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Rearrangement and expression of the antigen receptor α, β and γ genes in suppressor antigen-specific T cell lines

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The rearrangement and transcription of the antigen receptor α, β and γ genes were investigated in murine antigen-specific suppressor T cell lines, to establish whether the suppressor T cell subset expresses the same antigen receptor transcripts previously found in helper and cytotoxic T lymphocytes. The genomic organization of the α, β and γ chain loci was investigated using probes representative of the entire gene or fragments from variable, joining and constant regions. The present results show that in functional suppressor T cells the three antigen receptor genes are all rearranged. The β gene is expressed in all the tested cell lines, while the expression of the α and γ genes is variable. In one cell line (LH8) α and γ genes are not efficiently transcribed; in the other cell line (LA41) the γ mRNA is found in amounts similar to β mRNA, whereas the α gene is expressed at low levels. These data suggest that in suppressor T cells no direct correlation exists between the expression of α, β and γ antigen receptor genes and the effector function.

Key words: T cell antigen receptor genes/gene transcription/ suppressor murine T lymphoma cells

Introduction

T lymphocytes are a heterogeneous population of cells playing important roles in the immune system, such as the killing of cells displaying foreign antigens (cytotoxic cells, Tc) and the amplification (helper cells, Th) or inhibition (suppressor cells, Ts) of immune responses. These functions are thought to be mediated mostly by receptor molecules specifically expressed at the surface of T lymphocytes.

T cell-specific cDNA clones, which identify gene rearrangements in T lymphocytes, but not in other cell types, have been isolated from mice and humans (Chien *et al.*, 1984b; Hedrick *et al.*, 1984a, 1984b; Saito *et al.*, 1984a, 1984b; Yanagi *et al.*, 1984). The T cell-specific cDNA sequences fall into different classes. One is known to encode the α chain, whereas the second encodes for the β chain of the T lymphocyte antigen receptor. A third cDNA clone which satisfies many of the properties expected for polypeptide chains of the T cell antigen receptor, but whose function is still unknown, has also been identified (Saito *et al.*, 1984a) and recently designated as the γ gene (Hayday *et al.*, 1985; Kranz *et al.*, 1985).

T cell antigen receptor genes are structurally related to the immunoglobulin genes. Variable (V), diversity (D), joining (J) and constant (C) regions have been described (Caccia *et al.*, 1984; Chien *et al.*, 1984a, Gascoigne *et al.*, 1984; Kavaler *et al.*, 1984; Malissen *et al.*, 1984; Sims *et al.*, 1984; Siu *et al.*, 1984). A prerequisite for the expression of these T cell receptor genes is the genomic rearrangement of the several coding elements into a transcriptional unit. The occurrence of such rearrangements has been studied in all the T cell subsets. In Ts cells so far investigated, the rearrangement of the β gene has been analyzed (Hedrick *et al.*, 1985), but no data are available for the α and γ genes. Thus it is not known if this cell subset expresses a particular antigen receptor, distinct from that expressed by Th or Tc cells.

We have studied the rearrangement and transcription of the α, β and γ genes in murine antigen-specific Ts lymphomas. The results demonstrate that the three antigen receptor genes are all rearranged. However, only the β gene is always transcribed, whereas expression of α and γ genes is variable.

Results

Gene rearrangement

Two well characterized suppressor cell lines, named LH8 and LA41, were selected from our large collection of mouse T cell lymphomas, obtained by Radiation Leukemia Virus transformation of T cells from immunized C57BL/6 mice. Characterization of the two cell lines was not limited to phenotype, but also concerned the fine antigen specificity and the immunological function of the cells and their soluble products (Adorini *et al.*, 1982a, 1983a; Ricciardi-Castagnoli *et al.*, 1981, 1982a; Sinigaglia *et al.*, 1984). These products specifically suppress the antigen-induced *in vitro* T cell proliferation and *in vivo* antibody response (Adorini *et al.*, 1982b, 1983b), as well as the specific delayed-type hypersensitivity (DTH) (Adorini *et al.*, 1984). We also previously showed that disulfide-linked molecules of 42 kd were revealed by two-dimensional gel electrophoresis of radiolabeled LH8 cell surface proteins under non-reducing/reducing conditions (Ballinari *et al.*, 1985). These molecules were also immunoprecipitated by the xenoantiserum 8177, which recognizes T cell-receptor dimers on murine thymocytes (Allison *et al.*, 1982).

The genomic organization of the α, β and γ chain loci in LA41 and LH8 cells was investigated and compared with the germ line C57BL/6 configuration. Analyses were performed with the total probes representative of the V-J-C regions of each receptor chain gene (see Materials and methods). In the case of the α and γ chain genes, separate V, J and C probes were also used.

The study of the α chain gene in our Ts cells demonstrated the occurrence of somatic recombination. In fact, Southern blot analysis of DNAs from LA41, LH8 and parental C57BL/6 kidney cells, hybridized with the V_α probe, showed several common bands as well as rearranged bands present only in Ts DNAs (Figure 1). The restriction patterns obtained with *Hind*III showed a missing band of 4.4 kb in LH8 and LA41 DNAs, as well as two additional bands of 10.0 and 18.0 kb present only in LH8 DNA. Rearrangements were also detected using *Eco*RI digestions, which caused the appearance of a 4.8-kb band in both LH8 and LA41 DNAs, while *Bgl*II digestions led to the appearance

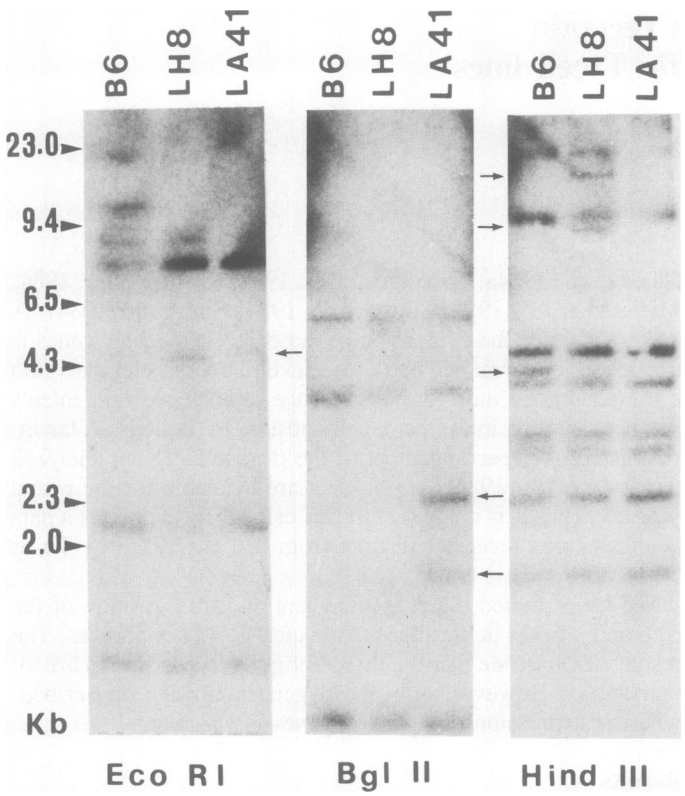

Fig. 1. Rearrangement of the α chain gene in Ts cells by Southern blot analysis. DNAs from C57BL/6 kidney (B6), LH8 and LA41 Ts cells were digested with the indicated restriction enzymes and hybridized with the V_α probe. Arrows indicate altered bands, arrowheads the mol. wt. markers (*Hind*III-digested λ DNA).

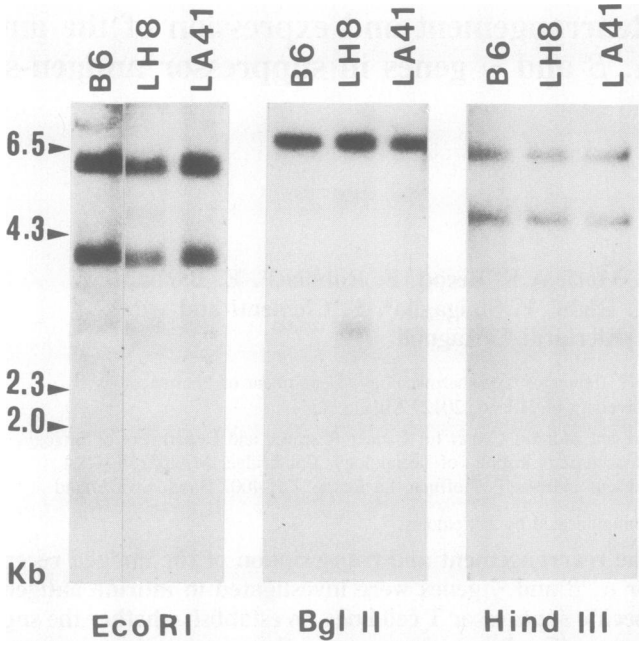

Fig. 2. Southern blot analysis of DNAs hybridized with the C_α probe. DNAs from C57BL/6 kidney (B6), LH8 and LA41 Ts cells were digested with the indicated restriction enzymes and hybridized with the C_α probe. Arrowheads indicate mol. wt. markers.

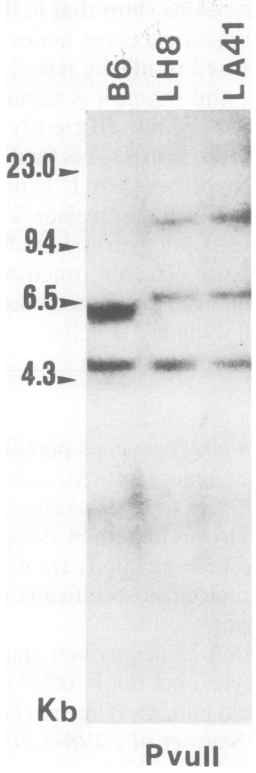

Fig. 3. Southern blot analysis of DNAs from C57BL/6 kidney (B6), LH8 and LA41 Ts cells digested with *Pvu*II and hybridized with the V-J-C_β probe. Arrowheads indicate mol. wt. markers.

of rearranged bands of 2.4 and 1.6 kb only in LA41 cells. Similar results were obtained also with a V-J-C_α probe (data not shown). On the contrary, the occurrence of rearrangement was not detected using a C_α probe after either *Eco*RI, *Bgl*II or *Hind*III digestions (Figure 2).

Southern blot analyses performed using the V-J-C probe of the β chain also demonstrated T cell-specific DNA rearrangements in both LA41 and LH8 cell lines. A common band of 4.8 kb was present in LA41 and LH8 as well as in control DNAs digested with *Pvu*II, while two rearranged bands of 7.0 and 13.0 kb appeared only in LH8 and LA41 DNA fragments (Figure 3).

Even for the γ gene our data demonstrate somatic recombination. As shown in Figure 4, compared with C57BL/6 kidney DNA, rearranged bands appeared in LH8 and LA41 DNAs digested with either *Pvu*II, *Hind*III or *Kpn*I endonucleases, and hybridized with the V-J-C_γ probe. The extent of the rearrangement of the γ gene in DNAs restricted with *Eco*RI was investigated in order to compare our data with those recently reported on BALB/c embryo and Tc cell clone DNAs (Hayday *et al.*, 1985). Southern blot analysis of C57BL/6 germ line DNA using genomic J_γ-specific probes, derived from different J_γ-C_γ clusters, detected three *Eco*RI fragments of ~17.0, 12.5 and 8.3 kb, different from those found in the germ line configuration of BALB/c mice (Hayday *et al.*, 1985). These results indicate the existence of strain polymorphism. *Eco*RI-digested DNAs from LH8 and LA41 cells hybridized either with the J_γ probes or with a C_γ probe showed only two rearranged fragments of 23.0 and 18.2 kb. The latter 18.2-kb fragment was similarly detected in LH8 and LA41 DNAs using the V_γ probe, whereas in the C57BL/6 germ line DNA two different fragments of 12.7 and 6.3 kb were found (Table I).

Gene expression

Expression of α, β and γ genes was investigated by Northern blot analysis of poly(A)$^+$ mRNAs from LH8 and LA41 compared with BW5147 T lymphoma. Figure 5 shows hybridization results obtained with the β probe. A prominent band of

1.3 kb was detected, with similar intensity in all the cell lines, showing that the LH8, LA41 and BW5147 cells expressed about the same amount of β mRNA.

Expression of the α chain mRNA was investigated using either the total probe or the separated V_α and C_α region probes. Northern blot analyses with either the V-J-C_α or C_α probes showed two specific bands of ~1.4 and 1.7 kb in BW5147. In contrast, α chain mRNA was not detected in LH8 cells, and showed a low level of transcription in LA41 cells (Figure 6a,b). As expected, no hybridization was observed between messengers from the cell lines tested and the V region of the α probe. To rule out the possibility that mRNA degradation accounted for the lack of hybridization in the LH8 and LA41 lanes, we dehybridized the blots and rehybridized them with the β chain probe, previously shown to hybridize to poly(A)$^+$ mRNAs from LH8, LA41 and BW5147 cells. A strong signal was obtained in the BW5147 as well as in the LH8 and LA41 lanes, demonstrating no degradation of these mRNAs.

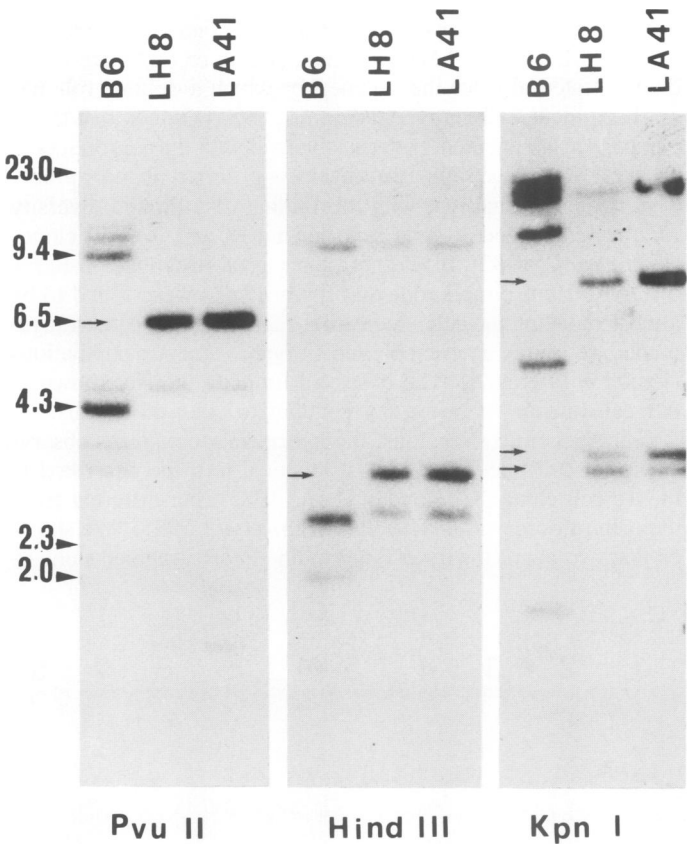

Fig. 4. Rearrangement of the γ chain gene in Ts cells by Southern blot analysis. Genomic DNAs were digested with *Pvu*II, *Hind*III or *Kpn*I restriction endonucleases. The appearance of rearranged bands, in comparison with germ line DNA (B6) in LH8 and LA41 lanes is indicated by arrows. Arrowheads indicate mol. wt. markers.

Expression of the γ chain mRNA was investigated by Northern hybridization using the total V-J-C_γ probe. Figure 6c shows a strong distinct band of ~1.7 kb in the LA41 lane, while in the LH8 and BW5147 lanes the band corresponding to this mRNA is barely visible even in a long exposure of the autoradiograph. The same result was obtained using either the V_γ (Figure 6d) or the C_γ probes (data not shown).

Taken together these data indicate that expression of receptor genes is quite variable in our Ts lines. The β gene is expressed in amounts comparable with those found in BW5147 cells, while the expression of the α chain gene is much lower in LA41, and almost undetectable in LH8 cells. In addition, the γ chain gene is transcribed to a considerable level, but only in LA41 cells (Table II).

Discussion

Rearrangement of receptor genes has been clearly documented in both Th and Tc lymphocytes (Hedrick *et al.*, 1985). Recent studies showed, however, that in Ts hybridomas the β receptor gene is not rearranged. These results suggested that suppressor cells are endowed with antigen receptors different from those expressed by Th and Tc subsets (Hedrick *et al.*, 1985; Kronenberg *et al.*, 1985).

Extensive evidence, obtained in our Ts murine cell lines, documents the somatic recombination of not only the β gene, but also the α and γ genes for which no information was yet available. In addition a variable degree of transcription of these genes has been demonstrated.

The previous data from other laboratories obtained with murine Ts hybridomas, showing deletion of the β locus derived from the Ts fusion partner, might be attributed to chromosomal loss or gene deletion, which are known to occur with high frequency in somatic hybrids. In contrast, our present data have been obtained using virally transformed lymphoma cells, having a stable karyotype and well characterized function. Our data are in agreement with the recent finding of Toyonaga *et al.* (1984) who showed a rearrangement of β genes in a human Ts clone.

Analyses with the separate V_α and C_α probes in our Ts cell lines revealed that the α chain is encoded by separate, non-

Table I. Pattern of rearrangement using different probes of the γ gene

Probes	V[a]			J1[b]			J2[b]			C[a]		
DNAs	B6	LH8	LA41	B6	LH8	LA41	B6	LH8	LA41	B6	LH8	LA41
					23.0	23.0		(23.0)	(23.0)		23.0	23.0
		18.2	18.2		(18.2)	(18.2)		18.2	18.2		18.2	18.2
				17.0			(17.0)			17.0		
Fragment size (kb)												
	12.7			(12.5)			12.5			12.5		
	6.3			(8.3)			(8.3)			(8.3)		

[a] V_γ and C_γ probes are those described in Materials and methods.
[b] $J1^\gamma$ probe is the 650-bp *Eco*RV-*Bgl*II fragment derived from the J-C 13.4 genomic clone while J2 probe is the 520-bp *Ava*I-*Ava*II fragment derived from the J-C 10.5 genomic clone (Hayday *et al.*, 1985). In brackets, fragments giving a fainter signal.

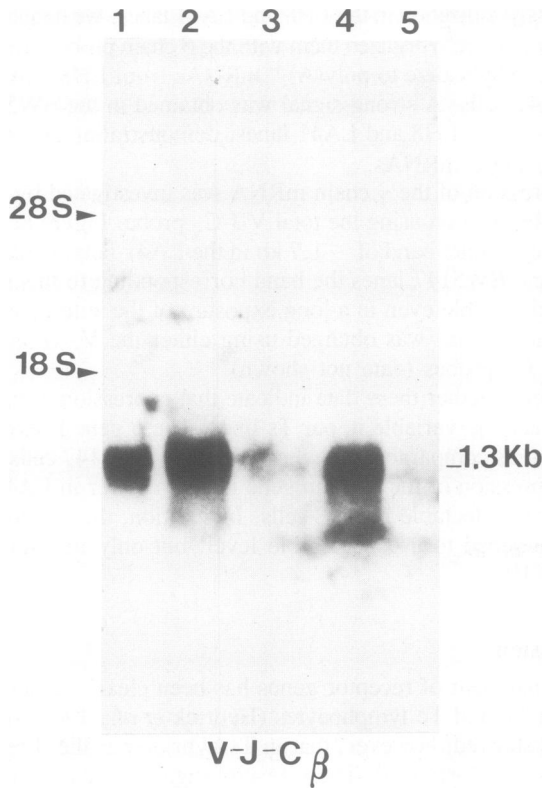

Fig. 5. Northern blot analysis with the β chain probe. 5 μg of poly(A)$^+$ RNA from BW5147 cells (**lane 1**); 5 and 0.5 μg, respectively, of LH8 poly(A)$^+$ RNA (**lanes 2** and **3**), 5 and 0.5 μg of LA41 poly(A)$^+$ RNA (**lanes 4** and**5**) were hybridized with the total β probe. Mol. wt. markers are 18S and 28S rRNA from BW5147 cells.

contiguous DNA segments, with a long intron between J and C regions containing restriction sites for *Eco*RI, *Bgl*II and *Hind*III endonucleases. In fact, while the C_α region probe was unable to detect rearrangements when the genomic DNA was digested with the above-mentioned restriction enzymes, somatic recombination was easily shown using the V_α region probe. These results are similar to those obtained recently using human T cell lines (Sim *et al.*, 1984). Thus the V_α probe we used shares extensive homology with the rearranged gene from our Ts lines, suggesting the presence of a limited number of V_α genes. The rearranged DNA fragments differed in size in LH8 and LA41 showing that the V_α gene might rearrange with different J_α segments. This could be one of the mechanisms by which the generation of a large repertoire of antigen recognition is obtained.

In both LA41 and LH8 Ts cells, somatic recombination was demonstrated also for the γ gene, for which a definite role has not been identified yet (Hayday *et al.*, 1985). In contrast to the complex rearrangement patterns obtained with the α probe, both Ts lines showed the same rearrangement pattern with the γ probe. This result is consistent with the finding of a limited diversity for the rearranged γ gene reported in Th and Tc cell clones (Kranz *et al.*, 1985). In addition, the γ gene rearranged patterns of DNAs from lymph-node and thymus cells were found to be almost indistinguishable, indicating that the γ gene rearrangements are highly restricted even in heterogeneous populations (Raulet *et al.*, 1985). The observed limited diversity shown in our cell lines is therefore not peculiar to cultured T cells.

Using genomic J_γ regions, the hybridization patterns observed in our cell lines were almost identical to those described in the Tc cell clone 2C (Hayday *et al.*, 1985), but different from the pattern observed in Th cells (Kranz *et al.*, 1985). We showed that two out of the three J_γ germ line *Eco*RI fragments under-

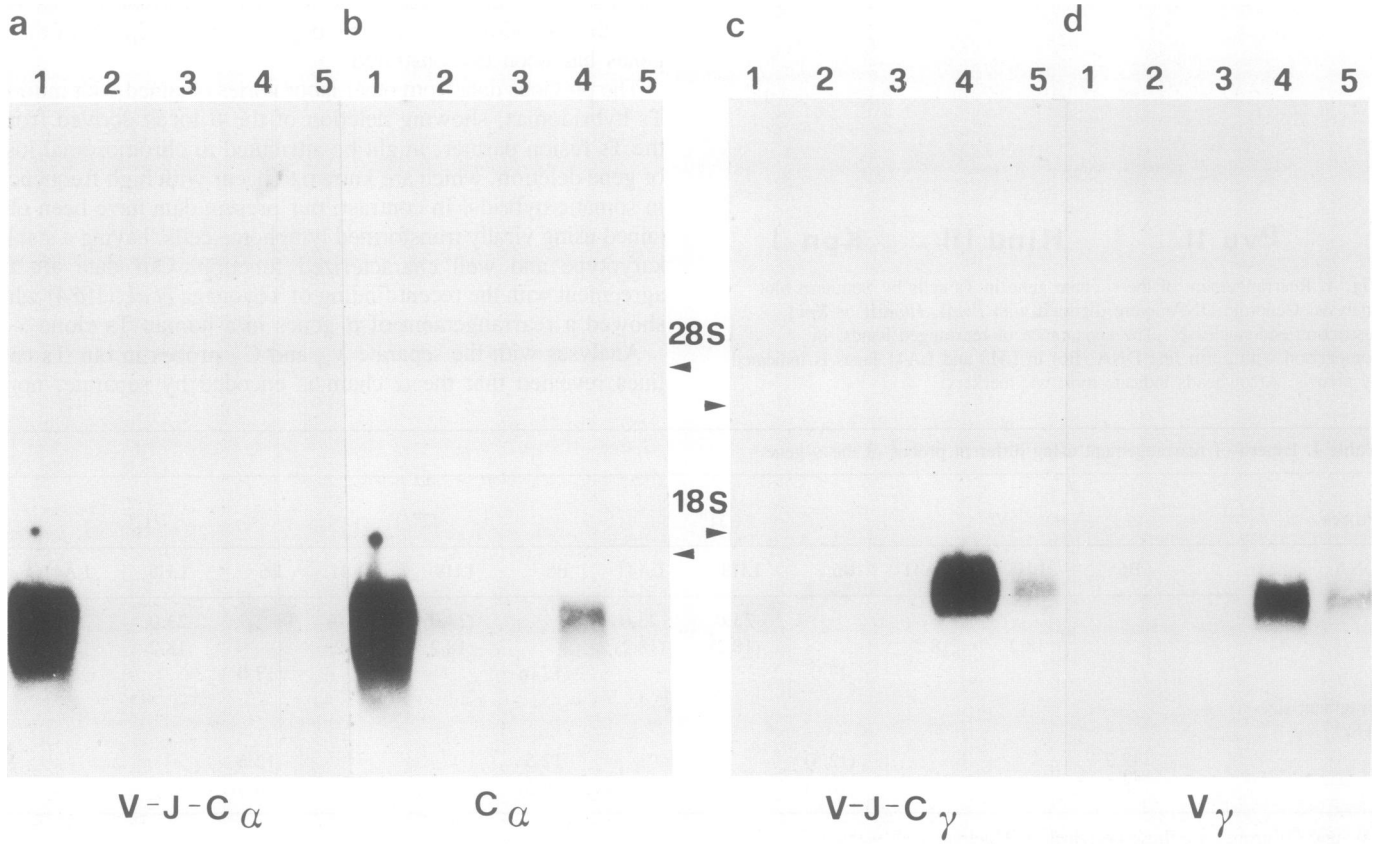

Fig. 6. Northern blot analyses of poly(A)$^+$ RNA. 5 μg of poly(A)$^+$ RNA from BW5147 cells (**lane 1**), 5 and 0.5 μg from LH8 (**lanes 2** and **3**), 5 and 0.5 μg from LA41 (**lanes 4** and **5**) were hybridized with: (**a**) the V-J-C_α probe; (**b**) the C_α probe; (**c**) the V-J-C_γ probe; (**d**) the V_γ probe.

Table II. Characteristics of the T suppressor cell lines used in the present studies

	LH8[a]	LA41[a]
Antigen receptor gene rearrangement		
α chain	+	+
β chain	+	+
γ chain	+	+
Antigen receptor gene transcription		
α chain	−	±
β chain	+	+
γ chain	−	+
Fine antigen specificity of suppressor factor[b]	+	+

[a]Surface phenotype Thy 1.2^+, Lyt 2.2^+, I-J^{b+}, sIg^-.
[b]Adorini *et al.*, 1983b; Sinigaglia *et al.*, 1984.

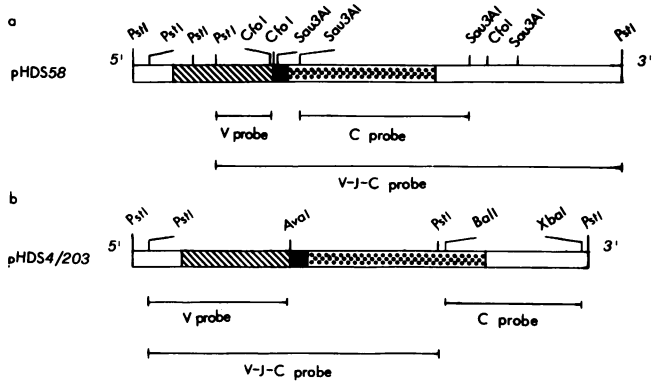

Fig. 7. Construction of C, V and V-J-C probes from cDNA clones. (**a**) pHDS58, representative of the α gene; (**b**) pHDS4/203, representative of the γ gene. Shaded, black, dotted and white areas represent respectively V, J, C and 5'- or 3'-untranslated regions.

went genomic rearrangement in both alleles, and that at least one contributed to a complete rearranged γ gene, as demonstrated by Southern analysis with the V_γ probe. The third fragment was apparently lost during somatic recombination. Thus rearrangements in these Ts and Tc clones surprisingly led to the formation of similar γ genes. Message expression of α, β and γ genes was investigated.

With the β probe a distinct band of 1.3 kb was observed with similar intensity in the tested cell lines. This 1.3-kb mRNA component [also described by Kronenberg *et al.*(1985) in BW5147 cells] represents the complete sequence of the β chain message. In contrast to the similar transcription levels observed for the β gene in our cell lines, transcription of the α and γ genes was found to be quite variable. The α chain mRNA, which is expressed in high levels in the reference BW5147 cells (Chien *et al.*, 1984b), was almost undetectable in LH8 cells regardless of the use of V_α, C_α and V-J-C_α probes. A similar result was obtained for γ gene expression in LH8 cells.

The results of message expression in LA41 cells revealed that the γ gene is transcribed in high levels in this Ts lymphoma. Expression of the γ gene has been recently demonstrated to occur in several Tc clones, and to be rare in Th cells (Kranz *et al.*, 1985). Moreover, indications suggesting changes of expression of γ mRNA level during T cell differentiation (high levels in immature, low levels in mature thymocytes) have been reported. Interestingly, the changes of α mRNA expression during differentiation of T lymphocytes were in the opposite direction with respect to γ mRNA (Raulet *et al.*, 1985). Our finding that the V_γ gene transcribed in LA41 cells is closely related to that expressed in the Tc cell clone 2C (Hayday *et al.*, 1985), confirms once more the presence of a limited number of V_γ gene segments.

In conclusion, our data demonstrate that, within the same suppressor T cell subset, transcription of the β gene can occur in association with the transcription of either the γ gene or another, unidentified gene. These data suggest that in Ts cells no direct correlation exists between the expression of α, β and γ genes and the effector function. The mechanisms by which the expression of T cell receptor genes is controlled and the physiological meaning of the different gene expression are still unclear and need further investigation.

Materials and methods

Cell lines

The characterization of LH8 and LA41 cell lines was described in previous publications (Ricciardi-Castagnoli *et al.*, 1981; Sinigaglia *et al.*, 1984). Briefly, antigen-specific suppressor lymphoma cells were cloned and their suppressive activity tested in several immune assays, such as the antigen-specific *in vitro* proliferation assay, the *in vivo* antigen-induced antibody production (Adorini *et al.*, 1983a), and the DTH response (Adorini *et al.*, 1984). Immunosuppression was also shown by cell-free translated products from LH8 mRNAs (Ricciardi-Castagnoli *et al.*, 1985). The fine antigen-specificity of the LH8 suppressor factor is for hen egg-white lysozyme (Ricciardi-Castagnoli *et al.*, 1982b) while that of LA41 cells is for *Torpedo* acetylcholine receptor (Sinigaglia *et al.*, 1984). BW5147 lymphoma cells are T cells widely used in many laboratories; they were chosen as a positive control for expression of α and β genes (Chien *et al.*, 1984a; Kronenberg *et al.*, 1985). All cell lines were grown in RPMI 1640 medium containing 10% fetal calf serum, 2×10^{-5} M 2-mercaptoethanol and 2 mM L-glutamine, at 37°C in a humidified 5% CO_2 incubator. For DNA or RNA extractions cells were grown at high density in Petri dishes.

Isolation of RNA

Cellular RNA was isolated by modification of the method of Glisin *et al.* (1974). A pellet of 5×10^8 cells was lysed with 20 ml of lysis buffer (4 M guanidine isothiocyanate, 25 mM sodium citrate, pH 7.6, 0.5% N-lauroylsarcosine and 100 mM 2-mercaptoethanol). The lysate was layered over a 15 ml cushion of 5.7 M CsCl, 0.1 M EDTA, and centrifuged at 20°C for 16 h at 22 000 r.p.m. in a Beckman SW27 rotor. After centrifugation, the supernatant was removed by suction and the tube was inverted to drain the remaining liquid. The RNA pellet was dissolved in diethylpyrocarbonate (DEP) treated water, and precipitated by adding in sequence 0.1 volumes of 2 M sodium acetate (pH 5.2) and 2.5 volumes of ethanol. After precipitation at −80°C the RNA was recovered by centrifugation and dissolved in DEP water; its optical density at 260 nm was then determined. Poly(A)$^+$ mRNA was separated by affinity chromatography on oligo(dT) (Collaborative Research) columns. The binding buffer was 0.01 M Tris-HCl, pH 7.5, 0.5 M NaCl, 0.5% SDS, 1 mM EDTA while the elution buffer was the same without NaCl. Poly(A)$^+$ mRNA was then precipitated as described above.

Electrophoresis, blotting and hybridization of RNA

Aliquots (0.5 or 5 μg) of poly(A)$^+$ mRNA were resolved by electrophoresis in 1% (w/v) agarose containing 2.2 M formaldehyde, 0.2 M morpholinopropanesulfonic acid (MOPS), pH 7.0, 50 mM sodium acetate, 1 mM EDTA, pH 8.0, according to Maniatis *et al.* (1982). RNA was transferred for 16 h to Biodyne A nylon membranes (Pall, Glencover, NY) in 20 x SSC (3 M NaCl, 0.3 M sodium citrate, pH 7.0). The filters backed for 1 h at 80°C and then pre-hybridized at 42°C for 1 h in hybridization buffer (50% v/v formamide, 5 x SSC, 5 x Denhardt solution, 50 mM sodium phosphate, pH 6.5, and 0.1% SDS) containing 250 μg/ml sonicated and heat-denaturated salmon sperm DNA. Hybridization was performed at 42°C for 48 h in the hybridization buffer containing ~ 10^6 c.p.m./ml of thermally denatured probe. The probes were obtained from DNA fragments by nick-translation, using [^{32}P]dCTP and commercial BRL kit reagents (Bethesda Research Laboratories, MD). Prior to autoradiography, filters were washed at room temperature for 20 min with 2 x SSC and 0.1% SDS, then at 50°C for 30 min with 0.1 x SSC and 0.1% SDS. Dried filters were exposed to X-ray films at −80°C for 4 − 24 h in the presence of intensifying screens. Long exposures were carried on for at least 1 week.

Isolation, electrophoresis and hybridization of DNA

DNA was extracted from cell culture pellets or kidney tissue by the phenol-chloroform method (Blin and Stafford, 1976). Restriction endonuclease-digested DNA was separated by size on 0.8% agarose gel (w/v) in 0.04 M Tris acetate, 0.02 M EDTA. The gel was denatured with 0.5 M NAOH and 1.5 M NaCl for 30 min and neutralized with 3 M sodium acetate, pH 5.5, for 30 min at room temperature. DNA was transferred overnight to Biodyne A nylon membrane in 20 x SSC and then backed for 1 h at 80°C. Pre-hybridization, hybridization with the radioactive probe and washings of the blots were performed by the procedures described above for RNA hybridization.

Probes

The pHDS58 cDNA clone representative of the α gene, and the pHDS11 cDNA clone representative of the β gene were generously provided by Dr. S. Tonegawa (MIT, Cambridge, MA). Plasmid DNAs were prepared by alkali lysis as described by Maniatis *et al.* (1982) and CsCl-ethidium bromide equilibrium centrifugation. The total (V-J-C) probes from each plasmid clone were obtained by *Pst*I restriction endonuclease digestions and separated by size on 1% agarose gels. Further digestion of the insert of the pHDS58 clone with *Cfo*I allowed us to obtain the 148-bp fragment representative of the V_α region, while a constant, transmembrane and cytoplasmic region probe of 497 bp (C_α) was obtained after *Sau*3AI digestion of the insert (Figure 7a). The V_γ probe was the 396-bp *Pst*I-*Ava*I fragment and the C_γ probe was the 387-bp, *Bal*I-*Xba*I fragment from the insert of the pHDS4/203 $^\gamma$cDNA clone (Figure 7b). The J_γ genomic probes J-C 13.4 and J-C 10.5 are described in Table I.

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