Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 8638–8642, December 1985
Immunology

Rearrangement and expression of the α- and β-chain genes of the T-cell antigen receptor in functional murine suppressor T-cell clones

(virus-transformed suppressor T-cell lines/antigen-specific recognition structures)

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Communicated by Elvin A. Kabat, August 8, 1985

ABSTRACT **Two different antigen-specific radiation leukemia virus (RadLV)-transformed suppressor T-cell clones, LH8.105 and LA41, exhibiting anti-lysozyme and anti-acetylcholine-receptor suppressor activity, respectively, have been examined for rearrangement and expression of genes encoding the α and β chains of the T-cell receptor for antigen. LH8.105 cells express the T-cell-receptor polypeptides, as shown by specific immunoprecipitation. In both cell lines, potentially functional transcripts of α- and β-chain genes are detected by RNA blot analysis. These suppressor T-cell clones exhibit α-chain gene rearrangements, deletion of both alleles of the constant-region (*C*) gene segment $C_{\beta 1}$, and rearrangement of the two alleles of $C_{\beta 2}$ when analyzed by Southern blot hybridization. Restriction analysis suggests that the DNA rearrangement is beyond the second joining-region (*J*) minigene of the $J_{\beta 2}$ cluster. These results establish that at least some mouse suppressor T-cell clones, like helper and cytotoxic T lymphocytes, rearrange and transcribe the genes coding for the α and β chains of the antigen-specific T-cell receptor.**

Three different classes of T-cell-specific cDNA clones, α (1, 2), β (3, 4), and γ (5), have been isolated recently from human and mouse cDNA libraries. The α and β cDNA clones code for the α and β T-cell antigen-receptor subunits, respectively, as demonstrated by partial amino acid sequence data from the purified receptor (6, 7), whereas the protein product of the γ gene has not been identified. The function of the γ gene remains unclear, although its pattern of expression suggests a possible role during T-cell differentiation (8). The T-cell genes show sequence homology and genomic-organization similarities with immunoglobulin genes. The genomic sequences corresponding to these cDNA clones are rearranged and expressed specifically in T cells.

The genomic organization of the β and γ genes has been studied extensively. The β chain of the human and mouse antigen-specific T-cell receptor is encoded in variable (*V*), diversity (*D*), joining (*J*), and constant (*C*) gene segments. The chromosomal locus of the β-chain genes appears to contain two linked constant genes ($C_{\beta 1}$ and $C_{\beta 2}$), each associated with its own cluster of *J* genes (9–11). For the γ class, three V_γ gene segments and three J_γ–C_γ clusters have been identified in mouse DNA (12). The genomic organization of the α genes is still unknown, but the cDNA sequences suggest that V_α–J_α and possibly D_α gene segments are present and rearrange specifically in T cells to generate the transcriptionally active gene (1, 2). An obvious question is whether the three major antigen-specific T-cell subsets (cytotoxic, helper, and suppressor), which express on their membrane surface the α–β heterodimeric structure, use either $C_{\beta 1}$ or $C_{\beta 2}$ in their rearranged genes. The data, so far, suggest that in human T-cell clones the $C_{\beta 1}$ genes are rearranged and used by either cytotoxic, helper, or suppressor clones, excluding the possibility that C_β isotypes are subset-specific markers (13). The situation is not clear with regard to mouse suppressor T cells. In most suppressor hybridomas, deletions of $C_{\beta 1}$ genes were observed whereas, in others, the rearranged C_β genes lacked *V* sequences (14–16). The genes coding for the α chain of the T-cell receptor also show genomic rearrangements and are expressed in human and mouse cytotoxic and helper T cells (1, 2, 17–19), but no data are yet available for mouse suppressor cells. We have analyzed two radiation leukemia virus (RadLV)-transformed suppressor T-cell clones, LH-8.105 and LA41, specific for lysozyme and acetylcholine receptor, respectively, and detected rearrangement and expression of both T-cell receptor α- and β-chain genes.

MATERIALS AND METHODS

Cell Lines. The characterization and antigen specificity of the cell lines used in this study are given in Table 1. The specific suppressor activity of the LH8.105 cells on anti-lysozyme response was previously demonstrated, both *in vitro* and *in vivo*, by using the cell culture supernatant, the purified T-suppressor factor (20–22), or mRNA translation products (23). Similar studies have been performed to assess the suppressor activity of anti-acetylcholine-receptor response by clone LA41 (24). RadLV-VL_3 (VL_3) is a permanent T-cell line, chronically infected with highly oncogenic RadLV, and produces large amounts of infectious particles that can be recovered from the culture supernatant (25). This line was included in the study because both the LH8.105 and the LA41 lines were immortalized by RadLV-transformation.

Immunoprecipitation. LH8.105 cells were labeled with ^{125}I by the lactoperoxidase method and lysed as described (26). Radioiodinated cell lysates were incubated with antisera and immunoprecipitated with formalin-fixed *Staphylococcus aureus* (Pansorbin, Calbiochem). For each analysis, 0.1 ml of lysate (5×10^6 cell equivalents) was immunoprecipitated overnight at 4°C with 30 μl of antiserum 8177 (27, 28) (a gift of J. P. Allison) or with a normal rabbit serum. The immunoprecipitates were analyzed by $NaDodSO_4$/10% PAGE (29) under reducing conditions. After electrophoresis, the gels were fixed, dried, and exposed to Kodak X-R5 film at −70°C with an intensifying screen (Cronex Lightning Plus, DuPont).

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Abbreviations: RadLV, radiation leukemia virus; kb, kilobase(s).

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Table 1. Characteristics and functions of mouse T-cell clones

Clone	Strain	Origin	Function	Antigen specificity	Phenotype
LH8.105	C57BL/6	RadLV-transformation	Suppressor	Hen egg white lysozyme	Thy-1$^+$ Lyt-2$^+$ Ig$^-$ I-J$^+$
LA41	C57BL/6	RadLV-transformation	Suppressor	Acetylcholine receptor	Thy-1$^+$ Lyt-2$^+$ Ig$^-$ I-J$^+$
RadLV-VL_3	C57BL/Ka	X-ray induction	Unknown	Unknown	Thy-1$^+$ Lyt-1$^+$ Lyt 2$^+$
V11.5	B10.A5R/AKR	Hybridoma	Produces interleukin 2	Cytochrome *c* peptide	Unknown

RNA and DNA Blot Analysis. RNA was extracted from cell pellets as described (30) and poly(A)$^+$ RNA was obtained by fractionation on oligo(dT)-cellulose. Poly(A)$^+$ RNA (10 μg) was electrophoresed through 1% agarose gel in formaldehyde as described (31) and transferred onto nitrocellulose. High molecular weight DNA was prepared from cell pellets as described (32). DNA was digested with restriction enzymes and electrophoresed in 0.8% agarose at 40 V for 20 hr. DNA was then denatured and transferred to nitrocellulose according to Southern (33). Nitrocellulose filters for both RNA and DNA were incubated with a ^{32}P-labeled *Eco*RI DNA fragment from the 86T5 cDNA clone (4, 34) or with a ^{32}P-labeled *Eco*RI–*Eco*RV DNA fragment from the TT11 cDNA clone (1) (gifts of M. M. Davis), nick-translated to a specific activity of 2–4 $\times$ 10^8 cpm/μg. Hybridizations were performed at 65°C for 16 hr in 0.9 M NaCl/0.09 M sodium citrate, pH 7/0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% bovine serum albumin/0.1% $NaDodSO_4$/herring sperm DNA (100 μg/ml). Washings were performed at 65°C, with a final wash in 30 mM NaCl/3 mM sodium citrate, pH 7/0.1% $NaDodSO_4$ for 30 min.

RESULTS

Immunoprecipitation of the T-Cell Antigen-Receptor Protein. Fig. 1 shows the analysis of ^{125}I-labeled LH8.105 surface molecules immunoprecipitated by rabbit antiserum 8177, specific for T-cell-receptor molecules (27, 28). This antiserum precipitates, under reducing conditions, a protein of ≈42 kDa. Under nonreducing conditions, this antiserum precipitates 84-kDa molecules from ^{125}I-labeled LH8.105 cells (data not shown). Further analysis by two-dimensional gel electrophoresis under nonreducing/reducing conditions has demonstrated the expression by LH8.105 cells of surface molecules of 84 kDa, composed of two disulfide-linked subunits of 42 kDa, which are immunoprecipitated by T-cell receptor-specific antiserum (35).

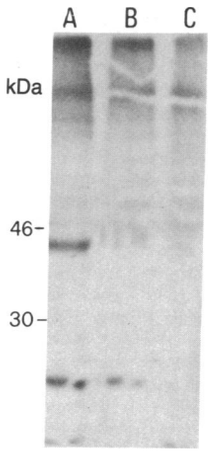

FIG. 1. $NaDodSO_4$/10% PAGE analysis of immunoprecipitates from ^{125}I-labeled LH8.105 cells. LH8.105 cell lysate was immunoprecipitated by antiserum 8177 (lane A), normal rabbit serum (lane B), or Pansorbin alone (lane C).

Gene Expression. Blot-hybridization analyses of poly(A)$^+$ RNA from T-cell lines was carried out using the TT11 α-chain cDNA clone (1) and the 86T5 β-chain cDNA clone (4, 34) as probes. Fig. 2*A* shows that clones LH8.105 and LA41, as well as the antigen-specific interleukin 2-producing hybridoma V11.5 (D. Hansburg, personal communication), express two mRNA species, of 1.3 and 1.1 kilobases (kb), that hybridize to the $C_{\beta 1}$ probe, whereas VL_3 contains only the 1.3-kb species (Fig. 2*A*). These mRNAs are absent from the SP2/0 B-cell hybridoma. The presence of two mRNA species, slightly different in size, has been reported for other T-cell lines (13, 36, 37). It has been established that the 1.3-kb transcript contains *V, D, J,* and *C* sequences encoding the complete protein, whereas the 1.1-kb mRNA does not contain *V* sequences (38). Fig. 2*B* shows that mRNA from all T-cell lines examined contains a single 1.8-kb band, absent in mRNA from the SP2/0 B-cell line, which hybridizes to the

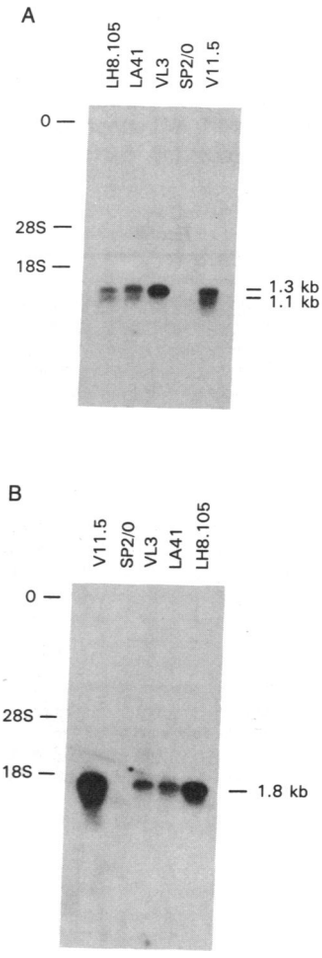

FIG. 2. Blot analysis of T- or B-cell mRNA probed with the β-chain-specific 86T5 cDNA clone *Eco*RI insert (*A*) or the α-chain-specific TT11 cDNA clone *Eco*RI–*Eco*RV insert (*B*). The SP2/0 B-cell hybridoma was used as a negative control. Ribosomal RNA (28*S* and 18*S*) and *Hae* III-digested ϕX174 DNA (not shown) were used as size markers. O, origin.

α-chain probe. The presence of two different bands of 1.3 and 1.8 kb, corresponding to a *D-J-C* and to a *V-D-J-C* transcript of the α-chain gene, respectively, has been described previously in some T-cell lines (1). From the α- and β-chain mRNA analyses, we conclude that the T-cell clones analyzed express potentially functional α- and β-chain mRNA.

Gene Rearrangements. To determine which one of the two C_β genes is expressed in these suppressor T-cell clones, DNA from these cell lines, which are of C57BL/6 origin, and from C57BL/6 liver cells were analyzed by Southern blot hybridization. Based on the restriction map of the mouse C_β locus (39), the DNA was digested with those enzymes having a cleavage site in a position that would be either changed (*Hpa* I, *Kpn* I, *Pvu* II) or maintained (*Eco*RI) after gene rearrangement (Fig. 3*B*). Southern blots were probed with the 86T5 cDNA insert containing the *C*- and *J*-region sequences. *Eco*RI digestion of C57BL/6 normal liver DNA (Fig. 3*A*, lane L) reveals the two germ-line bands of 2.5 kb ($C_{\beta 1}$) and 9.4 kb ($C_{\beta 2}$) predicted from the genomic restriction map. Since this restriction pattern would not be modified by gene rearrangement, the disappearance of the 2.5-kb band in the two suppressor clones LH8.105 (lane 3) and LA41 (lane 2) and in the VL_3 thymoma (lane 1) clearly indicates deletion of both alleles of the $C_{\beta 1}$ gene in these RadLV-transformed cell lines, whereas both *C* genes are present in the V11.5 hybridoma (lane 4).

To analyze gene rearrangement, DNA was digested with *Kpn* I, *Hpa* I, or *Pvu* II restriction enzymes. The hybridization pattern of *Kpn* I digests confirms the $C_{\beta 1}$-gene deletion in all three RadLV-transformed T cell lines (lanes 1, 2, and 3). In addition, the VL_3 thymoma shows a new single $C_{\beta 2}$ band, while the LH8.105 and the LA41 suppressor clones show two new bands. The hybridization pattern of *Hpa* I digests contains two new bands for the VL_3 line and a new broad band in both suppressor clones. Since $C_{\beta 1}$ is deleted, as demonstrated by the *Eco*RI digestion, the LH8.105, LA41, and VL_3 cell lines have both $C_{\beta 1}$ alleles rearranged. The broad band in the LH8.105 and LA41 *Hpa* I digests, as well as the single band in the *Kpn* I digests of the VL_3 clone are very likely due to poor resolution of two very close bands. *Pvu* II digestion of normal liver DNA shows a broad band, clearly containing the $C_{\beta 1}$ and $C_{\beta 2}$ bands, which were not resolved under these conditions. The hybridization pattern of the three T-cell lines definitively confirms the rearrangement of both $C_{\beta 2}$ alleles. Fig. 4 shows the Southern blot hybridization pattern in T-cell lines with the α-chain-specific probe (clone TT11, *Eco*RI–*Eco*RV insert) (1). Arrows indicate bands present in the liver germ-line configuration but absent in the T-cell lines. Although *Pvu* II and *Kpn* I restriction analyses fail to demonstrate any new band in the T-cell lines tested, their different hybridization pattern, as compared to liver DNA, suggests that there may be rearrangement of the α-chain gene.

DISCUSSION

The antigen receptor expressed by mouse helper and cytotoxic lymphocytes is composed of a disulfide-linked dimer of ≈84 kDa that is resolved by $NaDodSO_4$/PAGE, under reducing conditions into two subunits of ≈42 kDa (27, 28). The antigen-specific recognition structure expressed by suppressor T-cell clones is still controversial and although a heterogeneous array of antigen-binding soluble factors released by suppressor T cells has been described, their relationship to the cell surface receptor has not been determined.

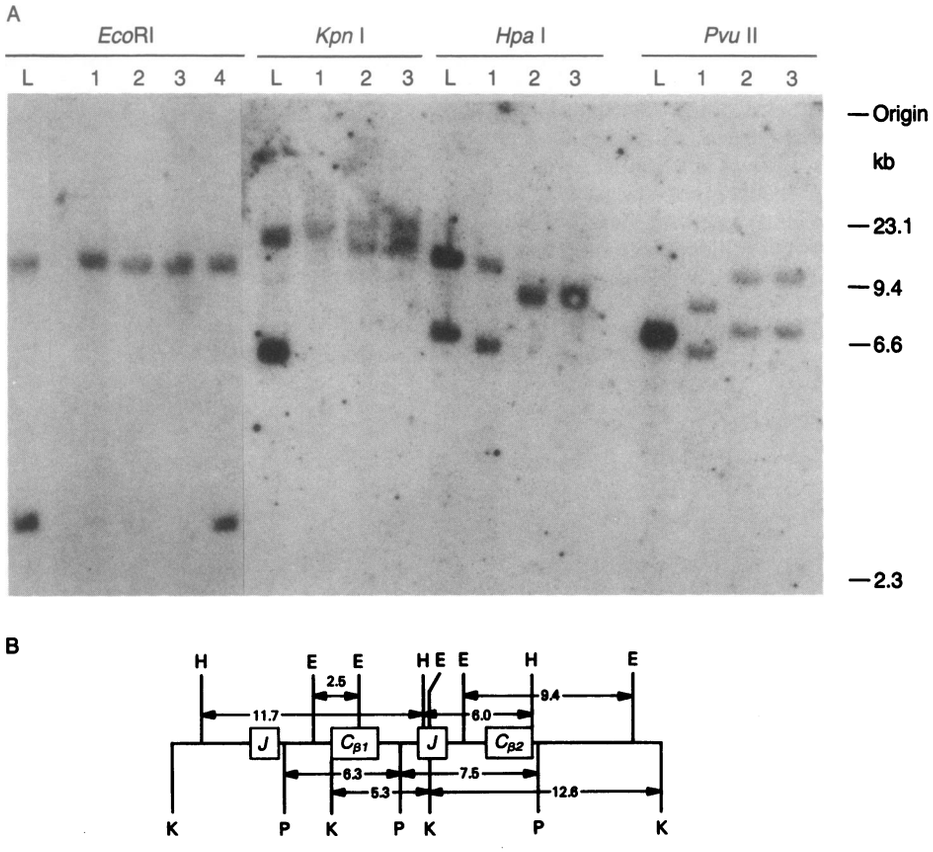

FIG. 3. (*A*) Southern blot analysis of the β-chain-specific gene rearrangement in T-cell clones. Lanes: L, liver; 1, VL_3; 2, LA41; 3, LH8.105; 4, V11.5. Markers at right represent *Hind*III-digested λ-phage DNA. (*B*) Partial restriction map of the mouse C_β locus (39). Restriction sites: E, *Eco*RI; H, *Hpa* I; K, *Kpn* I; P, *Pvu* II. Fragment sizes are indicated in kb.

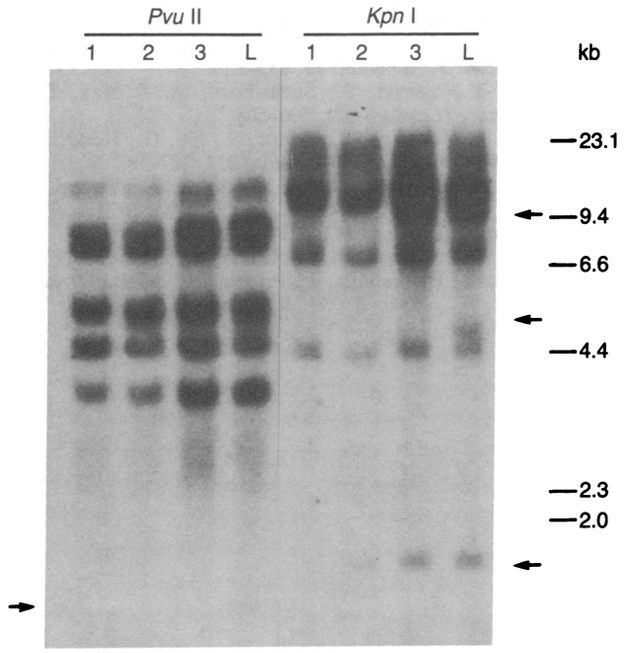

FIG. 4. Southern blot analysis of the α-chain-specific gene rearrangement in T-cell clones. Lanes: L, liver; 1, LH8.105; 2, LA41; 3, VL_3. Arrows indicate fragments detected in the liver DNA sample but absent from the T-cell samples. Markers at right represent *Hin*dIII-digested λ DNA.

We have examined two different mouse T-cell-lymphoma clones, LH8.105 and LA41, obtained by RadLV-induced transformation. These clones constitutively release, into the culture supernatant, products able to specifically suppress the immune response to antigen. Molecules of about 84 kDa composed of disulfide-linked 40–44 kDa subunits are immunoprecipitated from ^{125}I-labeled LH8.105 cells by a rabbit antiserum raised against a T-cell receptor structure (35). Therefore, LH8.105 suppressor T cells express a disulfide-linked protein similar to the receptor molecules described for helper and cytotoxic T cells.

Results in the present paper show that mouse suppressor clones express mature T-cell receptor α- and β-chain mRNAs and use the $C_{\beta 2}$ gene for their antigen receptor. The β-chain rearrangement pattern allows a partial analysis of the J segment used by these suppressor T-cell clones. The $J_{\beta 2}$ gene cluster contains six functional J segments, and the *Kpn* I site (Fig. 3*B*) is located between J_2 and J_3 minigenes (39). Since the *Kpn* I digests reveal rearrangement, it is very likely that the rearranged β-chain gene uses one of the J_3–J_6 segments in V–C joining. The rearranged bands in the two suppressor lines, LH8.105 and LA41, appear to be identical in size at this level of analysis. This might imply that the same V gene is used by both cell lines, but analysis of this point must await determination of the nucleotide sequences of the V genes.

Some mouse helper T-cell clones also rearrange $C_{\beta 2}$ genes (4, 5) and some human suppressor T-cell clones rearrange $C_{\beta 1}$ (13). Since no data on productive gene rearrangement in mouse suppressor T cells have been reported, our results provide further support to the idea that the two C_β genes are not class-specific isotypes.

The two RadLV-induced suppressor T-cell lines studied (LH8.105 and LA41) show deletion of both $C_{\beta 1}$ alleles and rearrangement of both $C_{\beta 2}$ alleles, in contrast to the deletion of the entire β-chain gene locus observed in most suppressor T-cell hybridomas (14–16).

Almost all T-cell suppressor hybridomas examined have been found to have lost both C_β genes, whereas T-suppressor clones from human (13) and mouse (this work) express a functional β-chain gene. These suppressor hybridomas, in contrast to the suppressor clones, might represent a cell subset that uses a presently unidentified receptor gene in place of the β-chain gene. Alternatively, it is possible that genotype instability in these hybridomas induces a heterogeneous hybridoma population, in which most cells have lost the β-chain gene and a small subpopulation, retaining a β-chain gene and suppressive activity, may not be detectable by molecular analysis. In conclusion, our results indicate that at least some mouse suppressor T cells use the same set of genes as helper and cytotoxic T cells for their antigen-specific receptor.

We thank Dr. M. Davis for the 86T5 and TT11 probes, Dr. J. P. Allison for the antiserum 8177, and Mrs. S. Butler for preparation of the manuscript.

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