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Sequence and expression of transcripts of the T-cell antigen receptor α-chain gene in a functional, antigen-specific suppressor-T-cell hybridoma

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ABSTRACT **We have detected rearrangement and expression of a gene encoding a T-cell antigen receptor α chain in the keyhole limpet hemocyanin (KLH)-specific, inducible suppressor-T-cell (T_s) hybridoma 34S-281 (BW5147 lymphoma–C57BL/6 T_s) by using α-chain cDNA clones isolated from this T_s hybridoma. The cDNA clones have a restriction length polymorphism in the constant region that identifies them as being of C57BL/6 origin. The cDNA sequence has an ATG start codon for an open reading frame including variable, joining, and constant gene segments. Furthermore, the T_s α-chain gene transcripts were detected on membrane-bound polysomes by RNA blot analysis using a variable-region fragment from one of the α-chain cDNA clones as probe, suggesting that they are actively translated in the T_s hybridoma. As the β-chain gene is deleted in all T_s hybridomas analyzed, and since a disulfide-linked dimer is detectable by two-dimensional NaDodSO$_4$/PAGE of lysates of surface-radioiodinated T_s, we suggest that T_s antigen receptors are either α-chain homodimers or heterodimers composed of the α chain in association with an undefined chain.**

Helper and cytotoxic T lymphocytes express an antigen-specific receptor composed of α and β subunits, which are encoded by separate genes (1–4). However, the subunit composition of the antigen receptor on suppressor T cells (T_s) remains to be solved; several investigators have reported discrepant results, demonstrating that some T_s lines and hybridomas express α- and β-chain genes, whereas some do not (5–9) despite having their T_s function. Thus, two points should be discussed in this regard. (*i*) T_s expressing α- and β-chain genes are likely to be cytotoxic T lymphocytes with immunoregulatory activity (because of their specificity for immunocompetent cells) but not factor (T_s factor, TsF)-producing T_s, which were described by Gershon and Kondo (10) and by Tada and associates (reviewed in ref. 11). (*ii*) Some T_s use the same set of genes as helper and cytotoxic T lymphocytes, whereas some use entirely different sets of receptor genes.

Two types of T_s hybridomas have been established in this laboratory and maintained for more than 7 years, after fusion of keyhole limpet hemocyanin (KLH)-primed T_s of C57BL/6 mice and BW5147 lymphoma cells derived from an AKR mouse (reviewed in ref. 12). One type is represented by hybridomas 34S-18 and 34S-704, which produce TsF composed of two chains (45 kDa and 28 kDa) with KLH-binding activity and mediating KLH-specific and H-2^b-restricted suppressor function. The other type is the inducible T_s hybridoma (34S-281) complementary to 34S-18 or 34S-704. The inducible hybridoma is activated and starts to produce another TsF with KLH-specific and H-2^b-restricted suppressor activity after stimulation by KLH-specific TsF of the two-chain type (13) or by an anti-KLH monoclonal antibody with suppressor activity (14).

Molecular studies on these T_s hybridomas have indicated that T_s do not use the β-chain gene, because only the BW5147-type rearrangement pattern was observed by Southern blot analysis. No additional rearranged bands or germline bands were detected (6). (In ref. 6, lines 281-31 and 281N3-1, 704F3, and 18F2 are the subcultured lines of 34S-281, 34S-704, and 34S-18, respectively. Line 281-31 is an activated, and 281N3-1 an unactivated, subcultured line of 34S-281.)

In this communication, we describe the isolation and nucleotide sequence of T_s α-chain cDNA clones. The nucleotide sequence corresponds to a potentially functional α-chain message composed of variable (V), joining (J), and constant (C) segments without a frameshift, a stop codon, or a deletion in the reading frame. The V segment of the T_s α-chain appears to be actively rearranged and expressed in the T_s hybridoma, but not in BW5147, and belongs to a V_α family distinct from those previously reported (15). As the β-chain genes are deleted in all T_s hybridomas tested, the possible involvement of an undefined chain in the formation of the T_s antigen receptor is discussed.

MATERIALS AND METHODS

Cell Lines. Characterization of the antigen-specific T_s hybridomas 34S-704, 34S-18, and 34S-281 has been described (reviewed in ref. 12). For DNA and RNA extractions, cells were grown to a density of 10^6 cells per ml in Petri dishes. After three washes with phosphate-buffered saline, cell pellets were frozen in liquid nitrogen and stored at −80°C.

C_α Probe. A cDNA clone (Tα-802) encoding the V-J-C α-chain message was isolated from a C57BL/6 thymus cDNA library by screening with three synthetic oligodeoxynucleotides (21-mers) corresponding to the C region of α-chain gene. A 590-base-pair (bp) *Nco* I fragment from this clone was used as the C_α probe (Fig. 2*b*).

DNA Blot Analysis. DNA was digested with restriction enzymes and separated by electrophoresis according to standard methods (16). DNA was blotted to nitrocellulose by the procedure of Southern (17). After hybridization, the filters were washed with 2× SSC/0.1% NaDodSO$_4$ (1× SSC is 0.15 M NaCl/0.015 M sodium citrate) at 65°C and then with 0.1× SSC/0.1% NaDodSO$_4$ at 65°C. Air-dried filters were exposed at −80°C to Kodak XAR-5 film with intensifying screens for one to several days. Long exposures were carried out for at least 1 week.

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Abbreviations: KLH, keyhole limpet hemocyanin; T_s, suppressor T cell(s); TsF, T_s factor(s); V, variable; J, joining; C, constant; bp, base pair(s); kb, kilobase(s).

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RNA Blot Analysis. RNA was isolated by a modification of the method of Glisin *et al.* (18). Poly(A)$^+$ RNA (2 μg), purified by oligo(dT)-cellulose column chromatography, was resolved by electrophoresis in a 1% (wt/vol) agarose gel containing 2.2 M formaldehyde, 20 mM 4-morpholinepropanesulfonate (pH 7.0), 15 mM sodium citrate, and 1 mM EDTA, as described (19). After hybridization, the filters were washed and exposed as described in the previous section. Membrane-bound polysomes were separated from free polysomes by differential centrifugation (20) and then collected by magnesium precipitation (21). Poly(A)$^+$ RNA of membrane-bound polysomes was isolated as described (18, 19).

cDNA Cloning. A cDNA library of 2.5 × 10^6 plaque-forming units was constructed in the λgt10 vector, using 1 μg of poly(A)$^+$ RNA from the hybridoma 34S-281 according to the method of Davis and coworkers (22), and screened with the C_α probe as described (23).

Sequence Determination. Restriction fragments were subcloned into both M13mp18 and M13mp19 vectors, and nucleotide sequences were determined by the dideoxy chain-termination method (24) (see Fig. 3).

Two-Dimensional Gel Electrophoresis. EL-4, 34S-281, and BW5147 cells (10^7) were radiolabeled with $Na^{125}I$ as described (25). After labeling, cells were lysed with Nonidet P-40 and radioactive materials were analyzed by two-dimensional NaDodSO$_4$/PAGE under nonreducing conditions in the first dimension and under reducing conditions in the second dimension according to the method of Goding and Harris (26).

RESULTS

T_s α-Chain Genes in Hybridomas. We first searched for a DNA restriction fragment length polymorphism on the genome around the C_α gene of C57BL/6 and AKR mice, because our T_s hybridomas were derived by fusion of C57BL/6 T_s with the AKR lymphoma BW5147. A *Bgl* I site was found to be polymorphic between these two strains. Southern blot analysis of *Bgl* I-digested DNA (Fig. 1) revealed that the C_α probe detected a 12-kilobase (kb) fragment in AKR and a 9.4-kb fragment in C57BL/6 kidney DNA, whereas both fragments were detected in DNA from the T_s hybridomas, indicating that the hybridomas had retained the α-chain genes of T_s origin in their genomes for more than 7 years. This is quite in contrast to the β-chain genes, which have been deleted in all these hybridomas (6).

Cloning of T_s α-Chain cDNA. To determine whether the α-chain gene of T_s origin that was detected is active or not, we prepared a λgt10 phage cDNA library from mRNA of T_s hybridoma 34S-281 and screened the library with the C_α probe. About 260 clones were isolated from the phage library of 2.5 × 10^6 plaque-forming units, and 130 positive clones were further analyzed to determine whether they were of C57BL/6 or AKR origin by detection of the polymorphic restriction site in the C_α region. The *Hpa* II site in this region is polymorphic between AKR and C57BL/6. Therefore, cDNA clones isolated were double-digested with *Nco* I and *Hpa* II restriction enzymes, and Southern hybridization was carried out using the C_α probe. As shown in Fig. 2, three clones, nos. 13, 26, and 46, were shown to be of C57BL/6 origin, whereas other clones, like nos. 3 and 58, were found to be of AKR origin. The results indicate that the α-chain gene of T_s origin is in fact transcribed and processed into mRNA in the T_s hybridoma 34S-281.

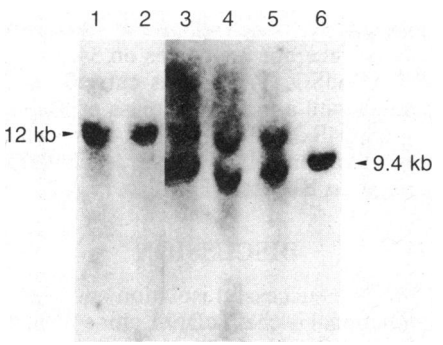

FIG. 1. Southern blot analysis of C_α genes in T_s hybridomas. Lanes: 1, AKR kidney; 2, BW5147; 3, 34S-281; 4, 34S-704; 5, 34S-18; 6, C57BL/6 kidney. DNAs were digested with *Bgl* I and hybridized with the C_α probe. Fragment sizes are indicated in kb.

Nucleotide Sequence Analysis of T_s α-Chain cDNA Clones. Restriction fragments of two overlapping T_s α-chain clones (nos. 46 and 104) were subcloned into M13 vectors and sequenced by the dideoxy method (Fig. 3). The nucleotide sequence (Fig. 4) shows an ATG start codon for a long open reading frame with V, J, and C segments. When the deduced amino acid sequence was compared with that of the TT11 α-chain cDNA clone of BW5147 origin (1), several common residues were observed in both V (30/93 residues) and J (9/22 residues) regions, which also contain most of the highly conserved residues (12/14 residues in the V region and 4/4 in the J region, as indicated by underlines) encoded by V- and J-region genes for the heavy and light chains of immunoglobulin or the β and other α chains of the T-cell receptor (2). The cysteine residues at positions 23 and 90 are conserved in the T_s α-chain clones. Furthermore, the C-region sequence is exactly the same as that of the TT11 cDNA clone at the amino acid level. However, there are two silent mutations (underlined) at the nucleotide level, one of which creates the C57BL/6-specific *Hpa* II site in the C region. In the 3′ untranslated region, there are five point mutations (underlined) and an 18-nucleotide long insertion (underlined) by which the *Hpa* II site present in 3′ untranslated region of AKR is lost in C57BL/6.

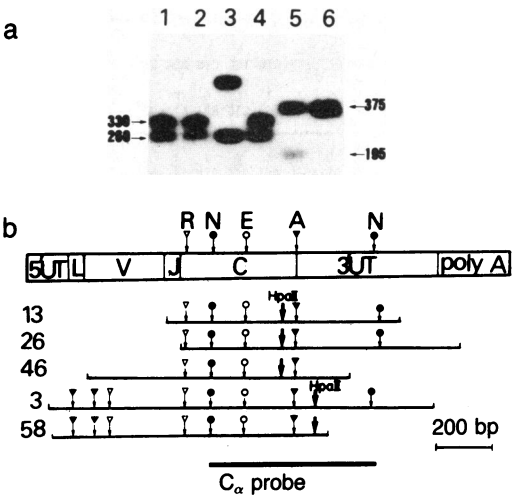

FIG. 2. (*a*) Southern blot analysis showing AKR vs. C57BL/6 strain polymorphism of *Hpa* II site within the C region. Lanes: 1, clone 13; 2, clone 26; 3, clone 46; 4, clone T_α-802 from C57BL/6 thymocyte library; 5, clone 3; 6, clone 58. DNAs were digested with both *Nco* I and *Hpa* II and hybridized with the C_α probe. Fragment sizes are indicated in bp. The larger band in lane 3 is the clone 46 *Hpa* II/*Eco*RI fragment with λgt10 vector sequence. (*b*) Restriction maps of cDNA clones. The *Eco*RI sites at the ends of clones were created by *Eco*RI-linker ligation. Restriction sites: R, *Rsa* I; N, *Nco* I; E, *Eco*RV; A, *Ava* II. *Hpa* sites are indicated by thick arrows. Open box at the top stands for the 1.7-kb, full-length cDNA structure. UT, untranslated region; L, region encoding the leader peptide. Solid line at the bottom indicates the C_α probe 590-bp *Nco* I fragment from clone T_α-802.

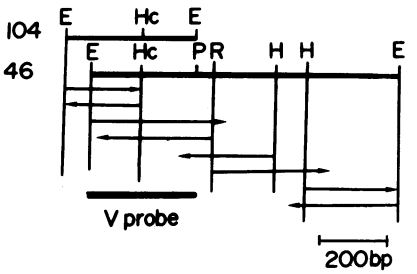

FIG. 3. Restriction map of overlapping cDNA clones 46 and 104 and strategy of sequencing. E, *Eco*RI; P, *Pvu* II; R, *Rsa* I; H, *Hin*dIII; Hc, *Hin*cII. Arrows indicate direction and extent of sequencing. *Eco*RI–*Pvu* II fragment of clone 46 used as the V46 probe is indicated by solid line. Clone 104 was isolated by using the V46 probe from the 34S-281 cDNA library.

The V region of T_s α-chain gene (designated V_α281) was examined for homology with known V_α gene families. The data indicate that the V_α281 gene has at most 34% homology (at the amino acid level) to other V_α gene families reported (15). Most intriguing is the finding that the V_α281 gene encodes an additional cysteine residue at amino acid position 19. As an identical V gene has not been reported, to our knowledge, the V_α281 gene belongs to a new V_α family. The J sequence of the T_s α-chain gene is the same as that used in two C57BL/6-derived helper-T-cell clones, LB2 and FN1-18 (27).

Expression and Rearrangement of the T_s α-Chain Gene. RNA blot analysis with total and membrane-bound mRNA

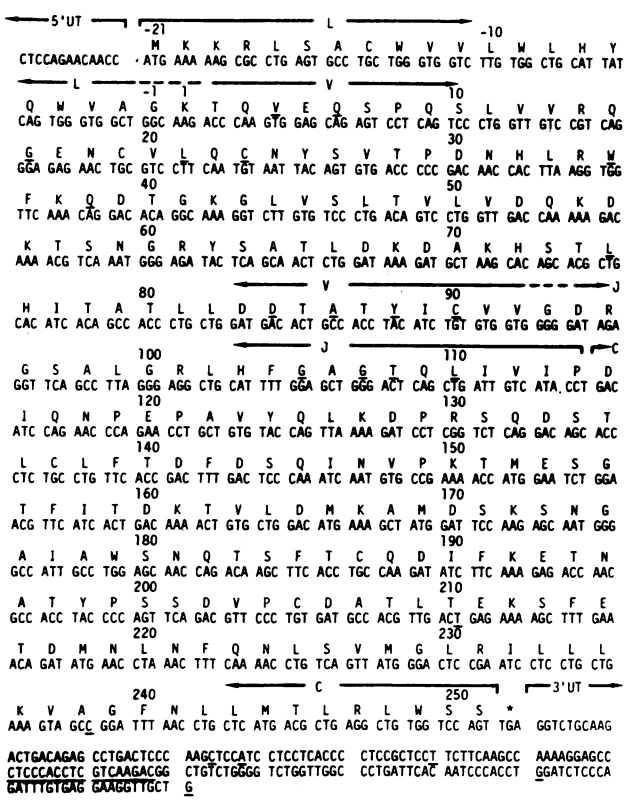

FIG. 4. Nucleotide sequence of T_s α-chain gene obtained from overlapping clones 46 and 104. Deduced amino acid residues are shown (standard one-letter abbreviations) above the DNA sequence. Underlined amino acid residues are highly conserved among immunoglobulin heavy and light chains and T-cell receptor α and β chains. 5'-Untranslated (5'UT), leader (L), V, J, C, and 3'UT regions are indicated. L/V and V/J boundaries are approximate. Underlined nucleotides are polymorphic between C57BL/6 and AKR.

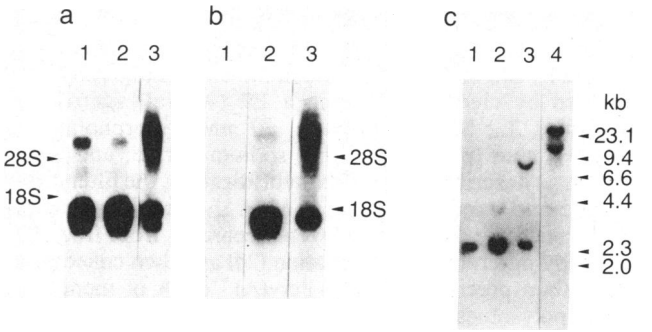

FIG. 5. (*a* and *b*) RNA blot-hybridization analysis of T_s hybridoma (34S-281) and BW5147 lymphoma mRNA with the C_α probe (*a*) and the V46 probe (*b*). Lanes: 1, BW5147 total poly(A)$^+$ RNA; 2, 34S-281 total poly(A)$^+$ RNA; 3, 34S-281 membrane-bound polysomal poly(A)$^+$ RNA. Size markers represent 28S and 18S ribosomal RNA. The same amount of RNA (5 μg) was loaded in each lane. The exposure time of the autoradiograms was 12 hr (*a*) or 1 week (*b*). (*c*) Southern blot analysis showing the V_α281 gene rearrangement in the T_s hybridoma detected by the V46 probe. Lanes: 1, AKR kidney; 2, BW5147; 3, 34S-281; 4, C57BL/6 kidney.

from the T_s hybridoma 34S-281 was carried out using the V46 (*Eco*RI/*Pvu* II fragment of clone 46, as shown in Fig. 3) and C_α probes. The C_α probe detects two RNA species, of 1.7 kb and 1.3 kb, in the hybridoma as well as in BW5147 cells (Fig. 5*a*). However, this T_s hybridoma expresses a 1.7-kb mRNA that hybridizes with the V46 probe, whereas this mRNA is absent from BW5147 (Fig. 5*b*). It is likely that the 1.7-kb mRNA contains V-J-C sequences, whereas the 1.3-kb mRNA lacks the V sequences, because it is not detected by the V46 probe. Moreover, the 1.7-kb mRNA is also present in mRNA from membrane-bound polysomes of 34S-281 (Fig. 5*b*, lane 3), suggesting that the mRNA hybridized with the V46 probe represents the productive V-J-C species. From these results, we conclude that the T_s α-chain gene transcript is potentially functional and is expressed in T_s. However, from RNA blot analysis and cloning efficiency of the T_s α-chain cDNA, the V46-positive (1.7-kb) mRNA is estimated to be about 10% as abundant as the V46-negative (1.3-kb) mRNA in the hybridoma.

Rearrangement of the V_α281 gene was analyzed by Southern blot hybridization with the V46 probe. The results show several common bands, as well as rearranged bands present only in the 34S-281 DNA (Fig. 5*c*). The *Bam*HI restriction patterns of the hybridoma DNA show missing bands at 12 kb and 23 kb, which are detected in C57BL/6 kidney DNA, as well as one additional band at 8.5 kb. Rearrangements were also detected with the restriction enzymes *Eco*RI, *Hin*dIII, and *Pst* I (data not shown). Thus, the V_α281 gene is rearranged in the T_s hybridoma 34S-281.

Two-Dimensional Gel Electrophoresis. To detect disulfide-linked T_s antigen-receptor molecules on 34S-281 cells, two-dimensional NaDodSO$_4$/PAGE was carried out. Disulfide-linked molecules with a molecular mass of 37–44 kDa were prominent in the 34S-281 T_s hybridoma, as well as in EL-4 lymphoma (positive control). However, such molecules were barely detectable in BW5147 (Fig. 6).

DISCUSSION

We report here the successful isolation and sequencing of a potentially functional α-chain cDNA clone from a functional KLH-specific T_s hybridoma. The hybridoma (34S-281) was derived by fusion of BW5147 cells (of AKR origin) with C57BL/6 T_s. The cDNA clones 46 and 104 were identified as α-chain clones of C57BL/6 T_s origin because they contain the C57BL/6 allele (absent in AKR) of a polymorphic *Hpa* II

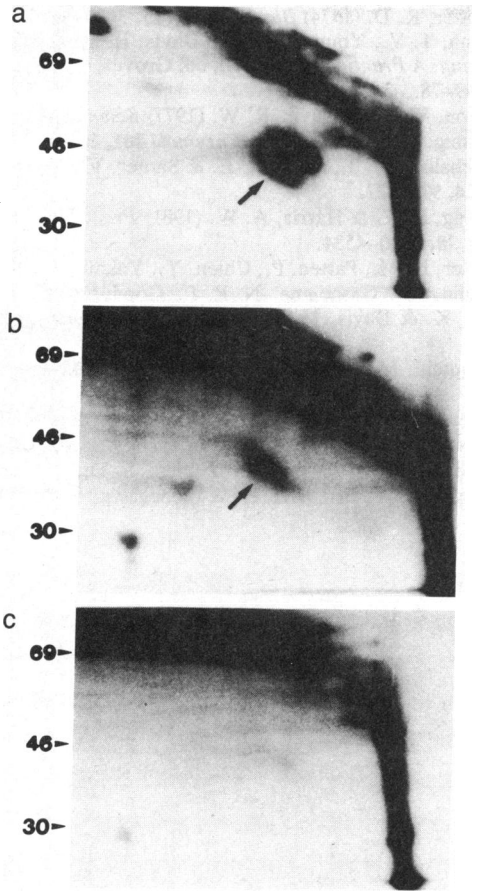

FIG. 6. Two-dimensional $NaDodSO_4$/PAGE of ^{125}I-labeled cell lysates of EL-4 (*a*), 34S-281 (*b*), and BW5147 (*c*). The samples (10^6 cpm) were electrophoresed in a $NaDodSO_4$/8% polyacrylamide gel under nonreducing conditions in the first dimension and then in a $NaDodSO_4$/10% polyacrylamide gel under reducing conditions in the second dimension. Arrows indicate the positions of disulfide-linked dimers with approximate molecular sizes of 36–48 kDa in EL-4 (*a*) and 37–44 kDa in 34S-281 (*b*). Positions and sizes (kDa) of markers are shown at left.

restriction site in the C region. By using the V46 probe (see Fig. 3), we detected specific DNA rearrangement and transcripts of the T_s α-chain gene in the hybridoma.

That the T_s α-chain gene we have detected is potentially functional and likely to be expressed is indicated by the following. First, the cDNA has an ATG start codon and encodes a V-J-C polypeptide without frameshifts, stop codons, or deletions in the reading frame, although nonproductive messages have been demonstrated to arise in T cells by joining events that cause a frameshift, by rearrangement of a pseudogene segment, or by initiation of transcription 5′ to the J gene segment (15). Second, transcripts were detected with the V46 probe in blot analysis of mRNA isolated from membrane-bound polysomes, suggesting that the mRNA is actually translated in the T_s hybridoma.

Analysis of the deduced amino acid sequence of the T_s α-chain cDNA indicates that the sequence of the C region is identical to that of the C_α gene segments obtained from helper and cytotoxic T lymphocytes (1, 2). The V region of the T_s α-chain gene (V_α281) shares many residues not only with the V and J regions of a BW5147 α-chain clone (TT11), but also with those of genes for immunoglobulin heavy and light chains and β chains and other α chains of the T-cell receptor. However, there are several notable properties of the V_α281 gene. (*i*) An additional cysteine residue is encoded at position 19. This residue may be responsible for a unique conformational change of the V-region structure or for interaction with another molecule to construct a dimeric T_s receptor. (*ii*) V_α281 belongs to a V_α family different from the V_α1–V_α10 families reported by Arden *et al.* (15). (*iii*) The copy number of the V_α281 family in C57BL/6 (three) is different from that in AKR (two) (Fig. 5*c*).

T_s hybridoma 34S-281 is quite stable and active. It can be induced to produce TsF, which suppresses an anti-dinitrophenyl (Dnp) IgG secondary response to KLH-Dnp. This induction occurs after simulation either by KLH-specific TsF of the two-chain type (13) or by a particular monoclonal anti-KLH antibody with suppressor activity (14). This activation of the hybridoma has been found to be mediated through the receptor on T_s, which works as a stereocopy of the KLH epitope predominantly seen by regulatory lymphocytes (28).

The cDNA clones isolated from the hybridoma contain the sequence of a productively rearranged α-chain gene. Moreover, disulfide-linked dimeric molecules of the apparent molecular mass expected for the T-cell receptor α and β chains are detected in the T_s hybridoma. It is thus quite likely that the T_s α chain is expressed in T_s as a functional molecule. As the T_s receptor expresses the specific idiotypic determinant, the possibility that the T_s α-chain is associated with the BW5147 β-chain to construct the functional T_s receptor seems unlikely.

If the α chain is responsible for construction of an antigen receptor on T_s, the T_s antigen receptor may be an α-chain homodimer or a heterodimer composed of the α chain in association with an undefined chain (other than the β-chain, because the β-chain gene is deleted on all T_s hybridomas). It has been reported that the gene encoding the putative "γ chain" is rearranged and transcribed in some T lymphocytes (29, 30). Therefore, it is possible that the γ chain is a subunit of the T_s antigen receptor, in association with the T_s α chain. However, the γ gene is not always expressed in KLH-specific T_s hybridomas. Low levels of γ-gene transcripts are detected in the 34S-281 T_s hybridoma, whereas none is observed in the other KLH-specific T_s hybridomas, 34S-704 and 34S-18 (unpublished observation). Therefore, in general, the T_s antigen receptor is not likely to be an α/γ heterodimer.

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1. Chein, Y., Becker, D. M., Lindsten, T., Okamura, M., Cohen, D. I. & Davis, M. M. (1984) *Nature (London)* **312,** 31–35.
2. Saito, H., Kranz, D. M., Takagaki, Y., Hayday, A. C., Eisen, H. N. & Tonegawa, S. (1984) *Nature (London)* **312,** 36–40.
3. Hedrick, S. M., Neilsen, E. A., Kavaler, J., Cohen, D. I. & Davis, M. M. (1984) *Nature (London)* **308,** 153–158.
4. Yanagi, Y., Yoshikai, Y., Leggett, K., Clark, S. P., Aleksander, I. & Mak, T. W. (1984) *Nature (London)* **308,** 145–148.
5. Kronenberg, M., Goverman, J., Haars, R., Malissen, M., Kraig, E., Phillips, L., Delovitch, T., Suciu-Foca, N. & Hood, L. (1985) *Nature (London)* **313,** 647–653.
6. Hedrick, S. M., Germain, R. N., Bevan, M. J., Dorf, M., Engel, I., Fink, P., Gascoigne, N., Herber-Katz, E., Kapp, J., Kaufmann, Y., Kaye, J., Melchers, F., Pierce, C., Schwartz, R. H., Sorensen, C., Taniguchi, M. & Davis, M. M. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 531–535.
7. Mori, L., Lecoq, A. F., Robbiati, F., Barbanti, E., Righi, M., Sinigaglia, F., Clementi, F. & Ricciardi-Castagnoli, P. (1985) *EMBO J.* **4,** 2025–2030.
8. Ballinari, D., Castelli, C., Traversari, C., Pierotti, M. A., Parmiani, G., Palmieri, G., Ricciardi-Castagnoli, P. & Adorini, L. (1985) *Eur. J. Immunol.* **15,** 855–860.

9. De Santis, R., Givol, D., Hsu, P., Adorini, L., Doria, G. & Appella, E. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8638–8642.
10. Gershon, R. K. & Kondo, K. (1971) *Immunology* **21,** 903–914.
11. Tada, T. & Okumura, K. (1979) *Adv. Immunol.* **28,** 1–87.
12. Taniguchi, M., Saito, T., Takei, I., Kanno, M., Tokuhisa, T. & Tomioka, H. (1982) in *Lymphokines*, eds. Feldmann, M. & Shreier, M. H. (Academic, New York), pp. 77–117.
13. Sumida, T., Takei, I. & Taniguchi, M. (1984) *J. Immunol.* **133,** 1131–1136.
14. Sumida, T. & Taniguchi, M. (1985) *J. Immunol.* **134,** 3675–3681.
15. Arden, B., Klotz, J. L., Siu, G. & Hood, L. E. (1985) *Nature (London)* **316,** 783–787.
16. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
17. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
18. Glisin, V., Crkvenjakov, R. & Byus, C. (1974) *Biochemistry* **13,** 2633–2637.
19. Goldberg, D. A. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5794–5798.
20. Ramsey, J. C. & Steele, W. J. (1977) *Biochem. J.* **168,** 1–8.
21. Palmiter, R. D. (1974) *Biochemistry* **13,** 3606–3615.
22. Huynh, T. V., Young, R. A. & Davis, R. W. (1985) in *DNA Cloning: A Practical Approach*, ed. Grover, D. (IRL, Oxford), pp. 49–78.
23. Benton, W. D. & Davis, R. W. (1977) *Science* **196,** 180–182.
24. Messing, J. (1983) *Methods Enzymol.* **101,** 20–78.
25. Marchalonis, J. J., Cone, R. E. & Santer, V. (1971) *Biochem. J.* **124,** 921–927.
26. Goding, J. W. & Harris, A. W. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4530–4534.
27. Becker, D. M., Patten, P., Chien, Y., Yakato, T., Eshhar, Z., Giedlin, M., Gascoigne, N. R. J., Goodnow, C., Wolf, R., Arai, K. & Davis, M. M. (1985) *Nature (London)* **317,** 430–434.
28. Taniguchi, M. & Sumida, T. (1985) *Immunol. Rev.* **83,** 125–150.
29. Saito, H., Kranz, D. M., Takagaki, Y., Hayday, A. C., Eisen, H. N. & Tonegawa, S. (1984) *Nature (London)* **309,** 757–762.
30. Hayday, A. C., Saito, H., Gillies, S. D., Kranz, D. M., Tanigawa, G., Eisen, H. N. & Tonegawa, S. (1985) *Cell* **40,** 259–269.