ₁ rearrangements that have occurred in this polyclonal T cell line. These findings imply that there is rearrangement of one TiC β ₁ allele and maintenance of the other allele in germline configuration.

Since T cells, like B lymphocytes, are known to undergo rearrangement of germline DNA segments in the assembly of their active receptor genes (14, 15),

TABLE I
Characterization and Function of Human T Cell Clones

Clone*	Phenotype	Function	Reference
AC3	T1 ⁺ T3 ⁺ T4 ⁺ T8 ⁻ T11 ⁺	Autoreactive MHC class II-specific inducer	9
RW16C	T1 ⁺ T3 ⁺ T4 ⁺ T8 ⁻ T11 ⁺	RWAGE/MHC class II-specific inducer	10
AT4 _{II}	T1 ⁺ T3 ⁺ T4 ⁺ T8 ⁻ T11 ⁺	Suppressor-inducer clone	11
T8AC6	T1 ⁺ T3 ⁺ T4 ⁺ T8 ⁺ T11 ⁺	Suppressor of Ig (noncytotoxic)	9, 12
T8AC7	T1 ⁺ T3 ⁺ T4 ⁺ T8 ⁺ T11 ⁺	Suppressor of Ig (noncytotoxic)	9, 12
T8RW	T1 ⁺ T3 ⁺ T4 ⁺ T8 ⁺ T11 ⁺	Suppressor of Ig (noncytotoxic)	9, 12
CT4 _{II}	T1 ⁺ T3 ⁺ T4 ⁺ T8 ⁻ T11 ⁺	Cytotoxic MHC class II specific	8
CT4 _{III}	T1 ⁺ T3 ⁺ T4 ⁺ T8 ⁻ T11 ⁺	Cytotoxic MHC class II specific	8
CT4 _{IV}	T1 ⁺ T3 ⁺ T4 ⁺ T8 ⁻ T11 ⁺	Cytotoxic MHC class II specific	8
CT4 _V	T1 ⁺ T3 ⁺ T4 ⁺ T8 ⁻ T11 ⁺	Cytotoxic MHC class II specific	8
CT8 _{III}	T1 ⁺ T3 ⁺ T4 ⁺ T8 ⁺ T11 ⁺	Cytotoxic MHC class I specific	8
CT8EL [‡]	T1 ⁺ T3 ⁺ T4 ⁺ T8 ⁺ T11 ⁺	Cytotoxic MHC class I specific	8

* All clones were derived from the same donor.

‡ CT8EL is a polyclonal T8⁺ cytotoxic T lymphocyte line.

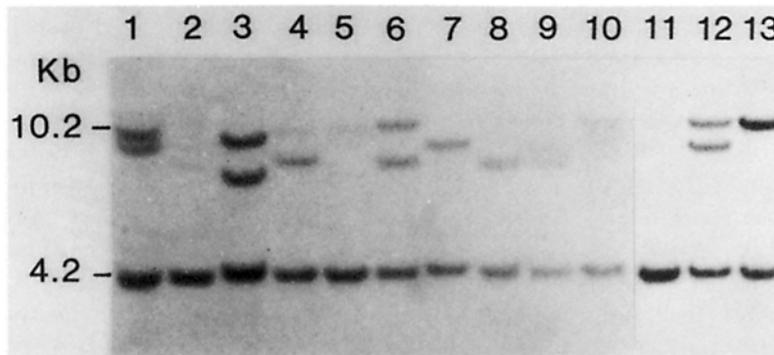


FIGURE 1. $TiC\beta_1$ gene rearrangements are present in inducer, suppressor, and cytotoxic T lymphocytes. Southern blot analysis of DNA from 12 T cell clones derived from a single individual (lanes 1-12) and an autologous B lymphoblastoid line Laz 509 (lane 13) using a nick-translated $Ti\beta$ C region probe. Size determinations were made using ³²P-labeled fragments of HindIII-digested phage λ DNA. Lanes 1-12 contain DNA from the following: (1) RW16; (2) CT4_{III}; (3) CT4_V; (4) CT4_{IV}; (5) AT4_{II}; (6) T8AC6; (7) T8AC7; (8) CT4_{II}; (9) CT8_{III}; (10) CT8EL; (11) T8RW; (12) AC3.

these results represent clearcut evidence for the use of the $TiC\beta_1$ C region in formation of the $Ti\beta$ molecules in the clones. The slight differences in molecular weight of the rearranged $TiC\beta_1$ bands are likely due to $TiC\beta_1$ gene fusion to other V, D, and/or J segments in individual clonal populations that yield different size fragments after restriction enzyme digestion. Perhaps more importantly, the functional diversity of the cell populations using $TiC\beta_1$ strongly suggests that this gene cannot encode their cytotoxic or regulatory repertoire.

A second pattern of $TiC\beta_1$ rearrangement is evident in the T4⁺ cytotoxic clones CT4_{II} and CT4_{III} (Fig. 1, lanes 8 and 2), the T8⁺ cytotoxic clone CT8_{III} (lane 9), and the T8⁺ suppressor clone T8AC7 (lane 7). In this pattern, one germline $TiC\beta_1$ allele is deleted and the remaining $TiC\beta_1$ gene is rearranged. In the case of the T8⁺ CTL clone, the appearance of two new $TiC\beta_1$ bands suggests that both alleles are rearranged, one of which may be nonfunctional. The latter appears likely since only one T3-associated Ti molecule is known to exist on the

surface of that cell population, as defined by anticlonotypic monoclonal antibodies.

A third and fourth set of alterations of the $TiC\beta_1$ gene is found in the $T4^+$ suppressor-inducer T cell clone, AT4_{II} (Fig. 1, lane 5) and the $T8^+$ suppressor clone, T8RW (lane 11). In the former, there is a germline configuration of both $TiC\beta_1$ and $TiC\beta_2$ genes. However, the intensity of the $TiC\beta_1$ band is reduced by ~50%, suggesting again that one allele has been deleted. In the case of T8RW, both $TiC\beta_1$ genes have been deleted (lane 11). Since T8RW expresses a defined surface Ti molecule, the findings imply that either an additional $TiC\beta$ gene which does not cross-hybridize with the C β REX probe is used by these clones or, alternatively, that some T cell clones may use a $TiC\beta$ gene without undergoing detectable rearrangements.

Northern blot analysis of T8RW mRNA provided a means to examine this question. RNA was obtained from the T8RW clone, size fractionated by agarose gel electrophoresis, transferred to nitrocellulose, and hybridized to the C β REX probe. If no $TiC\beta$ -specific mRNA was detected, it would imply that a different $TiC\beta$ gene was used by T8RW. On the other hand, a signal on Northern blot would demonstrate that the $TiC\beta_2$ gene was transcribed in the absence of detectable gene rearrangement.

Fig. 2 shows a Northern blot of T8RW RNA (lane 4). For purposes of comparison, mRNA was obtained from other T cell populations, including human thymus (lane 1), the human thymic tumor REX (lane 2), and the $T4^+$ inducer clone AC3 (lane 3). As shown, T8RW contained mRNA that hybridized to the C β REX probe. In fact, two different species of mRNA could be identified: the prevalent species of ~1.3 kb and a less abundant species of a slightly smaller molecular weight. Moreover, these were identical in size to the two species of mRNA of the REX tumor and human thymus identified by the C β REX probe. Note that the lower molecular weight form of the Ti β RNA is more abundant in thymus (~50% of the hybridizing materials) than in either REX or T8RW (~10%). Furthermore, the AC3 inducer clone (Fig. 2, lane 3) lacks this size class of mRNA completely. Such differences in the abundance of the lower molecular

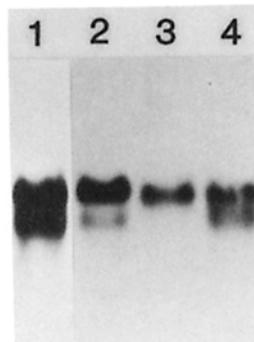


FIGURE 2. The $TiC\beta_2$ gene is transcribed in the absence of detectable gene rearrangement. Northern blot analysis of mRNA from various human T lineage cells with the nick-translated $TiC\beta$ probe. RNA was isolated as described in Materials and Methods from human thymocytes (1), the T lineage thymus tumor REX (2), the AC3 inducer clone (3), and the T8RW suppressor clone (4). Note that two species of mRNA are detected in all but AC3.

weight mRNA are not due to degradation since ethidium bromide staining of agarose gels before probe hybridization indicated that the RNA samples were intact. Importantly, the lower molecular weight forms all contain a transmembrane exon as defined by a specific oligonucleotide probe and thus do not encode a secreted form of $Ti\beta$ (Acuto, Royer, and Reinherz, manuscript in preparation).

Fig. 2 demonstrates rather unequivocally that the $TiC\beta_2$ gene is used in T8RW. Whether the $TiC\beta_2$ gene is also used in the suppressor inducer clone AT4_{II} is not known at present. That a C region gene could be used in the formation of an active receptor structure in the apparent absence of gene rearrangement certainly has precedent in the IgD gene system of the B lymphocyte (16). In the IgD system, VDJ-C μ -C δ transcription occurs in the absence of gene rearrangement and, subsequently, the intervening C μ segment is deleted by an RNA splicing mechanism. It is also possible that restriction enzymes other than EcoRI will elucidate some form of C β_2 gene rearrangement. Further studies are necessary in this regard. The present results strongly suggest that functional T cell isotypy is not encoded within the $Ti\beta$ genes. Thus, the possibility remains that it will either be encoded within the C region genes of the $Ti\alpha$ subunit or elsewhere.

Summary

Human T cell clones and a cDNA probe specific for constant regions of the β subunit of the antigen/major histocompatibility complex (MHC) receptor, $TiC\beta_1$ and $TiC\beta_2$, were employed to determine whether these genes were differentially used by functional classes of T lymphocytes. DNA from 10 interleukin-2-dependent T cell clones including class I and class II MHC-specific cytotoxic T lymphocytes ($n = 6$), T4⁺ inducer T lymphocytes ($n = 2$), and T8⁺ suppressor T lymphocytes ($n = 2$) showed rearrangement of the $TiC\beta_1$ gene on Southern blot analysis with or without deletion of the other $TiC\beta_1$ allele. In contrast, $TiC\beta_2$ always remained in germline configuration. Moreover, the finding that one additional suppressor clone deleted both $TiC\beta_1$ alleles, maintained a germline $TiC\beta_2$ configuration, and yet actively transcribed $TiC\beta_2$ message suggested that $TiC\beta_2$ is not a pseudogene. Rather, it appeared to be used less frequently than the $TiC\beta_1$ gene and in the absence of detectable DNA rearrangements. Together, these results demonstrate that the functional repertoire (or isotype) of a given subclass of T cells is not encoded within the $Ti\beta$ genes.

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References

1. Meuer, S. C., O. Acuto, R. E. Hussey, J. C. Hodgdon, K. A. Fitzgerald, S. F. Schlossman, and E. L. Reinherz. 1983. Evidence for the T3-associated 90KD heterodimer as the T cell antigen receptor. *Nature (Lond.)* 303:808.
2. Acuto, O., S. C. Meuer, J. C. Hodgdon, S. F. Schlossman, and E. L. Reinherz. 1983. Peptide variability exists within the α and β subunits of the T cell receptor for antigen. *J. Exp. Med.* 158:1368.
3. McIntyre, B. W., and J. P. Allison. 1983. The mouse T cell receptor: structural heterogeneity of molecules of normal T cells defined by xenoantiserum. *Cell* 34:739.
4. Kappler, J., R. Kubo, K. Haskins, C. Hannum, P. Marrack, M. Pigeon, B. McIntyre,

- J. Allison, and I. Trowbridge. 1983. The major histocompatibility complex-restricted antigen receptor on T cells in mouse and man: identification of constant and variable peptides. *Cell*. 35:295.
5. Acuto, O., M. Fabbi, J. Smart, C. B. Poole, J. Protentis, H. D. Royer, S. F. Schlossman, and E. L. Reinherz. Purification and N-terminal amino acid sequencing of the β subunit of a human T cell antigen receptor. *Proc. Natl. Acad. Sci. USA*. 81:3851.
 6. Yanagi, Y., Y. Yoshikai, K. Leggett, S. P. Clark, I. 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.
 7. Hedrick, S. M., D. I. Cohen, E. A. Nielsen, and M. M. Davis. 1984. Isolation of cDNA clones encoding T cell specific membrane associated proteins. *Nature (Lond.)*. 308:149.
 8. Meuer, S. C., S. F. Schlossman, and E. L. Reinherz. 1982. Clonal analysis of human cytotoxic T lymphocytes: T4⁺ and T8⁺ effector T cells recognize products of different MHC regions. *Proc. Natl. Acad. Sci. USA*. 79:4395.
 9. Bensussan, A., S. C. Meuer, S. F. Schlossman, and E. L. Reinherz. 1984. Delineation of an immunoregulatory amplifier population recognizing autologous Ia molecules: analysis with human T cell clones. *J. Exp. Med.* 159:559.
 10. Meuer, S. C., D. A. Cooper, J. C. Hodgdon, R. E. Hussey, K. A. Fitzgerald, S. F. Schlossman, and E. L. Reinherz. 1983. Identification of the antigen/MHC receptor on human inducer T lymphocytes. *Science (Wash. DC)*. 222:1239.
 11. Meuer, S. C., D. A. Cooper, J. C. Hodgdon, R. E. Hussey, C. Morimoto, S. F. Schlossman, and E. L. Reinherz. 1983. Immunoregulatory human T lymphocytes triggered as a consequence of viral infection: clonal analysis of helper, suppressor inducer, and suppressor effector populations. *J. Immunol.* 131:1167.
 12. Bensussan, A., O. Acuto, R. E. Hussey, C. Milanese, and E. L. Reinherz. Autoregulation of T8⁺ suppressor T cell growth: T3-Ti receptor triggering leads to IL-2 unresponsiveness. *Nature (Lond.)*. In press.
 13. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 545 pp.
 14. Chien, Y., N. R. J. Gascoigne, J. Kavalier, N. E. Lee, and M. M. Davis. Somatic recombination of a murine T cell receptor gene. *Nature (Lond.)*. In press.
 15. Siu, G., S. P. Clark, Y. Yoshikai, M. Malissen, Y. Yanagi, E. Strauss, T. W. Mak, and L. Hood. The human T cell antigen receptor is encoded by variable, diversity, and joining gene segments that rearrange to generate a complete V gene. *Cell*. In press.
 16. Maki, R., W. Roeder, A. Traunecker, C. Sidman, M. Wabl, W. Raschke, and S. Tonegawa. 1981. The role of DNA rearrangement and alternative RNA processing in the expression of immunoglobulin delta genes. *Cell*. 24:353.