Brief Definitive Report

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, TiCβ$_1$ and TiCβ$_2$.[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× 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 (pβ REX). Probes specific for the constant region gene of Ti β REX (C β REX) were generated after digesting pβ 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× standard sodium citrate (SSC) for 30 min. After transfer, nitrocellulose membranes were hybridized to radioactively labeled C β REX probes (6× SSPE, 50% formamide; 42°C, 2×10^6 cpm/ml).

Results and Discussion

To ascertain whether different functional T cell populations selectively used one or another of the two genes, termed TiCβ_1 and TiCβ_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 studies[1] 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 TiCβ_1 and TiCβ_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 TiCβ_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 TiCβ_1 gene. Note that the diffuseness of the pattern of the TiCβ_1 gene rearrangements in CT8EL is probably secondary to multiple distinct TiCβ_1 rearrangements that have occurred in this polyclonal T cell line. These findings imply that there is rearrangement of one TiCβ_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.

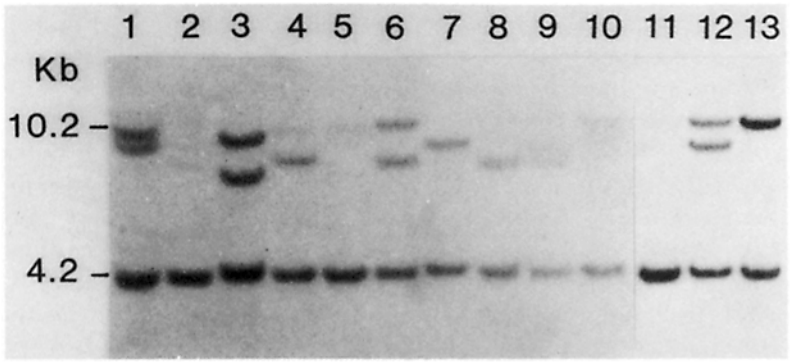

FIGURE 1. $TiC\beta_1$ gene rearrangements are present in inducer, suppressor, and cytotoxic T lymphocytes. Southern blot analysis of DNA from 12 T cell clones derived from a single individual (lanes *1–12*) and an autologous B lymphoblastoid line Laz 509 (lane *13*) using a nick-translated Ti β C region probe. Size determinations were made using ^{32}P-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 β 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 TiCβ_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 TiCβ_1 and TiCβ_2 genes. However, the intensity of the TiCβ_1 band is reduced by ~50%, suggesting again that one allele has been deleted. In the case of T8RW, both TiCβ_1 genes have been deleted (lane *11*). Since T8RW expresses a defined surface Ti molecule, the findings imply that either an additional TiCβ 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 TiCβ 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 TiCβ-specific mRNA was detected, it would imply that a different TiCβ gene was used by T8RW. On the other hand, a signal on Northern blot would demonstrate that the TiCβ_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

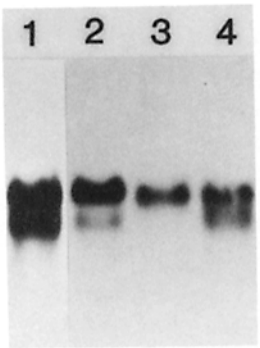

FIGURE 2. The TiCβ_2 gene is transcribed in the absence of detectable gene rearrangement. Northern blot analysis of mRNA from various human T lineage cells with the nick-translated TiCβ probe. RNA was isolated as described in Materials and Methods from human thymocytes (*1*), the T lineage thymus tumor 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 β (Acuto, Royer, and Reinherz, manuscript in preparation).

Fig. 2 demonstrates rather unequivocally that the TiCβ_2 gene is used in T8RW. Whether the TiCβ_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-Cμ-Cδ transcription occurs in the absence of gene rearrangement and, subsequently, the intervening Cμ segment is deleted by an RNA splicing mechanism. It is also possible that restriction enzymes other than EcoRI will elucidate some form of Cβ_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 β genes. Thus, the possibility remains that it will either be encoded within the C region genes of the Ti α 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, TiCβ_1 and TiCβ_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 TiCβ_1 gene on Southern blot analysis with or without deletion of the other TiCβ_1 allele. In contrast, TiCβ_2 always remained in germline configuration. Moreover, the finding that one additional suppressor clone deleted both TiCβ_1 alleles, maintained a germline TiCβ_2 configuration, and yet actively transcribed TiCβ_2 message suggested that TiCβ_2 is not a pseudogene. Rather, it appeared to be used less frequently than the TiCβ_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 β genes.

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