The Journal of Immunology

RESEARCH ARTICLE | JANUARY 15 1991

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J Heuer; ... et. al

J Immunol (1991) 146 (2): 775–782.

https://doi.org/10.4049/jimmunol.146.2.775

0022-1767/91/1462-0775$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 146, 775–782, No. 2, January 15, 1991
Printed in U.S.A.

T CELL RECEPTOR α AND β GENE EXPRESSION IN A MURINE ANTIGEN-SPECIFIC T SUPPRESSOR LYMPHOCYTE CLONE WITH CYTOLYTIC POTENTIAL[1]

JOACHIM HEUER,[2] JOACHIM DEGWERT, HANS-GERD PAUELS, AND ECKEHART KÖLSCH

From the Department of Immunology, University of Münster, Domagkstrasse 3, W-4400 Münster, FRG

The composition of α and β TCR genes was analyzed in a murine BSA-specific Ts cell clone with cytolytic potential. The isolated poly(A)$^+$ mRNA from Ts cell clone BVI/5 was used to construct a cDNA library in the bacteriophage λgt11. Full-length cDNA clones specific for TCR α and TCR β genes have been detected and isolated by hybridization with specific oligonucleotide probes. The functional rearranged TCR α gene is composed of a member of the Vα1 family, the junctional gene JαTT11 and Cα. The gene segments Vβ13, Dβ2.1, Jβ2.4, and Cβ2 form the functional rearranged TCR β gene. Furthermore, a nonfunctional TCR α gene transcript has been detected, where a Vα8 gene is rearranged to a so far not described Jα gene segment (JαBVI). A stop codon in its junctional region is responsible for this nonfunctional transcript. By using Southern blot analysis, the described rearranged TCR genes can be detected in the Jα junctional region and in the Jβ2 cluster on the genomic DNA level. Immunoprecipitation studies with the KT3 anti-CD3 mAb and flow microfluorimetry analysis with the H57-597 anti-TCR-α/β mAb show that TCR/CD3 complexes are synthesized and expressed on BVI/5 Ts cells. Taken together, the cDNA sequencing data, the protein studies, and the specificity of Ag recognition demonstrate that the BVI/5 Ts cell clone not only transcribes the TCR α and β genes but also expresses a functional BSA-specific receptor.

The Ag receptors of Th cells and CTL have been analyzed in detail with respect to structural and biochemical properties (1–4). The TCR of these T cell types recognizes foreign Ag in the context of class II or class I molecules of the MHC. It is a heterodimer composed of two disulfide bond-linked polypeptide chains (5, 6), which are responsible for Ag and MHC recognition (7). The TCR is associated with a cluster (CD3) of polypeptide chains which are necessary for signal transduction through the cell membrane (8). The α and β genes of the TCR (9–13) and the genes of the CD3 complex have been cloned (13–16).

In contrast to the progress in analyzing TCR-α/β structures of Th cells and CTL, the nature of the Ag-recognition structure of Ag-specific Ts cells is not unequivocally clear. Human Ts cells specific for *Mycobacterium leprae* Ag (17) or keyhole limpet hemocyanin (18) and murine Ts cells specific for lysozyme have TCR-α/β structures (19). On the other hand, most Ts hybridomas lack rearranged TCR α and β genes (20–22). On this ground still other TCR structures have been postulated for Ts cells, but convincing experimental data are lacking.

BSA-specific Ts cell clones have in the past been isolated in our laboratory from mice tolerized to BSA by low doses of Ag. The fact that these cell clones have been characterized extensively, in vitro and in vivo, provide the rational basis for addressing the question of TCR expression on Ag-specific murine Ts cells. BSA-specific Ts cell clones have originally been isolated from tolerant CBA/J mice and were cultured in vitro in the presence of the nominal Ag and APC. They retain over years exquisite Ag specificity, recognize BSA in association with I-A or I-E class II molecules, and discriminate between cross-reacting mammalian serum albumins. Suppressor activity of these Ts cells has been documented in suppressor cell assay systems both in vitro and in vivo. Results from these experiments have led to the conclusion that, in principle, the activity of these Ts cells can lead to clonal anergy or clonal deletion of Th cells in peripheral lymphoid organs (23–27).

To determine whether Ts cells of the above type use the TCR-α/β structure for Ag recognition, we have looked for α and β genes. The following report presents the sequence data for rearranged TCR α and β genes, an analysis of gene rearrangements in the genomic DNA, and an evaluation of TCR/CD3 complex expression in BVI/5 Ts cells.

MATERIALS AND METHODS

Ts cells. The BSA-specific Ts cell clone BVI/5 was used in the experiments. The in vitro culture conditions for the maintenance of this cell clone have been described earlier (25). BVI/5 Ts cells have routinely been tested for Ts cell activity in vitro during the course of the present study.

Cytotoxicity tests. ^{51}Cr-release assays were performed as previously described (27) with 2×10^4 EL4 (H-2^b) thymoma cells/well as targets at a cell concentration of 1×10^5 EL4 cells/ml in absence or presence of BSA (200 μg/ml) or Con A (5 μg/ml). Effector and target cells were cocultured for 16 h.

cDNA library. Total RNA was isolated from cells with the use of guanidinium isothiocyanate (28, 29), and poly(A)$^+$ mRNA was purified by two passages over an oligo(dT) column. The cDNA library was constructed by oligo(dT)-priming of cDNA synthesis by reverse transcriptase (GIBCO BRL, Eggenstein, FRG). The cDNA was ligated to *Eco*RI linkers and inserted into the *Eco*RI site of λgt11 bacteriophages. The bacteriophages were packaged in vitro according to the instructions of the commercially available Packagene extract (Pro-

Received for publication February 20, 1990.
Accepted for publication October 24, 1990.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by the Deutsche Forschungsgemeinschaft through Sonderforschungsbereich 310.
[2] Address correspondence and reprint requests to: Joachim Heuer, Institut für Immunologie, Universität Münster, Domagkstr. 3, D-4400 Münster, FRG.

mega, Heidelberg, FRG). The resulting library contained 6×10^5 independent cDNA clones. After amplification, plaques were transferred to nitrocellulose filters and hybridized.

DNA sequencing. The sequence of both strands of DNA was determined by the dideoxy-chain-termination method (30) in M13mp18 and M13mp19 (31) with a modified T7 DNA polymerase as outlined in the Sequenase Kit (U.S. Biochemical Corp., Cleveland, OH). Oligonucleotide primers used for sequencing reactions were synthesized on Gene Assembler (Pharmacia-LKB, Freiburg, FRG) by using phosphoramidite. Crude material was purified on a 20% polyacrylamide gel. Sequence information was read directly into an IBM computer via an 2017 Macroread sonic digitizer (Pharmacia-LKB).

Southern hybridization analysis. High m.w. DNA from the cultured Ts cell clone BVI/5 and CBA/J liver tissue were prepared by standard techniques (31). Briefly, DNA was prepared by cell lysis with proteinase K digestion, extraction with phenol, and precipitation with ethanol followed by removal of RNA by DNase-free RNase A. Ten micrograms of DNA were digested with the appropriate restriction endonuclease, electrophoresed in a 1.0% agarose gel, denatured, neutralized, and transferred to a Nytran-N nylon membrane filter (Schleicher & Schuell, Dassel, FRG) by a vacuum blotting unit (Pharmacia-LKB). Hybridization was performed at 42°C in 1 M NaCl, 50% formamide, 10% dextransulfate, 50 mM Tris (pH 7.5), 0.1% $Na_4P_2O_7$, 0.1% SDS, 10 × Denhardt's solution (0.2% each: BSA, polyvinylpyrrolidone, Ficoll), and 150 μg/ml sheared and denatured salmon sperm DNA. For hybridization, samples of 1×10^6 cpm/ml of the probe to be hybridized were added to the hybridization buffer. The membranes were washed once in 3 × SSC, 0.1% SDS at room temperature for 3 min, then twice in 3 × SSC, 0.1% SDS at 65°C for 30 min, and finally twice in 0.3 × SSC, 0.1% SDS at 65°C for 30 min and then were exposed to Kodak XAR-5 x-ray Film (Kodak, Stuttgart, FRG) with two intensifying screens at −70°C.

Probes. The probes for screening the λgt11 cDNA library were a 23-bp oligonucleotide probe (5′TATAGGGTGGCCTTCCCCAGTAG 3′) specific for the Cβ2 region (32) and a 23-bp oligonucleotide probe (5′ GTGCTGTCCTGAGACCGAGGATC 3′) specific for the Cα region (33) of TCR genes. Oligonucleotides were end-labeled with T4 polynucleotide kinase (GIBCO BRL) and [γ-^{32}P]ATP (Amersham Buchler, Braunschweig, FRG). The J15 probe specific for the intron between Jβ2 and Cβ2 corresponds to a 900-bp *Hin*dIII-*Eco*RI fragment derived from the genomic clone CSpλ4 from the CTL line CSp2 (34). The Vα1-specific probe was a 377-bp *Spe*I-FoKI fragment of the cDNA clone BVI/5.α1. The Vα8 specific probe was a 387-bp *Eco*RI-FoKI fragment of the cDNA clone BVI/5.α2. The Vβ13-specific probe was a 279-bp *Hin*fI-*Hpa*II fragment of the cDNA clone BVI/5.β11. The probes 9 and 10 described in the literature (35), specific for the TCR Jα cluster correspond to cosmid clones from a cosmid genomic library constructed from B10.WR7 liver DNA. Probe 9 corresponds to a 2-kb *Bam*HI-*Hpa*I fragment isolated from cosmid 55.1W7. Probe 10 corresponds to a 1.5-kb *Kpn*I-*Pst*I fragment isolated from cosmid 19.1W7. Probes were labeled to high sp. act. with [α-^{32}P]dCTP by the random primer method (36).

Immunoprecipitation of TCR/CD3 complexes. For metabolic labeling, BVI/5 Ts cells were harvested 10 days after the last antigenic stimulation, washed three times, and preincubated for 1 h in MEM (GIBCO BRL) without methionine and cysteine. Cells were pulsed with a mixture of 70% [^{35}S]methionine (1091 Ci/mmol) and 15% [^{35}S] cysteine (Tran ^{35}S-label, ICN Biochemicals, Irvine, CA) for 4 h (200 μCi/10^7 cells). Then the cultures were chased with cold methionine (15 mg/ml MEM) and cysteine for 30 min. For radioiodination BVI/5 Ts cells were harvested 18 to 21 days after the last antigenic stimulation and purified over a Lymphoprep (Nycomed AS, Oslo, Norway) gradient. Radioiodination was catalyzed by Iodo-Gen (Pierce Chemical Corp., Rockford, IL) coated to the walls of a glass test tube with 100 μg of the reagent for 10^7 cells. BVI/5 Ts cells (10^7) were added to the tube in a volume of 100 μl, followed by the addition of 200 μCi Na^{125}I (Amersham-Buchler). Iodination was carried out on ice for 30 min with gentle agitation. Before lysis, the labeled cells were washed three times in PBS. Lysis was carried out overnight in 150 mM NaCl, 50 mM Tris, 1 mM PMSF (Sigma, St. Louis, MO) containing 1% of NP-40 or 1% digitonin (Serva, Heidelberg, FRG), respectively. The lysates were cleared by centrifugation at 1,000 × *g* for 10 min and 10,000 × *g* for 30 min followed by two preclearing steps with mouse serum-coupled Sepharose for 2 h and overnight. For immunoprecipitation of the TCR/CD3 complex, the purified KT3 anti-CD3 mAb (37) was coupled to CNBr-activated Sepharose (Pharmacia-LKB) (approximately 1 mg mAb/ml swollen gel). The remaining active groups of the gel were hydrolyzed with 0.1 M Tris (pH 9.0) and nonspecific binding sites of the matrix were saturated with normal mouse serum. Samples of 50 μl of the matrix were mixed with the precleared lysate from 10^7 cells at 4°C overnight. After washing the matrix five times in the respective lysis buffer, the bound radioactive material was eluted with nonreducing SDS sample buffer at 80°C for 10 min. For analysis under reducing conditions 5% 2-ME was added to an aliquot of the eluate followed by heating to 80°C for additional 10 min. The samples were analyzed by one- and two-dimensional SDS-PAGE under nonreducing and reducing conditions in 12.5% polyacrylamide gels followed by autoradiography.

Immunofluorescence staining. Ag receptors on BVI/5 Ts cells were characterized by incubation of the cells with H57-597 (anti-TCR-α/β) mAb (38) followed by staining with fluoresceinated rabbit anti-hamster Ig as described elsewhere (24). Flow microfluorimetry analysis was performed with the use of a FACScan apparatus (Becton Dickinson, Mountain View, CA).

RESULTS

BSA-specific Ts cells from low dose tolerized CBA/J mice. Earlier studies (39) have shown that low doses of Ag preferentially activate Ts cells and thus induce an Ag-specific immune paralysis. Such Ts cells are also found after primary immunization, but under these conditions their activation cannot be demonstrated, because in situ Th cells once primed are refractory to suppression. Below we summarize functional data on BSA-specific Ts cell clones from tolerant and immune CBA/J mice as far as they are relevant for the analysis of TCR expression. Evidence that relates these Ts cells to or distinguishes them from the class II-restricted CTL (40) will also be evaluated.

One important feature is the class II-restricted Ag-specific proliferation of Ts cell clones. The endogeneous expression of class II-molecules gives the potential to present Ag and provides interaction molecules for contacting Th cells which are their likely target for suppression. Suppression is Ag-specific in the sense that the Ts cell function is activated only by the nominal Ag. But if the TCR is occupied by its nominal Ag, then Th cells with specificity for another Ag are in vitro also influenced and only if the nominal Ag is simultaneously present in the culture system (23–27) (for summary, see Ref. 41). The molecular process of suppression is not yet understood, but it is under further investigation with the appropriate mAb. Under certain conditions, a cytolytic potential can be observed (27). Such an example is given in Figure 1 for the Ts cell clone used in this study for TCR expression. EL4 (H-2^b) target cells release radioactivity in a 16-h ^{51}Cr-release assay if incubated with BVI/5 (H-2^k) Ts cells. This lysis can be observed only in the presence of BSA and Con A. At the moment, such a finding is only so far of limited interest, as it demands a demarcation between Ts

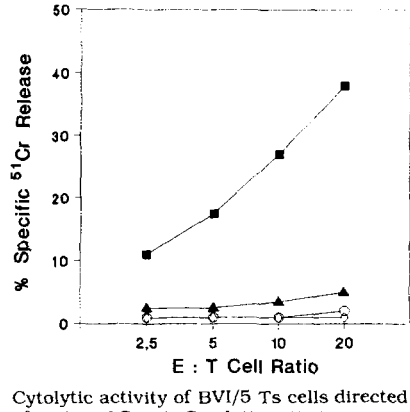


Figure 1. Cytolytic activity of BVI/5 Ts cells directed against EL-4 in the presence of BSA and Con A. Cytolytic activity was measured in a ^{51}Cr-release assay at various E:T ratios and is given as percent specific release. BVI/5 Ts cells (H-2^k) were used as effector cells against EL4 (H-2^b) targets in 16-h ^{51}Cr-release assays in medium alone (◇——◇), medium with Con A (▲——▲), BSA (○——○), or BSA and Con A (■——■).

cells with cytolytic capacity and class II-restricted CTL. If one defines class II-restricted CTL as a cell type which uses recognition of class II molecules plus Ag on the target to lyse it in the way class I-restricted CTL operate, then the BSA-specific Ts cells studied by us are not members of the above family (stringent definition of class II-restricted CTL). They fall into the category of class II-restricted CTL if the activation of Ts cells by class II molecules plus Ag on APC remains a necessary primary event. Effector functions could then operate through Ag-presentation to Th cells and subsequent lysis of the target Th or even bystander cells (permissive definition). The latter type is based on a rather flexible definition and would underline only the existence of a lytic potential, leaving it open whether it is used at all as Ts cell effector mechanism in vivo. It is also not known to what extend the immunologic paralysis induced by BVI/5 Ts cells in vivo is a consequence of clonal deletion or clonal anergy of Th cells.

Isolation of TCR α and β gene-specific cDNA clones. To study the primary structure of TCR genes of Ag-specific Ts lymphocytes, we used the Ts cell clone BVI/5, characterized by in vitro and in vivo functional activity (25, 26).

A cDNA library was constructed by using isolated poly(A)$^+$ mRNA from the cell clone and about 30,000 recombinant cDNA clones had been screened with the 23-bp oligonucleotides specific for the constant region of the TCR α and β genes as described elsewhere (33). We determined 0.042% Cα-specific and 0.032% Cβ2-specific cDNA clones among the total number of recombinant clones. Such a frequency is usually detected in cDNA libraries for TCR genes (42). Figure 2 shows an overview of the TCR cDNA clone types isolated.

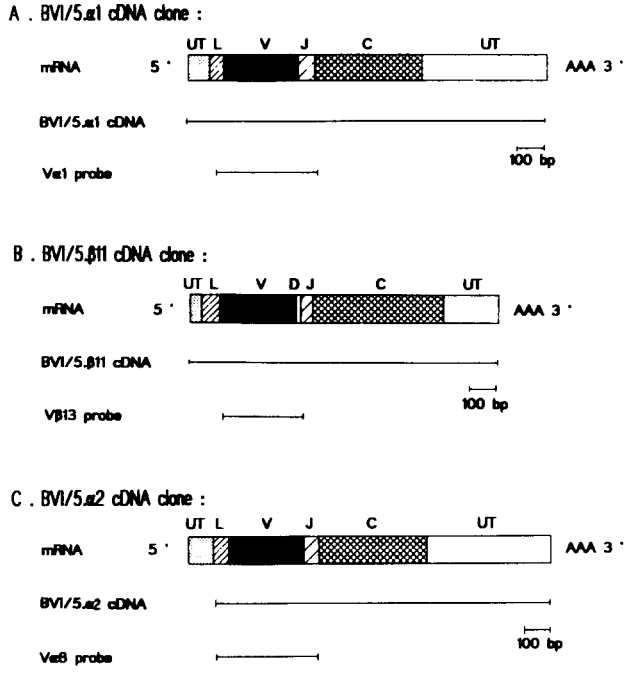


Figure 2. Schematic overview of TCR cDNA clones analyzed in this paper. The total length of the isolated cDNA clones BVI/5.α1, BVI/5.β11, and BVI/5.α2 with regard to the predicted mRNA is shown. Furthermore, the position of the three variable region specific DNA probes used in the Southern hybridization analysis are given. The following abbreviations are used: *L*, leader; *V*, variable gene segment; *J*, joining gene segment; *D*, diversity gene segment; *C*, constant region gene segment; *UT*, untranslated.

Sequencing analysis of TCR α and β gene-specific cDNA clones. The longest cDNA clones containing Cα- and Cβ2-specific nucleotide sequences were chosen for further sequence analysis. cDNA clones which could contain a full length transcript of TCR-α and -β composition were cloned into M13mp18 and M13mp19 bacteriophages. Figure 3*A* shows the nucleotide sequence of the cDNA clone BVI/5.α1, which represents the functional transcript of the α-chain of the TCR consisting of the elements Vα1, JαTT11, and Cα. The analyzed Vα1-specific sequence is with the exception of the nucleotide position 61 of the Vα1 gene (cytosine instead of thymidine) and the nucleotide position 43 of the leader region (thymidine instead of guanosine) identical to the Vα1 sequence of the TNP-specific T cell clone E1 (43). Figure 3*B* shows the nucleotide sequence of the cDNA clone BVI/5.β11. The clone BVI/5.β11 represents the complete functional transcript of the β-chain of the TCR consisting of the elements Vβ13, Dβ2.1, Jβ2.4, and Cβ2. Figure 3*C* shows a nonfunctional TCR α gene transcript composed of a part of the leader sequence and a variable region Vα8 gene, a so far not described Jα segment named Jα BVI and Cα. Figure 4 documents the joining region between Vα8 and Cα. The sequences corresponding to conserved amino acids of Jα segments are present and prove it as a Jα gene segment. A stop signal in the Jα region defines it as a nonfunctional transcript.

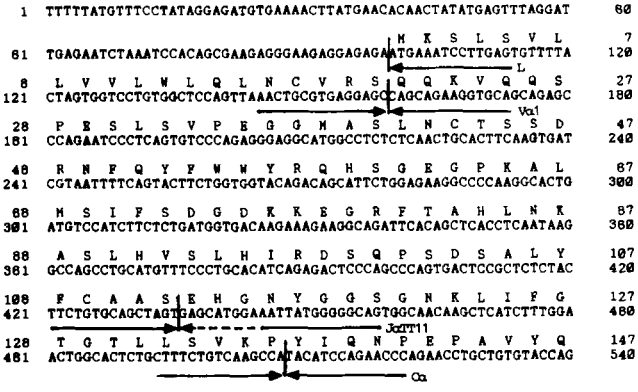
Southern analysis of the rearrangements of TCR α genes. Ts cell DNA was hybridized with a set of TCR α and β gene-specific probes in Southern blot analysis to study the events which led to functional rearrangement at the genomic DNA level (44). First we detect with the Vα1-specific probe (Fig. 5*a*) a new band of 3.6 kb by comparing the DNA of the Ts cell clone BVI/5 with the germ-line configuration by an *Bam*HI digestion. Because the position of the JαTT11 element of the TCR cDNA clone BVI/5α1 was mapped within the Jα gene cluster, the germ-line-specific probes 9 and 10 up- and downstream to the position of the JαTT11 gene were used to analyze the mechanism of rearrangement. After *Bam*HI digestion, probe 9 detects one band of 7.4 kb in the germ-line configuration and the rearranged band at 3.6 kb which was also detected with the Vα1 probe (Fig. 5*c*). The identity of both bands was further documented by a second hybridization of the same filter with probe 9 at the first step followed by a hybridization with the Vα1 probe (*lane 3*, Fig. 5*c*). Hybridization of *Bam*HI-digested DNA with probe 10 proves a deletion mechanism for the rearrangement of the Vα1 to the JαTT11 gene, based on the fact that the germ-line band of 7.4 kb is detected with the half of intensity in the BVI/5 Ts cell DNA in comparison with liver DNA (Fig. 5*b*, *lane 3*, *4*).

In Figure 5*b*, *lane 2*, the rearranged band of 3.0 kb documents the nonfunctional Vα8-JαBVI gene combination, because the identical band could be detected after hybridization of *Eco*RI-digested DNA with a Vα8-specific probe (Fig. 5*d*). The rearranged band could not be detected with probe 10 after *Bam*HI digestion meaning that the germ-line position of the JαBVI gene is in the 5′ boundary of the germ-line *Bam*HI fragment of 7.4 kb detected by probe 10 (Fig. 5*b*).

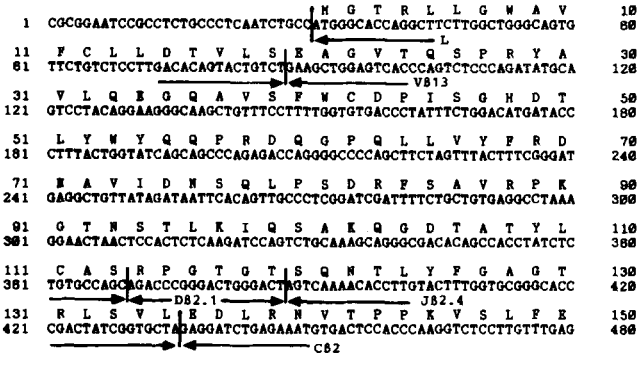
Southern analysis of the rearrangement of TCR β genes. From the sequencing data we know that the Vβ13 gene from the cDNA clone BVI/5.β11 is rearranged to the

Jβ2.4 gene. Two rearranged bands are visualized after hybridization with a Jβ2 cluster-specific probe which detects the 5.4-kb band within the germ-line DNA after *Hin*dIII digestion (Fig. 6*a*). The additional hybridization with a Vβ13-specific probe characterizes the rearranged band at 2.7 kb as a functional rearrangement (Fig. 6*b*).

A. BVI/5.α1 cDNA clone:



B. BVI/5.β11 cDNA clone:



C. BVI/5.α2 cDNA clone:

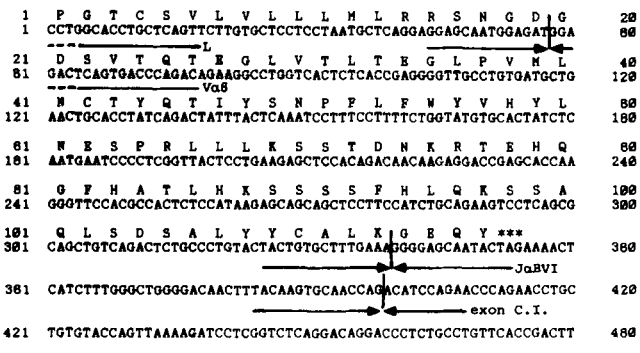


Figure 3. Nucleotide sequences and amino acid compositions of the isolated TCR α and β gene-specific cDNA clones. These sequence data were obtained from α and β gene-specific cDNA clones isolated from a λgt11 cDNA library. The encoded amino acids are given in one-letter code. Dotted lines between the Vα and Jα region indicate uncertainty over the exact 3′ boundary of the Vα1 and 5′ boundary of the JαTT11 because corresponding germ-line sequences are unknown (*A*). These cDNA clones represent full length transcripts. The encoding regions start with an initiating methionine. The leader, variable, diversity, joining, and constant regions are marked and underlined. In (*C*) a stop codon within the joining region is marked with asterisks.

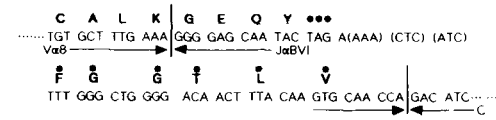


Figure 4. Jα region of the nonfunctional cDNA clone BVI/5.α2. The nucleotide and the amino acid (single letter code) sequence of a part of the Vα8, JαBVI, and the beginning of the constant region are given. The stop codon in the Jα region is marked with three asterisks, and the conserved amino acid positions of the Jα region are labeled with one asterisk. The possible triplet code after the stop codon in the joining region is given in parentheses.

The other rearranged band which is detectable with the J15 probe has not been further analyzed.

Expression of TcR genes in BVI/5 Ts cells. Immunoprecipitation of TCR/CD3 proteins is demonstrated in Figures 7 and 8. Figure 7, *a* and *b*, represents autoradiographs from biosynthetically labeled proteins precipitated with KT3 anti-CD3 mAb after lysis of BVI/5 Ts cells in either NP-40 or digitonin. The latter allows precipitation of the complete TCR/CD3 complex (45). The precipitated proteins were run under nonreducing (*a*) and reducing (*b*) conditions. Figure 7, *c* and *d*, represents autoradiographs of proteins immunoprecipitated from radioiodinated BVI/5 Ts cells lysed in digitonin and run under nonreducing (*c*) and reducing (*d*) conditions. The biosynthetic label gives a better resolution for the CD3 proteins, whereas the radioiodination results in clearer bands for the TCR-α,β proteins. Figure 8 represents autoradiographs of samples analyzed by two-dimensional SDS-PAGE under nonreducing (*horizontal*) and reducing (*vertical*) conditions of two immunoprecipitations used also in Figure 7. Figure 8*a* represents the same immunoprecipitate as run on Figure 7*a*, *slot 4*, and was performed with KT3 anti-CD3 mAb from a digitonin lysate of metabolically labeled BVI/5 Ts cells. Figure 8*b* represents the same immunoprecipitate as shown in Figure 7*a*, *slot 3*, from a NP-40 lysate. The migration pattern of the precipitated proteins under reducing and nonreducing conditions is the same as described for the 145-2C11 mAb (46). By using the H57-597 anti-α/β TCR mAb it can be demonstrated by flow microfluorimetry analysis that BVI/5 Ts cells express this type of Ag receptor (Fig. 9).

In separate experiments (H.-G. Pauels and E. Kölsch, manuscript in preparation) BALB/c × BALB.K F_1 mice were immunized with BVI/5 Ts cells and mAb were derived from those mice. These mAb specifically stimulate proliferation of this cell clone and immunoprecipitate a 90-kDa band under nonreducing and a 45-kDa band under reducing conditions which are identical with the bands coprecipitated with the KT3 anti-CD3 mAb. These mAb are presumably directed against a specific determinant on the BVI/5 TCR.

Taken together, the cDNA sequencing data and the gene expression studies demonstrate that the BVI/5 Ts cell clone does not only transcribe the TCR α and β genes but also expresses a functional BSA-specific TCR.

DISCUSSION

The BSA-specific Ts cell clone BVI/5 from a CBA/J mouse tolerized to low doses of this Ag has been studied with regard to several functions. Two of the essential features of BVI/5 Ts cells are the capacity to suppress in vitro the proliferation of BSA-primed lymphnode cells in an adequate assay (25), and the capability of inducing in

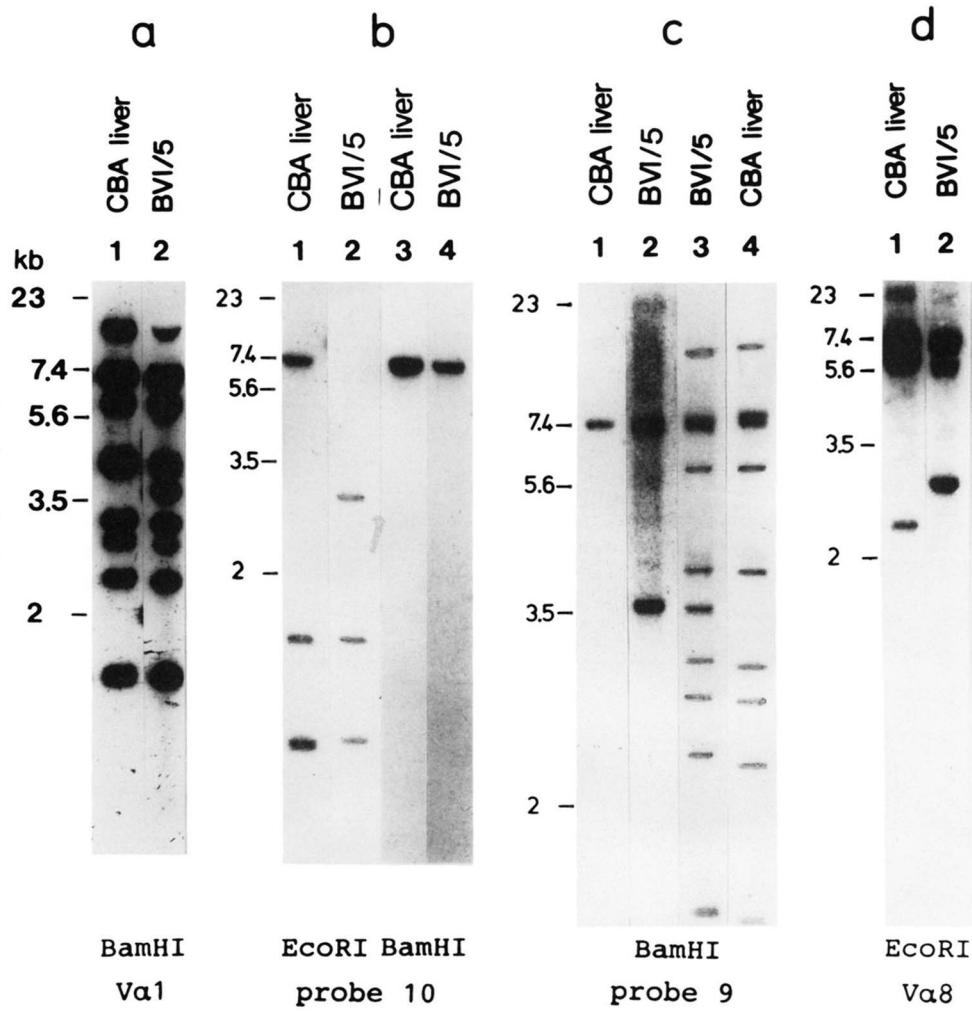


Figure 5. Rearrangements of TCR α genes in BVI/5 Ts cells. DNA from CBA/J liver and BVI/5 Ts cells digested with *Bam*HI (*a,b* (*lanes 3* and *4*) and c), or *Eco*RI (*b* (*lanes 1* and *2*), and *d*), electrophoresed, transferred to a nylon membrane, and hybridized with the specific probes: Vα1, probe 10, probe 9, and Vα8. In (c) (*lane 3* and *4*) the same membrane was hybridized with the Vα1 probe in a second step. The five size markers (23; 7.4; 5.6; 3.5; 2) are given in kb.

vivo BSA-specific T cell unresponsiveness in syngeneic recipient mice when BVI/5 Ts cells are transferred at the time of immunization with the nominal Ag. These animals are truly tolerant because they are also unable to mount a humoral anti-hapten response when primed at the time of cell transfer with hapten-conjugated BSA (26). We do not know the actual mechanism of effector to target cell interaction. However, it can be excluded that BVI/5 Ts cells are class II-restricted CTL in the stringent sense as defined in *Results*.

In the present study we have demonstrated, that cells of the Ag-specific Ts lymphocyte clone BVI/5 contain in frame full length functional transcripts of the TCR α- and β-chains of a conventional TCR-α/β heterodimer. The sequence data from the rearranged TCR α gene show its functionality by an in frame joining of a member of the Vα1 family to the joining gene segment JαTT11. A nonfunctional rearranged TCR α gene composed of a Vα8 gene and a so far not described gene segment (JαBVI) has been found in addition. The rearranged Vα1-JαTT11 gene and the nonfunctional rearranged Vα8-JαBVI gene have been mapped in the Jα gene cluster by using the Vα region-specific Vα1, Vα8, and the germ-line-specific probes 9 and 10 in Southern blot analysis. Probe 9 detects a germ-line fragment in the 3′ boundary position of JαTT11 to Cα and is therefore useful for detection of rearrangement events toward the JαTT11 gene. Because probe 10 is specific for germ-line sequences in the 5′ boundary of JαTT11, the mechanism of the rearrangement of Vα region genes to JαTT11 can be studied. After *Bam*HI digestion, subsequent hybridization with probe 9 detects one germ-line band (7.4 kb) and one rearranged band at 3.6 kb; probe 10 detects the same germ-line band in the BVI/5 DNA with half of the intensity in comparison with the germ-line band. Therefore, we conclude that the deletion events resulting in the rearranged Vα1-JαTT11 gene segment occur within one chromosome. The mechanism which leads to the Vα8-JαBVI composition has not been further analyzed. But it must have taken place on the other chromosome.

The mechanisms of allelic exclusions at the level of TCR α and β gene expression had been previously analyzed in several laboratories (7, 35, 47–51). In one report two productive Vα-Jα rearrangements could be detected in a single Ag-specific Th hybridoma (35). In our case a productive and a nonproductive TCR α gene rearrangement occurs within one cell.

The BSA-specific BVI/5 Ts cells recognize their nominal Ag and some cross-reacting serum albumins in the context of I-E^k class-II molecules (25) on APC. This finding already suggested involvement of a membrane-associated TCR in the Ag recognition process. By using the KT3 anti-CD3 mAb and the H57-597 anti-TCR-α/β mAb it has now been demonstrated that endogeneous TCR α and β chains are expressed on functionally active BVI/5 Ts cells.

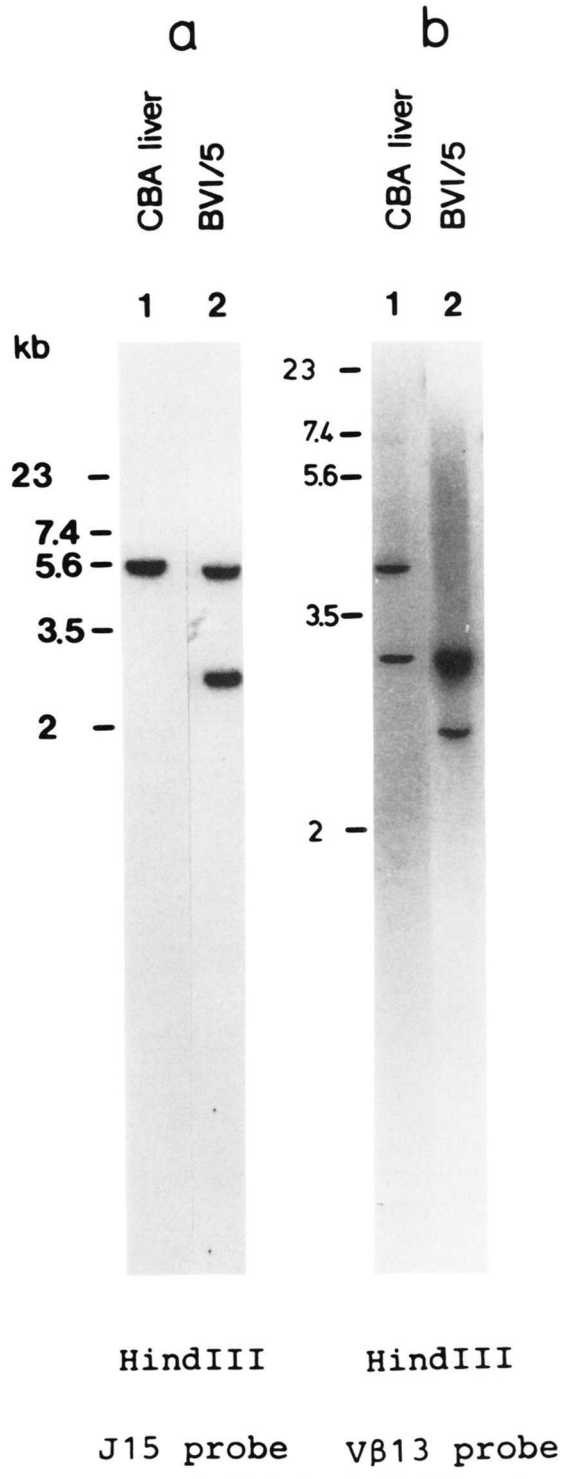


Figure 6. Rearrangement of TCR β genes in BVI/5 Ts cells. DNA from CBA/J liver and BVI/5 Ts cells were digested with *Hin*dIII, electrophoresed, transferred to a nylon membrane, and hybridized with the specific probes J15 and Vβ13. The five size markers (23; 7.4; 5.6; 3.5; 2) are given in kb.

The first data from Ts hybridoma suggested that Ag recognition structures on Ts cells would be different from the one on Th cells or CTL (20–22). At present still limited information is available about the Ag-recognition structures of properly characterized Ts lymphocytes (52). In contrast to previous results, new reports document that TCR gene structures are present in some Ts hybridomas (53, 54) and suggest involvement of the TCR-α/β structure in antigenic recognition by some Ts cells (55–59).

The TCR sequence data in this report and the demonstration of TCR-α/β heterodimer expression on Ag-specific Ts lymphocytes could be a step forward in understanding Ts cell-mediated specific immune paralysis in peripheral lymphoid organs. One of the major problems that needs to be solved is the evaluation of the mechanism by which Ts cells with in vitro cytolytic capacity (defined above as class II-restricted CTL in the permissive definition) induce specific immunologic paralysis in vivo. The ascribed lytic capacity to some Ts cell clones may guide (60) or may not guide (61) to the solution of the problem, and it will be necessary to investigate whether suppressive activity or cytolytic capacity can be separated in Ts cells of the BVI/5 type.

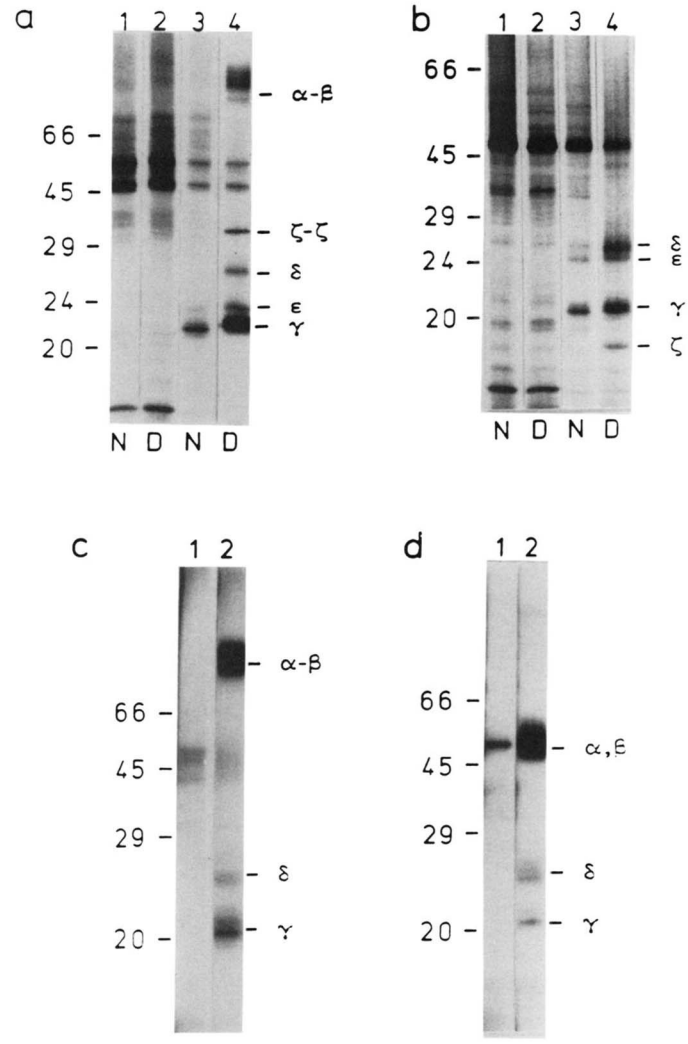


Figure 7. Immunochemical analysis of the TCR/CD3 complex expressed on BVI/5 Ts cells. (*a* and *b*) BVI/5 Ts cells were metabolically labeled with a mix of L-[^{35}S]methionine and L-[^{35}S]cysteine which gives a good signal for the CD3 proteins but only a weak signal for the TCR α- and β-chains. The labeled BVI/5 Ts cells were lysed with NP-40(*N*) or digitonin (*D*). *Lanes 1* and *2*: precipitation with mouse serum-coupled Sepharose 4B (corresponding to the last preclearing step), *lanes 3* and *4* precipitation with Sepharose 4B coupled with KT3 mAb. The samples were run in 12.5% polyacrylamide gels under nonreducing (*a*) and reducing (*b*) conditions. (*c* and *d*) Immunoprecipitation of digitonin lysates from radioiodinated BVI/5 cells, giving a strong signal for the TCR α- and β-chains but only a weak signal for the CD3 proteins. *Lane 1*: mouse serum-coupled Sepharose 4B, *lane 2*: KT3-coupled Sepharose 4B. Samples were run in 12.5% polyacrylamide gels under nonreducing (*c*) and reducing (*d*) conditions. Molecular weights (×1000) of standard proteins are indicated on the left, the expected positions of the proteins of the TCR/CD3 complex are indicated on the right side of each autoradiography.

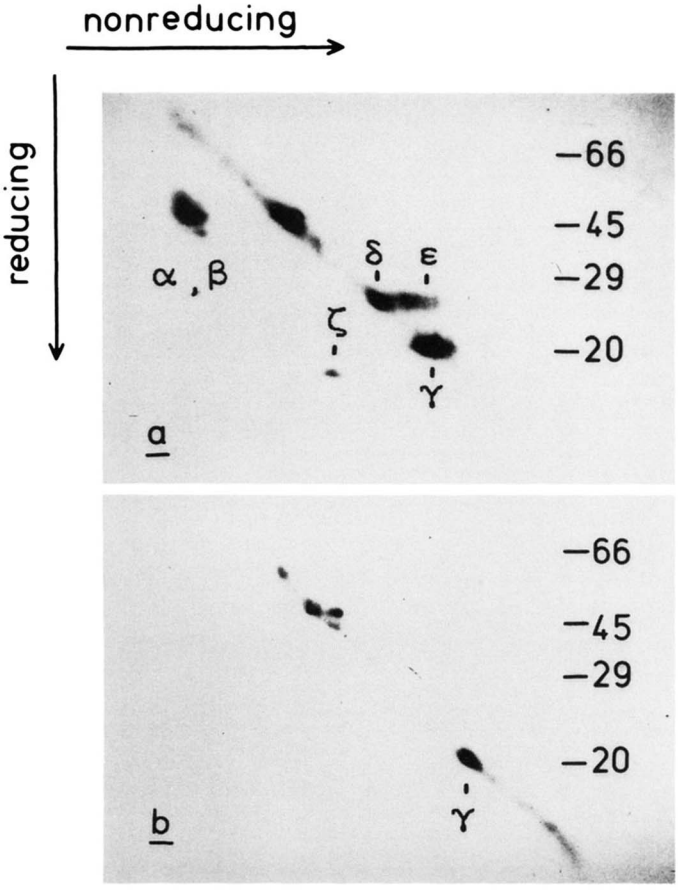


Figure 8. Immunochemical analysis with KT3 anti-CD3 mAb of the TCR/CD3 complex by two-dimensional SDS-PAGE under nonreducing and reducing conditions. The samples run represent those used for the experiment in Figure 7. (*a*) Corresponds to the sample of Figure 7*a*, *slot 4*, and (*b*) corresponds to the sample of Figure 7*a*, *slot 3*, and are from digitonin or NP-40 lysates, respectively. In the first dimension samples were run in 12.5% polyacrylamide gels under nonreducing conditions. In the second dimension they were run under reducing conditions. The expected positions of the proteins of the TCR/CD3 complex are indicated.

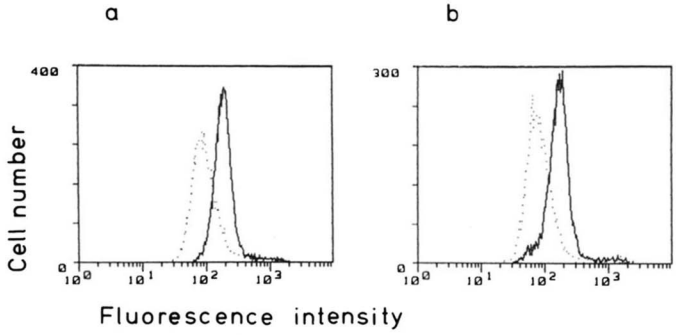


Figure 9. Flow microfluorimetry analysis of TCR-α/β expression on BVI/5 Ts cells by using the H57-597 anti TCR-α/β mAb. BVI/5 Ts cells were stained with indirect immunofluorescence. Cells were harvested and purified 5 (*a*) or 6 (*b*) days after antigenic stimulation. *Full lines*, BVI/5 Ts cells incubated with primary and secondary antibodies; *dotted lines*, incubation with secondary antibody only.

Acknowledgments. We thank Dr. M. Malissen for the kind gift of cosmids 55.1W7 and 19.1W7. We further acknowledge Drs. Z. Dembic and M. Steinmetz for their generous gift of the genomic clone CSpλ4. The KT3 mAb was a kind gift of Dr. K. Tomonari. The H57-597 anti-α/β TCR mAb was kindly given to us by Dr. R. T. Kubo.

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