Immunological Reviews 1991, No. 120

Published by Munksgaard, Copenhagen, Denmark

Recognition of MHC TL Gene Products by $\gamma\delta$ T Cells

LUC VAN KAER*, MIN WU*, YOSHIAKI ICHIKAWA*, KOUICHI ITO*+, MARC BONNEVILLE*¶, SUZANNE OSTRAND-ROSENBERG&, DONAL B. MURPHY§ & SUSUMU TONEGAWA*

INTRODUCTION

In the immune system of vertebrates, lymphocytes recognize antigen via cell-surface immunoglobulin (Ig) and T-cell receptors (TCR). The most intriguing property of these molecules is their structural variability, much of which originates from the ability of the gene segments encoding these receptors to undergo somatic DNA rearrangements (Tonegawa 1983, Davis & Bjorkman 1988). All TCR were originally thought to be disulfide-linked heterodimers composed of variable α and β chains, which are associated with the non-variable CD3 complex. However, the search for genes encoding TCR α and β chains revealed a third T cell-specific gene, γ, which also undergoes somatic rearrangement during T-cell differentiation (Saito et al. 1984). The discovery of the γ gene led to the identification of a second TCR which is a heterodimer composed of the variable γ chain disulfide-linked to a fourth variable δ chain (Brenner et al. 1986, Lew et al. 1986). The $\gamma\delta$ TCR is also associated with the CD3 complex. Lymphocytes expressing $\gamma\delta$ TCR appear before those expressing $\alpha\beta$ TCR during thymic ontogeny (Bank et al. 1986, Lew et al. 1986, Nakanishi et al. 1987, Itohara et al. 1989), but represent only a minor

*Howard Hughes Medical Institute at Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139;
&University of Maryland, Baltimore County Campus, Department of Biological Sciences, Baltimore, Maryland 21228-5398;
§The Wadsworth Center, New York State Department of Health, Empire State Plaza, Albany, New York 12201, U.S.A.
+Present Address: Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki-ku, Kanagawa 210, Japan.
¶Present Address: Laboratoire Immunologic Clinique, INSERM U211, 44035 Nantes Cedex, France.
Correspondence: Susumu Tonegawa, Howard Hughes Medical Institute at Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Room E17-353, 40 Ames Street, Cambridge, Massachusetts 02139, U.S.A.

population of T cells in adult thymus and peripheral lymphoid organs (< 5%) (Bluestone et al. 1987, Borst et al. 1988, Itohara et al. 1989). However, $\gamma\delta$ T cells predominate (50–100%) in mouse epidermis (Koning et al. 1987, Kuziel et al. 1987), in epithelial layers of the small intestine (Bonneville et al. 1988, Goodman & Lefrancois 1988), in lung (Augustin et al. 1989), and in female reproductive organs (Itohara et al. 1990). Particularly intriguing is the observation that $\gamma\delta$ T cells that reside in these different epithelial locations compose distinct subsets with different TCR repertoires and diversity. Thus, most T cells associated with mouse epidermis (dendritic epidermis cells, DEC, or skin-associated intraepithelial lymphocytes, s-IEL) bear homogeneous TCR encoded by V_5 γ and V_1 δ gene segments (Asarnow et al. 1988; see Heilig & Tonegawa 1987 for the nomenclature of mouse γ gene segments, and Elliott et al. 1988 for the nomenclature of δ gene segments), intraepithelial lymphocytes of the gut (i-IEL) bear diverse TCR that are preferentially encoded by V_7-containing γ genes (Bonneville et al. 1988, Asarnow et al. 1989), and $\gamma\delta$ cells associated with the mucosal epithelia of uterus, vagina, and tongue (vut-IEL) use homogeneous TCR encoded by V_6 γ and V_1 δ genes (Itohara et al. 1990).

To elucidate the biological role of $\gamma\delta$ T cells, it is essential to obtain information on the ligands that they recognize. In this review, we will summarize our recent studies on the role of MHC TL region-encoded class I molecules as ligands for $\gamma\delta$ T cells and discuss these findings with respect to the possible functions of $\gamma\delta$ T cells.

1. THE KN6 $\gamma\delta$ TCR RECOGNIZES A TL REGION-CONTROLLED DETERMINANT

In order to study the ligand specificity of $\gamma\delta$ T cells, we prepared hybridomas from fetal and adult C57BL/6 thymocytes expressing a variety of $\gamma\delta$ TCRs (Ito et al. 1989). These hybridomas were first tested for their ability to respond when their TCRs were crosslinked. It was previously shown that crosslinking of TCR $\alpha\beta$ usually induces the proliferation of normal T cells, while the same stimulus promotes growth inhibition in hybridomas (Ashwell et al. 1987). Thus, hybridomas were cultured with Fc receptor-positive A-20 B-lymphoma cells in the presence of anti-CD3 mAb and growth inhibition was measured by incorporation of [^{3}H]-thymidine into the DNA of the hybridomas. Hybridomas that tested positive in this assay were then screened for their reactivity with stimulator cells from various origins. We identified one hybridoma, KN6, containing rearranged $V_4J_1C_4$ γ and $V_3D_1D_2J_1C$ δ genes, whose growth was inhibited by syngeneic (C57BL/6) but not by allogeneic (BALB/c) cells (Bonneville et al. 1989). KN6 growth was inhibited efficiently by thymocytes, splenocytes, and peritoneal exudate cells. The response to syngeneic cells was observed in all CD3-positive subclones of the KN6 hybridoma but not in CD3-negative subclones, indicating that growth inhibition was

mediated by an interaction with the KN6 γδ TCR. Further evidence for this came from the observation that KN6 growth inhibition could be specifically reversed by antibodies directed against the KN6 γδ TCR (Bonneville et al. 1989). Finally, genes encoding the γ and δ chains of hybridoma KN6 were cloned and introduced into the germline of mice (Ishida et al. 1990). Most of the γδ T cells in these animals expressed the KN6 γδ TCR and proliferated in mixed lymphocyte culture in response to C57BL/6 but not BALB/c cells (Table I; Bonneville et al. 1990). These data formally prove that KN6 recognition of syngeneic C57BL/6 cells is controlled by γ and δ TCR genes.

Specificity of the KN6 γδ TCR was further examined by analyzing the ability of peritoneal exudate cells from a variety of mouse strains to stimulate KN6 proliferation. Three levels of proliferative response were observed: strong (e.g. strains C57BL/6, 129, and SM), intermediate (e.g. strain AKR), or no response (e.g. strain BALB/c) (Table I). Most strains tested in this assay clearly stimulated a KN6 response, demonstrating that the KN6 ligand is expressed in many different mouse strains. This suggested that the KN6 ligand is not very polymorphic and is therefore not encoded by any of the highly polymorphic class I or class II genes. A more likely candidate was one of the large number of low-polymorphic class I genes of unknown function that map in the Q, TL, or M (formerly called Hmt) regions of the H-2 gene complex (Fischer Lindahl et al. 1989, Flaherty et al. 1990). That the KN6 ligand is controlled by a gene linked to the MHC was demonstrated by results with congenic and recombinant strains (Table I). In particular, data with recombinants B6-*Tla*^{a} and A-*Tla*^{b} (Fig. 1.A) demonstrated

TABLE I

The KN6 ligand is expressed in peritoneal exudate cells from various strains of mice and is controlled by the MHC

Stimulator cells*	KN6 proliferative response
C57BL/6 (H-2^b), C57BL/10 (H-2^b), 129 (H-2^b)**, C3H.SW (H-2^b), SM (H-2^v)	strong
A (H-2^a), B10.A (H-2^a), A.CA (H-2^f), B10.M (H-2^f), AKR (H-2^k), CBA (H-2^k), C3H (H-2^k), B10.BR (H-2^k), P (H-2^p), B10.G (H-2^q), B10.RIII (H-2^r), B10.S (H-2^s), B10.PL(73NS) (H-2^u)	intermediate
BALB/c (H-2^d), B10.D2 (H-2^d)	none

Approximately 10^4 CD4$^-$, CD8$^-$ spleen T cells (>95% KN6 γδ TCR$^+$ and no detectable αβ cells) from KN6 transgenic mice (H-2^d) were cultured with irradiated (1500 rads) peritoneal exudate cells from the indicated strains of mice. After 72 h of culture cells were pulse-labeled with [^{3}H]-thymidine (1 μCi per well) for 6 h.

*The H-2 haplotype origin of the different strains of mice is indicated in parentheses.

**Strain 129 is usually listed as H-2^b but differences in the expression of TL determinants relative to the strains C57BL/6 and C57BL/10 have been observed (Shen et al. 1982).

that the KN6 ligand is controlled by a gene mapping distal to the Q region, and results with recombinant strain MA.R1 (Fig. 1.B) showed further that this gene maps proximal to the M region gene *Pgk*-2. Together with the molecular data below, this places the gene controlling the KN6 ligand in the TL region.

2. THE GENE ENCODING THE KN6 LIGAND IS A NOVEL TL^b CLASS I GENE (*T22*^{b})

In order to further characterize the gene controlling the KN6 ligand, we searched for cell lines that activate KN6 proliferation. Among other cell lines, KN6 cells responded to an embryonic carcinoma (EC) cell line, PCC3 (Bonneville et al. 1989, Ito et al. 1990). This is particularly interesting since it has been shown that PCC3 cells do not express any detectable or significant levels of class I H-2K, -D, or -L molecules (Maher & Dove 1984) or mRNA (Morello et al. 1982), respectively, and at least some EC cell lines are known to express TL region class I-like genes (Stern et al. 1986, Ostrand-Rosenberg et al. 1989), including PCC3 (Oudshoorn-Snoek & Demant 1990). This confirmed our previous data that KN6 cells recognize a determinant distinct from the classical MHC class I molecules. In addition, this result suggested that PCC3 cells would be a good source for

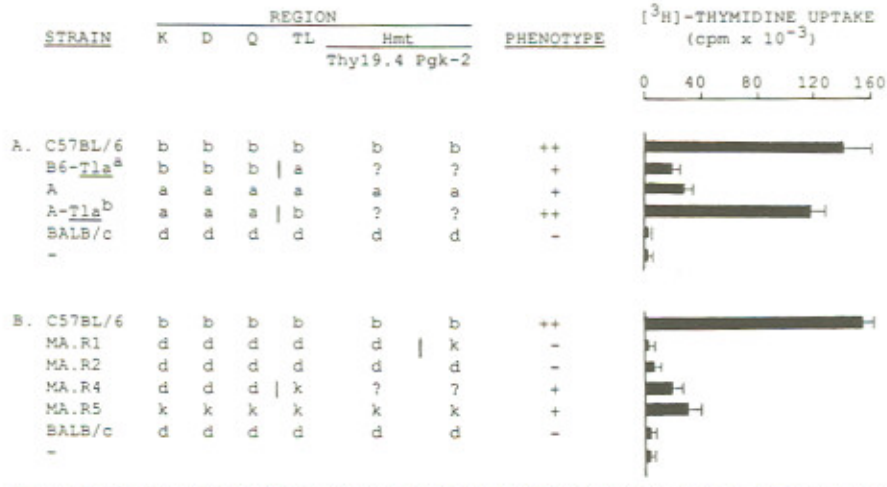


Figure 1. The ligand recognized by KN6 is controlled by the H-2 TL region. Approximately 10^4 CD4$^-$, CD8$^-$ spleen T cells from KN6 transgenic mice were cultured with 2.5×10^4 irradiated (1500 rads) peritoneal exudate cells from the listed strains of mice. Cells were cultured for 3 d and then pulsed with [^{3}H]-thymidine (1 μCi/well) for 6 h. The data are expressed as mean cpm and standard deviations of triplicate experiments. KN6 stimulatory responses are classified into three types: ++, strong response; +, intermediate response; and −, no response detected. Crossover positions in the recombinant strains are indicated by vertical bars. The Hmt region is now referred to as the M region.

construction of a cDNA library to isolate a cDNA encoding the KN6 ligand. Such a library was constructed and screened with a probe, pH2-IIa (Steinmetz et al. 1981), that hybridizes with exon IV of all known class I genes (Weiss et al. 1984). Eight cDNA clones were isolated and sequenced. Five different types of cDNA were identified (Table II): two types of clone corresponded to known Q region transcripts, the third type exhibited a high degree of nucleotide sequence homology with Q region sequences and therefore most probably corresponds to a novel Q region transcript, and the remaining two types of clone (clones PCC3-4 and -27, hereafter referred to as PCC3-4/27, and clone PCC3-24) were highly homologous (>88%) with other class I sequences in the fourth exon, but differed substantially elsewhere from all known class I sequences (Ito et al. 1990). Since this pattern of nucleotide sequence homology is expected for genes encoded by the TL region (Flaherty et al. 1990), we speculated that these cDNAs most likely are derived from hitherto unsequenced TL region genes. The two types of cDNA clone were 96% homologous in the coding regions.

Formal proof that the PCC3-4/27 cDNA encodes the KN6 ligand came from studies with mouse L cells transfected with plasmids containing cDNA under the control of the metallothionein promoter. Stably transfected cells were tested for their KN6-stimulatory capacity. L cells transfected with PCC3-4 or PCC3-27 were recognized by KN6, whereas non-transfected cells, vector-transfected cells, or PCC3-24 transfectants were not (Fig. 2). This interaction could be inhibited in a dose-dependent fashion by preincubation of the responder KN6 cells with anti-KN6 mAb (Ito et al. 1990), demonstrating that it is γδ TCR-mediated.

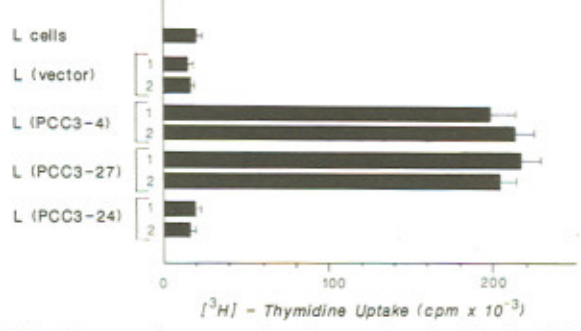
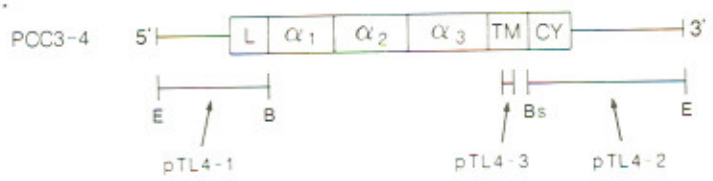


Figure 2. KN6 γδ T cells recognize mouse L cells transfected with the PCC3-4 and PCC3-27 cDNA clones. Approximately 10^4 responder cells from a short-term KN6 γδ T-cell line (>99% KN6 γδ T cells) were cultured with 2.5×10^4 irradiated (3000 rads) L cells or L cell transfectants. For each transfectant the result for two independent clones is presented. KN6 proliferation was assessed by the incorporation of [^{3}H]-thymidine (1 μCi/well) into the DNA of the responder cells. The data are presented as mean cpm and standard deviations of triplicate samples.

A.



B.

C.

STRAIN	REGION (K D Q TL Hmt Tpx-1)	FRAGMENT LENGTH (kb) pTL4-2	pTL4-1
C57BL/6		3.0, 2.5	4.7, 4.3
A/Boy		2.7	-
C3H		-	4.7, 4.3
C.SW		-	4.7, 4.3
B6.CAS3		-	5.1
A-Tla^b/Boy		3.0, 2.5	-
B6-Tla^a/Boy		2.7	-
CAS3(R9)		-	5.1
CAS3(R4)		-	4.7, 4.3

The TL region of strain 129/Sv, from which the PCC3 cell line was derived, has not been characterized at the molecular level. This strain is usually listed as H-2^b but differences have been observed in expression of TL region determinants in these mice relative to strains C57BL/6 and C57BL/10 (Shen et al. 1982). To isolate the C57BL/6 counterpart of PCC3-4/27, a cDNA library from this strain was screened with a probe (pTL4-2, see Fig. 3.A) that hybridized only with the PCC3-4/27 and PCC3-24 clones among all class I cDNAs isolated from PCC3 cells. Two types of cDNA were isolated and a representative clone of each type (clone B6-6 and clone B6-8) was sequenced.

Clone B6-8 was highly homologous to PCC3-24 (Table III), indicating that it is the C57BL/6 counterpart of this cDNA clone. However, B6-8 contains an 11 bp duplication in exon III, causing a frameshift and premature termination of the putative polypeptide product, and therefore cannot encode a complete class I molecule (Ito et al. 1990). Hybridization studies with cosmid clones that contain TL region class I genes previously isolated from C57BL/10 DNA and nucleotide sequencing showed that cDNA clone B6-8 corresponds to *T10*^{b} (Ito et al. 1990).

Clone B6-6 differed from PCC3-4/27 only in a single nucleotide, which did not cause an amino acid substitution (Table III, and Ito et al. 1990). Thus, the KN6 ligands in strains 129/Sv and C57BL/6 are identical at the amino acid level. Hybridization studies with pTL4-1 and pTL4-2 probes (Fig. 3.A) and cosmids containing 13 of the 15 previously described TL^b region class I genes (*T1*^{b}-*T13*^{b}, see Fig. 4) (Weiss et al. 1984, Brown et al. 1988) suggested that the gene encoding the KN6 ligand was unique. Although *T14*^{b} and *T15*^{b} genes were not available for this analysis, it is unlikely that either encodes the KN6 ligand because the *T14*^{b} gene is known to be defective and a *T15*^{b}-derived probe did not hybridize with *T10*^{b} (Brown et al. 1988). The gene encoding the KN6 ligand gene was isolated by screening a newly prepared C57BL/6 cosmid library with an oligonucleotide probe, pTL4-3 (Fig. 3.A), that hybridizes with the KN6 ligand gene but not with the closely related *T10*^{b} gene. We isolated one cosmid, mapped the ligand gene within the cosmid, and determined its nucleotide sequence. As expected, the

---

Figure 3. Restriction fragment length polymorphism (RFLP) analysis maps the *T22*^{b} gene in the H-2 TL region. (A) Schematic illustration of hybridization probes pTL4-1 and pTL4-2 used for the RFLP mapping, and probe pTL4-3, used for the cloning of gene *T22*^{b}. L, leader peptide; α_1, α_2, and α_3, three extracellular domains; TM, transmembrane domain; CY, cytoplasmic domain. Restriction sites: B, *Bam*HI; Bs, *Bsp*MI; E, *Eco*RI.

(B) Southern blot analysis of genomic DNA from parental and H-2 recombinant strains. *Bam*HI-digested genomic DNA isolated from the thymus of the indicated strains of mice was analyzed with the pTL4-2 (left panel) or pTL4-1 (right panel) probes.

(C) Schematic representation of the H-2 region carried by recombinant and parental strains, and summary of the detected DNA fragments. The Hmt region is now referred to as the M region.

TABLE II
Class I transcripts in PCC3 embryonic carcinoma (EC) cells

Clone	Identity	Full length?
PCC3-30 PCC3-116 PCC3-122	1 nucleotide difference with $Q7^b$ in the exons	no
PCC3-8	identical to $Q8^b$ in the exons	no
PCC3-10	a novel Q region gene transcript?	no
PCC3-4 PCC3-27	a novel TL region gene transcript?	yes
PCC3-24	a novel TL region gene transcript?	yes

sequence was identical to the B6-6 sequence in its exons. This novel TL^b region class I gene was originally designated 27^b (Ito et al. 1990), but under the newly proposed H-2 class I gene nomenclature it is now called $T22^b$ (Fig. 4, and Klein et al. 1990).

To demonstrate unambiguously that the $T22^b$ gene maps within the TL region, Southern blot analysis was conducted with genomic DNA from several MHC recombinant strains. Based on detection of restriction fragment length polymorphisms (RFLPs) with specific hybridization probes (pTL4-1 and pTL4-2, see Fig. 3.A), data with recombinant strains A-Tla^b/Boy and B6-Tla^a/Boy show that $T22^b$ maps distal to the Q region, and data with recombinants B6.CAS3(R9) and B6.CAS3(R4) show further that the gene maps proximal to the M region, within the TL region.

3. THE KN6 LIGAND GENE BELONGS TO A NOVEL TL^b GENE CLUSTER

In the BALB/c mouse, two clusters of TL region genes, TL^d-A ($T1^d$-$T11^d$) and TL^d-B ($T16^d$-$T23^d$) (Fig. 4; Fisher et al. 1985, Transy et al. 1987) were previously identified. The TL^d-A and TL^d-B clusters are related by duplication. The TL^b-A but not the TL^b-B counterpart had been detected in strain C57BL/6 (Weiss et al. 1984). It was previously shown that genes $T10^b$ and $T10^d$ are allelic (Brown et al. 1988), and $T10^d$ and $T22^d$ are believed to be related by duplication (Fisher et al. 1985). Since $T22^b$ and $T10^b$ are closely related, we suspected that $T22^d$ may be the BALB/c allele of $T22^b$. We sequenced a part of both $T10^d$ and $T22^d$ and isolated and sequenced cDNAs (represented by clones BALB-1 and BALB-2) corresponding to these genes. Clone BALB-1 was identical to gene $T22^d$ in its exons and differed by only 5 nucleotides from the B6-6 cDNA (which is identical to gene $T22^b$ in its exons) (Table III). However, the BALB-1 cDNA contained a small deletion at the start of exon II, which leads to a frameshift and premature

TABLE III

Number of nucleotide differences among PCC3-4/27-related cDNA clones

	PCC3-4/27 ($T22^{129}$)	PCC3-24 ($T10^{129}$)	B6-6 ($T22^{b}$)	B6-8 ($T10^{b}$)	BALB-1 ($T22^{d}$)	BALB-2 ($T10^{d}$)	SM-1 ($T22^{v}$)	AKR-3 ($T22^{k}$)
PCC3-4/27 ($T22^{129}$)	0							
PCC3-24 ($T10^{129}$)	43	0						
B6-6 ($T22^{b}$)	1	42	0					
B6-8 ($T10^{b}$)	43	8	42	0				
BALB-1 ($T22^{d}$)	6	45	5	45	0			
BALB-2 ($T10^{d}$)	23	46	22	43	23	0		
SM-1 ($T22^{v}$)	1	42	0	42	5	22	0	
AKR-3 ($T22^{k}$)	4	45	3	45	8	25	3	0

Only the protein-encoding region was compared. Deletions and duplications (see Ito et al. 1990; and Ichikawa et al., in preparation) were not counted as nucleotide differences. The designations (following the new nomenclature proposed by Klein et al. 1990) for the genes corresponding to the cDNAs are indicated in parentheses.

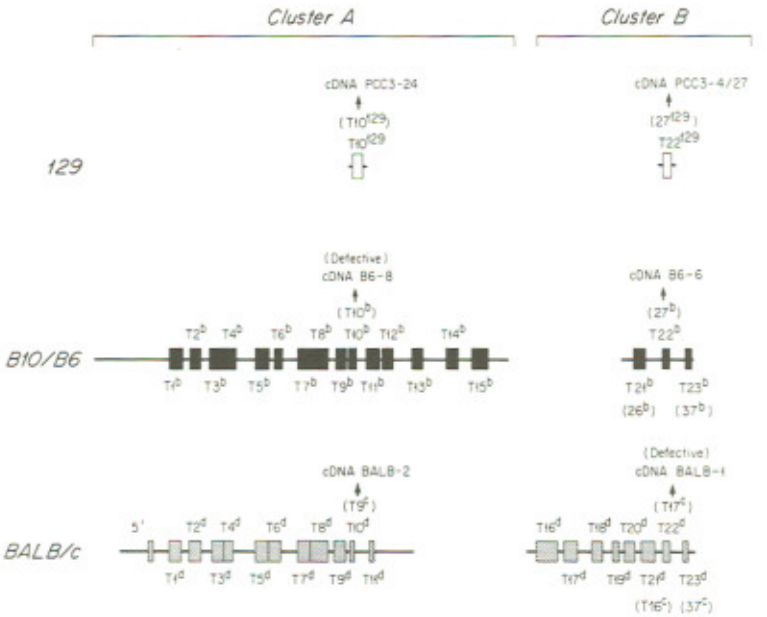


Figure 4. TL region genes in 129, C57BL/10(6) and BALB/c mice. The newly identified TL region gene cluster of strain C57BL/10(6), cluster TL^b-B, is indicated along with previously identified TL gene clusters in C57BL/10(6) and BALB/c. The designation for the genes follows the nomenclature proposed by Klein et al. (1990), old designations for the genes discussed in this paper are indicated in parentheses. The cDNA clones described in this study are also shown.

termination of translation (Ito et al. 1990), demonstrating that BALB-1 cDNA cannot encode a complete class I molecule. This deletion was shown to be due to a defect in the splice acceptor site in the intron I-exon II boundary of the *T22*^{d} gene (Ito et al. 1990), indicating that this gene is structurally defective (Fig. 4). This result also explains the molecular basis for the failure of TL^d strains (BALB/c, B10.D2; Table I) to stimulate proliferation of KN6 cells. Clone BALB-2 was identical to the *T10*^{d} gene in its exons and did not contain any structural defects (Figs. 4 and 6; Ito et al. 1990).

The pan-class I probe, pH2-IIa, detected 2 other genes in the *T22*^{b}-containing cosmid, genes *26*^{b} and *37*^{b} (Fig. 4). By nucleotide sequencing and comparison to TL gene sequences from BALB/c (Fisher et al. 1985, 1989, Transy et al. 1987, Brorson et al. 1989a), *26*^{b} and *37*^{b} were shown to be allelic to the BALB/c genes *T21*^{d} and *T23*^{d}, respectively (Fig. 4, and Ito et al. 1990, unpublished results). Because of this allelic relationship, *26*^{b} and *37*^{b} were renamed *T21*^{b} and *T23*^{b}, respectively (Fig. 4, and Klein et al. 1990). Thus, TL^b C57BL/6 DNA contains at least some of the homologues (*T21, T22, T23*) of the BALB/c TL^d-B cluster.

4. STRUCTURAL FEATURES OF THE *T22*^{b} PRODUCT

4.1. α_1 and α_2 domains – peptide binding

The role of conventional MHC class I products as antigen-presenting molecules to CD8$^+$ TCR $\alpha\beta^+$ cells has been well-established (for review see Townsend & Bodmer 1989). The three-dimensional structure of one of these MHC class I molecules, human HLA-A2, has recently been determined by X-ray crystallography (Bjorkman et al. 1987a, 1987b). Of the three extracellular domains, α_1, α_2, and α_3, the first two domains consist of a platform of eight antiparallel β-strands topped by two α-helices, forming a deep groove (Fig. 5). It has been proposed that this groove forms the binding site for antigen-derived peptides (Bjorkman et al. 1987b). The third domain (α_3) constitutes a classical immunoglobulin-like domain, which is non-covalently linked with β2-microglobulin.

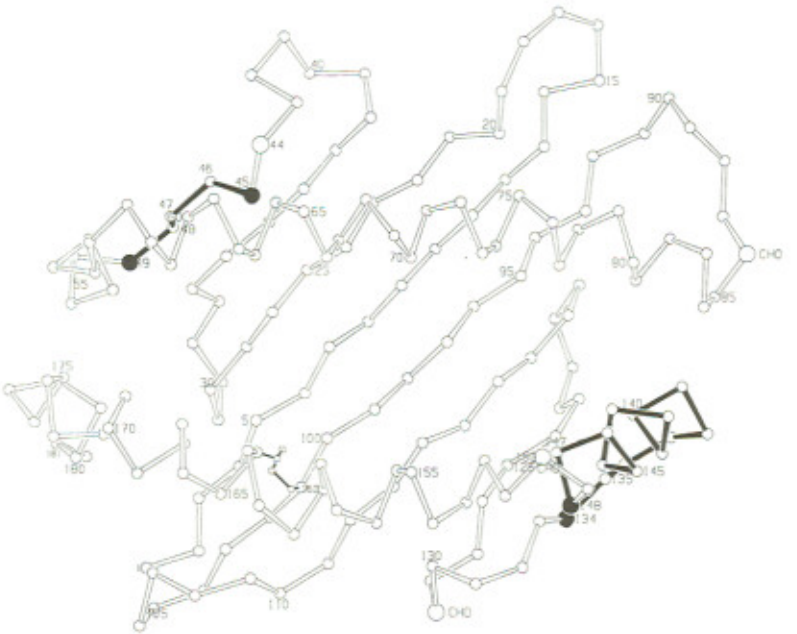
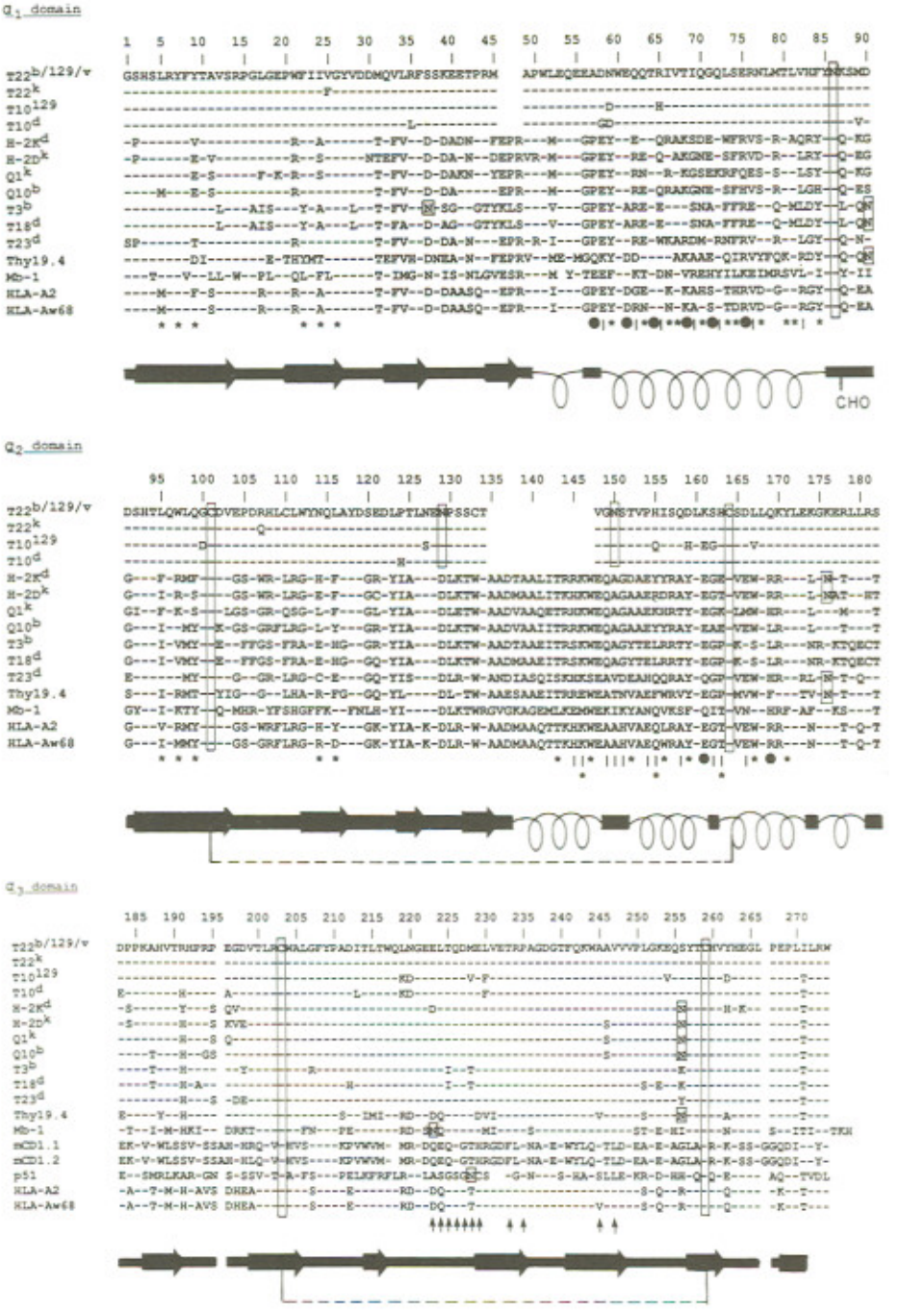


Figure 5. Schematic representation of the α_1 and α_2 domain structure of HLA-A2 and the probable locations of deletions and N-linked glycosylation sites in the T22^b protein. Numbers designate α-carbons of HLA-A2 amino acids. Assuming the polypeptide chain folding of the T22^b protein is similar, the possible locations of the deletions present in T22^b are indicated on the HLA-A2 structure (solid bonds). Large filled circles indicate the ends to be joined after deletion. Large open circles with a label CHO indicate the locations of potential N-linked glycosylation sites in the T22^b protein.


Q_1 domain
Q_2 domain
Q_3 domain
CHO

The amino acid sequence homology between $T22^b$ and HLA-A2 in the α_1 and α_2 domains is 54% and 39%, respectively (Fig. 6). Secondary structure analysis by the Chou & Fasman (1978) method suggests that the α_1 and α_2 domains of the $T22^b$ protein could fold into a β-sheet and α-helices structure not grossly different from that of HLA-A2. In addition, $T22^b$ contains the two conserved cysteine residues, in its α_2 domain, that are involved in an intradomain disulfide bond. This raises the possibility that $T22^b$ might function as a peptide-presenting molecule. However, several differences between the $T22^b$ and HLA-A2 amino acid sequences are noteworthy. First, there is a 13 amino acid deletion in the middle of the $T22^b$ α_2 domain (Fig. 6). However, the two ends remaining after this deletion (positions 134 and 148) can be near each other should the $T22^b$ protein adopt the HLA-A2 structure (Fig. 5). Thus, the deletion may not disrupt the remainder of the putative peptide-binding site, but rather only one end. Second, there is another deletion of three amino acids in the α_1 domain (Fig. 6), which might disrupt the 45 pocket at the left end (Fig. 5) of the site (Garret et al. 1989). Third, there are two new N-linked oligosaccharide sites at residues 129 (*N*PS) and 150 (*N*ST) (Figs. 5 and 6). The former would probably direct the oligosaccharide away from the site and the latter could direct an oligosaccharide into the region of the deletion covering the binding groove at the right end (D.

Figure 6. Comparison of the α_1, α_2, and α_3 domain amino acid sequences of the $T22^b$-related proteins with those of some mouse, rat and human class I or class I-like molecules. Amino acid sequences of the $T22^b$, $T22^{129}$, $T22^v$, $T22^k$, $T10^{129}$ and $T10^d$ proteins are aligned with those of one H-2K (H-$2K^d$), one H-2D (H-$2D^k$), two Q ($Q1^k$ and $Q10^b$), three TL ($T3^b$, $T18^d$, and $T23^d$), and two M (Thy19.1 and Mb-1) region products, two mouse CD1 molecules (mCD1.1 and mCD1.2), the neonatal rat Fc receptor (p51), and two human class I molecules (HLA-A2 and HLA-Aw68). The α_1 and α_2 domains of the mCD1.1, mCD1.2 and p51 products were not included in the comparison because of their low homology with the same domains in other class I molecules. A dash indicates that a residue is identical to that found in he $T22^b$ sequence. Open spaces indicate gaps introduced to optimize the alignment. Amino acid numbering follows the HLA-A2 sequence. Asparagine (N) residues of potential N-linked glycosylation sites (*N*XS or *N*XT) and the conserved cysteines (C) in the α_2 and α_3 domains are boxed. The symbols under the amino acid sequences of the α_1 and α_2 domains designate HLA-A2 residues that have been judged by X-ray crystallography (Bjorkman et al. 1987b) to be in the vicinity of the recognition site for processed foreign antigen: asterisks, residues pointing toward the site; vertical lines, residues on an α-helix pointing up, away from the membrane-proximal immunoglobulin-like domain; filled circles, residues on an α-helix pointing away from the site. The arrows under the amino acid sequences of the α_3 domain indicate residues in HLA-A2 that contribute to the binding of CD8 (Salter et al. 1990). Below the sequences is a schematic representation of the secundary structure of HLA-A2 (Bjorkman et al. 1987a): arrows, β-strands; loops, α-helices; bars, turns and bends. The sequences were obtained from Bradbury et al. (1988), Parham et al. (1988), Singer et al. (1988), Brorson et al. (1989b), Simister & Mostov (1989), Watts et al. (1989), Ito et al. (1990), and Ichikawa et al. (in preparation).

Wiley, personal communication) (Fig. 5). Finally, the 12 residues after the 13-residue deletion are very different from most class I sequences. Moreover, these residues contain a proline (position 154, Fig. 6), and the Chou & Fasman analysis does not give a high α-helix propensity in this region. This could mean that the lower α-helix is partially disrupted at the right end (see Fig. 5). Despite these differences, $T22^b$ could still function by presenting peptides.

4.2. α_3 domain – β2-microglobulin and CD8 binding

One interesting issue concerning the *T22*^{b} gene and its alleles is whether their products bind β2-microglobulin. Many class I and MHC class I-like molecules are known to bind β2-microglobulin. These include the classical class I molecules H-2K, D, and L (Klein et al. 1981), other class I products controlled by genes mapping in the Q (e.g., Qa-2 [Michaelson et al. 1981] and Q10 [Devlin et al. 1985]), TL (e.g., the classical thymus leukemia antigens [Yokoyama et al. 1982] and $T23^d$ [Cochet et al. 1989]), and M (e.g., Hmt [Fischer Lindahl & Langhorne 1981]) regions, human CD1 (Terhorst et al. 1981), and the neonatal rat Fc receptor p51 (Simister & Mostov 1989). Based on the proposed structure of the HLA-A2 molecule, β2-microglobulin is paired with the α_3 domain, making several interdomain contacts, and interacts with some parts of the α_1 and α_2 domains (Bjorkman et al. 1987a). In its α_3 domain, $T22^b$ and its allelic products are highly homologous with HLA-A2 and with many other class I molecules known to bind β2-microglobulin (Fig. 6). In addition, many of the residues in HLA-A2 which have been proposed to be in contact with β2-microglobulin are shared by these *T22* gene products (residues 6, 8, 10, 12, 25, and 35 in α_1, residues 94, 96, 115, 117, and 119 in α_2, and residues 188, 204, 206, 231, 233, 234, 237, 238, 242, and 244 in α_3; Bjorkman et al. 1987a). These results suggest that $T22^b$ (and its allelic products) binds β2-microglobulin. This hypothesis is supported by our recent observation that the cells from β2-microglobulin-deficient mice (Zijlstra et al. 1990) fail to stimulate KN6 cells *in vitro* (P. Pereira, M. Zijlstra, R. Jaenisch & S. T., unpublished observations).

CD8 molecules on T cells facilitate the interaction of $\alpha\beta$ TCR with the peptide-MHC class I molecular complex by binding with the α_3 domain of the class I molecule. The amino acid residues within the α_3 domain of HLA-A2 that contribute to the CD8 binding have been identified and shown to be in three clusters (Fig. 6, and Salter et al. 1990), the most critical being in the negatively charged loop at positions 223–229 (Fig. 6). Many of these residues are shared by $T22^b$. The conservation of the amino acids in these clusters is even greater between T22 and classical MHC class I molecules such as $H\text{-}2K^d$ and $H\text{-}2D^k$ in the mouse (Fig. 6). This suggests that T22 has the capacity to bind CD8. Interestingly, however, the majority of $\gamma\delta$ T cells are known to be CD8-negative, and the rest seem to express the CD8 α chain but not the CD8 β chain (Lefrancois et al.,

unpublished observations; Utsunomiya et al., unpublished observations). Possibly, γδ T cells express an as yet unidentified co-receptor structurally related to CD8, or they express CD8 transiently during a particular phase of activation.

5. LIMITED POLYMORPHISM IN THE *T22* GENE PRODUCT

As mentioned above, the nucleotide sequences of the *T22* gene and its cDNA clones indicate that there is no amino acid sequence difference between the products of this gene from strains C57BL/6 and 129/Sv. In agreement with this, peritoneal exudate cells from 129/Sv mice stimulate KN6 cells as strongly as cells from C57BL/6 (Table I). Strain SM ($H\text{-}2^v$) is yet another strain that stimulates KN6 strongly (Table I). Nucleotide sequencing of *T22* cDNA clones from this strain demonstrated that the deduced amino acid sequence is identical to that of C57BL/6 and 129/Sv (Y.I., unpublished data). In contrast to these strains, peritoneal exudate cells from many other strains stimulated KN6 cells moderately (Table I). The intermediate stimulatory activity may be due to structural polymorphism in the *T22* gene products. Indeed, the *T22* cDNA sequence of strain AKR ($H\text{-}2^k$), one of the many intermediate stimulatory strains (Table I), differed from that of C57BL/6 by three nucleotides (Table III), two of which caused amino acid replacements in the α_1 and α_2 domains (Val to Phe and Arg to Gln at positions 25 and 107, respectively [Fig. 6]). However, neither of these replacements has occurred at positions which would be predicted to interact with a bound peptide or a TCR (Bjorkman et al. 1987b). Cells from BALB/c mice failed to stimulate KN6 cells (Table I). Gene cloning and sequencing studies demonstrated that this is due to a defective splice acceptor site in the $T22^d$ gene (Ito et al. 1990). In addition to this defect, $T22^d$ differed in its protein-encoding region by five nucleotides from $T22^b$ (Table III). Taken together, these results suggest that the *T22* gene, like other TL region genes, is much less polymorphic than classical MHC class I (and class II) genes (Chen et al. 1985, Transy et al. 1987, Flaherty et al. 1990).

6. THE $T22^b$ GENE IS WIDELY EXPRESSED

$T22^b$ mRNA was detected in many different tissues (spleen, thymus, liver, muscle, intestine, brain, etc.) and cell types (spleen B and T cells, thymocytes, peritoneal macrophages, etc.), indicating that the gene is broadly expressed. These results are in agreement with functional assays showing that KN6 cells react with C57BL/6 cells of various origin: peritoneal exudate cells, $Ly1^-$ peritoneal exudate cells, splenocytes, thymocytes, $CD4^-$ thymocytes and $CD8^-$ thymocytes (Fig. 7, and Bonneville et al. 1989).

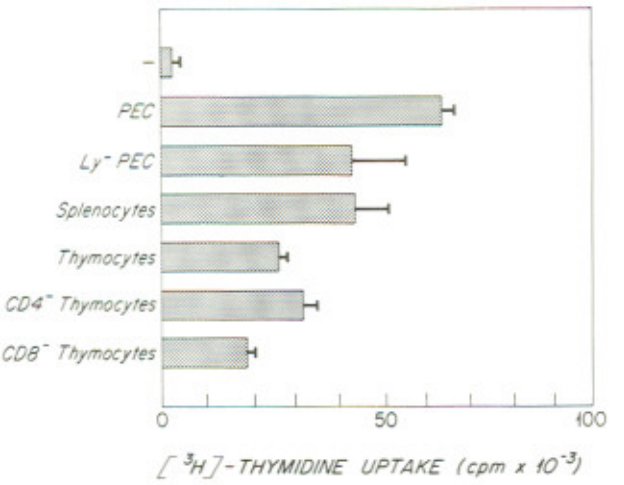


Figure 7. KN6 reactivity against various cell populations. Approximately 10^4 CD4$^-$ 8$^-$ spleen cells from KN6 transgenic mice were cultured alone (−) or in the presence of the indicated irradiated (1500 rads) stimulator cells. KN6 proliferation was assayed after 3 d of culture by pulsing the cells with [^{3}H]-thymidine. Data are presented as mean cpm and standard deviations of triplicate experiments.

7. ANOTHER TL CLASS I GENE, *T3*^{b}, IS SELECTIVELY EXPRESSED ON THE SURFACE OF EPITHELIAL CELLS OF SMALL INTESTINE

As discussed in subsequent sections, we speculate that TL class I genes have co-evolved with TCR γ and δ genes to interact with each other. Since $\gamma\delta$ T cells associated with some of the different peripheral sites utilize distinct V_γ and/or V_δ gene segments, one might expect, according to this hypothesis, that at least some TL class I genes are expressed in a highly tissue-specific (and cell type-specific) fashion. While previous studies (Boyse et al. 1965) suggest that this may indeed be the case, these studies were not, understandably, focused on the tissues and cell types with which $\gamma\delta$ T cells are now known to be associated. We therefore have begun to re-examine the expression of TL region class I genes, including *T3*^{b} (see Fig. 4).

T3 and *T18* (formerly designated *T13*^{c}) appear to encode the classical thymus leukemia (TL) antigens (Chen et al. 1987, Chorney et al. 1989, Flaherty et al. 1990). TL antigens are restricted in their tissue distribution to thymocytes (Boyse et al. 1965) and to certain leukemias (Old et al. 1963), and have been detected on mitogen-activated T cells. Strain C57BL/6 mice lack the *T18* gene and do not express (or express very low levels of) TL antigens. In contrast, leukemias derived from strain C57BL/6 (e.g. ERLD) express high levels of TL antigens, presumably encoded by *T3*^{b}.

We initially examined expression of *T3*^{b} by the RNAase protection assay. As expected, *T3*^{b} RNA was not detected in the thymus of C57BL/6 mice and in many other organs including spleen, liver, kidney, lung, brain, and vagina (Wu et al., in preparation). However, significant levels of *T3*^{b} RNA were detected in the small intestine and in a purified preparation of intestinal epithelial cells. Tissue sections of small intestine were then examined by the immunogold-silver staining method using the anti-T3^b monoclonal antibody, HD168. As shown in Fig. 8, the *T3*^{b} product is expressed on the surface of the columnar epithelium cells of the epithelium lining of the intestine. A small amount of staining was also observed in the epithelium of the glands. Similar staining was seen in both jejenum (stomach-proximal) and ileum (stomach-distal). In the large intestine, no expression was detected, except for a weak signal in the epithelium of the glands (Wu et al., in preparation).

Previous study had shown that γδ cells in the small intestine (i-IEL) are located within the villi, in contact with the columnar epithelial cells (Tonegawa et al. 1989). They are not detected in the intestinal crypts nor in the lamina propria. Based on these data, it is tempting to speculate that the *T3*^{b} product might be recognized by at least some of these i-IEL.

DISCUSSION

Data presented here have important implications for the function of non-classical MHC class I products as well as for the possible function of γδ T cells. The identification of a TL region-controlled class I molecule that is recognized by a γδ TCR raises the possibility that other TL region-encoded products might function as ligands for γδ T cells. Data in several systems support this notion and provide evidence that γδ T cells recognize other non-classical class I molecules. First, an alloreactive γδ CTL line was isolated that recognizes a TL region-controlled determinant (Bluestone et al. 1988, Houlden et al. 1989). Interestingly, the strain distribution of this ligand closely resembled that of T22^b. Second, a γδ T-helper hybridoma was shown to recognize the Qa-1 antigen (Vidović et al. 1989), known to be encoded by a gene mapping in the TL region (Stanton et al. 1981). Third, human γδ T cells that are reactive with non-MHC-linked class I molecules, CD1, have been identified (Porcelli et al. 1989). Fourth, many mouse and human γδ T cells were shown to react with mycobacterial heat-shock proteins (Augustin et al. 1989, Haregewoin et al. 1989, Holoshitz et al. 1989, Janis et al. 1989, Modlin et al. 1989, O'Brien et al. 1989, Fisch et al. 1990), and except for one case (Haregewoin et al. 1989), these γδ cells did not seem to be restricted by the classical polymorphic MHC products. Since some of these mouse γδ T cells also react with purified protein derivative (a source of hsp65 that is denatured and probably degraded) or a peptide corresponding to residues 180-196 of mycobacterial hsp65, it was suggested that this recognition involves non-classical

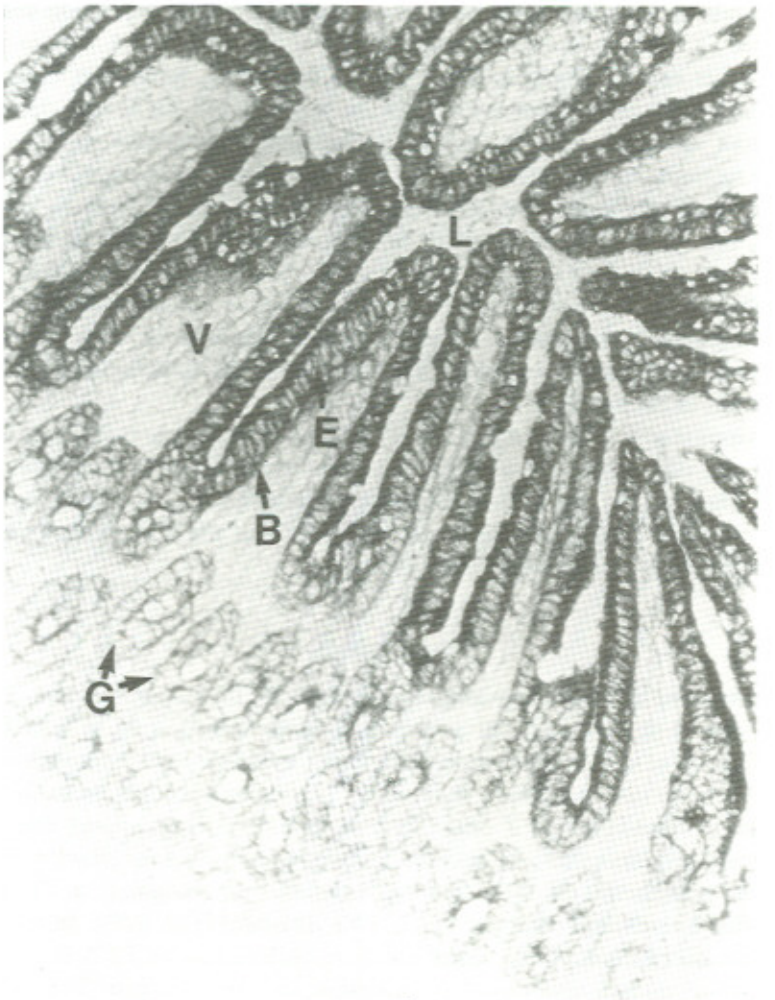


Figure 8. Demonstration that the *T3*^{b} gene product is expressed in the epithelium of the small intestine. Fresh intestinal tissue from a 12-wk-old C57BL/6 mouse was snap-frozen, 8 μm sections were fixed with cold acetone, and then stained with an anti-T3^{b} mAb (HD168) (kindly provided by E. Stockert, Memorial Sloan-Kettering Cancer Center, NY), followed by affinity-purified goat anti-rat Ig linked to colloidal gold particles. The signal was enhanced with silver reaction. A transverse section is shown. Abbreviations: B, basement membrane; E, epithelium; G, glands; L, lumen; V, villus.

antigen-presenting molecules (O'Brien et al. 1989, Born et al. 1990a). We propose that these molecules may be encoded by low-polymorphic class I-like genes. Finally, when a large number of γδ T-cell hybridomas was tested against a panel of allogeneic cells, no alloreactive hybrids could be identified, while such reactivity was readily detected with αβ TCR$^+$ hybridomas (O'Brien et al. 1989). This suggests that these γδ hybridomas were not specific for polymorphic restriction elements.

Although the aforementioned findings support the hypothesis that non-classical MHC class I molecules are recognized by γδ TCR, γδ T-cell clones reactive with allogeneic H-2 class I and class II molecules have also been reported (Matis et al. 1987, Bluestone et al. 1988). These clones were obtained from nude mice after strong selection by allogeneic stimulation, and exhibited a broad cross-reactivity for the products of different alleles of class I and class II MHC genes. These clones may have been derived from rare cells that are specific for non-classical MHC proteins but were selected by *in vivo* and/or repeated *in vitro* stimulation on the basis of their structural similarity and cross-reactivity with classical MHC proteins.

Recently, mice deficient in β2-microglobulin have been analyzed for γδ T cells and no apparent differences in the number of γδ T cells in the spleen, thymus or lymph node were observed between these mice and normal mice (Koller et al. 1990, Zijlstra et al. 1990). Although this observation seems to indicate that an interaction with a β2-microglobulin-associated molecule is not required for the maturation of γδ T cells, an obligatory step for CD8$^+$ αβ T cells, it does not necessarily mean that γδ T cells do not recognize non-classical class I molecules during maturation or during the expression of their effector functions.

The *T22*^{b} product itself may be recognized by the KN6 γδ TCR, or the *T22*^{b} product may be recognized together with bound peptide. We favor the latter on the basis of the general structural similarities between the TL region-encoded and the classical (i.e. H-2K, H-2D, and H-2L) class I products and between the αβ TCR and γδ TCR. The third possibility, that a T22^b-derived peptide is presented by a classical H-2 molecule to the KN6 γδ TCR, is unlikely since PCC3 stimulates KN6 cells and does not express any surface classical H-2 molecules, and because the functional assay did not reveal any influence of these polymorphic molecules on the response (Ito et al. 1990). Support for the peptide "presentor" function of non-classical class I molecules can be drawn from several studies. In the maternally-transmitted antigen (Mta) system, it appears that an M region class I molecule presents a mitochondrial peptide to cytolytic T lymphocytes (Fischer Lindahl et al. 1989) (some of these CTL clones were recently shown to be αβ TCR$^+$ [Fischer Lindahl et al. 1989]). In addition, the aforementioned Qa-1 T-helper hybridoma apparently recognized a Glu-Tyr (GT) copolymer as part of its ligand (Vidović et al. 1989). Studies in the Qc-1 system are also consistent with presentation of a self peptide by the product of a non-classical Q region class I gene to cytolytic

T lymphocytes (Murphy & Homer 1987). Host recognition of allogeneic class I-like molecules or possibly of class I-like molecules complexed with peptides of embryonal origin may also be responsible for anti-tumor immunity in studies using EC cell lines (Ostrand-Rosenberg et al. 1989).

If non-classical class I molecules can present antigen-derived peptides, what then is the nature of these peptides? It is unlikely that non-classical class I molecules would present a large variety of antigens to $\gamma\delta$ T cells, as the restriction elements of the $\alpha\beta$ cells do. While conventional class I molecules show extensive polymorphism to ensure the recognition of virtually any foreign peptide (e.g. derived from highly variable viral proteins), the non-classical MHC molecules are not very polymorphic. Perhaps the class I molecules recognized by TCR $\gamma\delta$ have evolved to recognize a particular set of relatively homogeneous endogenous and common microbial antigens. In their membrane-distal domains, non-classical class I molecules have diverged considerably from each other, and much more so than have the classical class I molecules (Fig. 6, and Stroynowski 1990). Since these domains most probably constitute the sites for antigen binding and TCR recognition, each member of the non-classical class I molecules might be specialized for the presentation of different sets of antigens and for the recognition of different $\gamma\delta$ TCR subsets. In line with this hypothesis, the sequence homology among V_γ gene segments is relatively low (Saito et al. 1984, Hayday et al. 1985, Garman et al. 1986, Heilig et al. 1986, Traunecker et al. 1986, Pelkonen et al. 1987). $\gamma\delta$ cells in the epidermis of the skin (DEC or s-IEL) and those in the mucosal epithelia of the vagina, uterus and tongue (vut-IEL) express homogeneous TCR, and, while sharing the same δ chain, utilize different V gene segments for the expression of the γ chain with a low amount of sequence homology (35% at the amino acid level [Garman et al. 1986]). It is tempting to speculate that the occurrence of a specific homogeneous $\gamma\delta$ T-cell subset in an organ or tissue might correlate with the tissue-specific expression of a specific non-classical class I molecule and the recognition of a particular endogenous antigen.

The intraepithelial lymphocytes of the gut (i-IEL) are more diverse and use TCRs encoded by yet another V region gene, $V_\gamma 7$ (Bonneville et al. 1988, Asarnow et al. 1989). These cells probably recognize more diverse ligands that are presented by the gut epithelial cells. Antigens recognized by the i-IEL may be derived from mycobacteria or from endogenous proteins that are induced in the epithelial cells by enteric inflammation. Our observation that the *T3*^{b} gene is specifically expressed in the gut epithelium supports the notion that at least some of the i-IEL might recognize the product of this gene as an antigen-presenting molecule. However, in a recent report (Lefrancois et al. 1990), it was suggested that some $\gamma\delta$ i-IEL may recognize class II antigens. This hypothesis was based on the observation that $V_\delta 4^+$ cells, while constituting on average 30% of the i-IEL population, were about 2-fold enriched in H-2^k mice. This enrichment was appar-

ently due to a determinant mapping in the H-2 I-E locus, indicating that the $V_\delta4^+$ i-IEL subset is selected by class II molecules. Although this observation could be interpreted to indicate that these cells are capable of class II-restricted antigen recognition, alternative explanations are possible. One possibility would be that only the selection process of $V_\delta4^+$ i-IEL requires the interaction with class II molecules while the true ligands of these cells in the intestinal epithelium are different. Another possibility is that the selection of these $V_\delta4^+$ i-IEL is mediated by class II-restricted $\alpha\beta$ TCR^+ T-helper cells, and therefore does not require direct interaction of the $\gamma\delta$ TCR with class II molecules.

Finally, $\gamma\delta$ cells from the peripheral lymphoid organs express diverse TCR, encoded by multiple combinations of γ and δ gene segments (Cron et al. 1988). These cells are likely to recognize a more diverse repertoire of endogenous and bacterial antigens, presented by multiple non-classical class I molecules. KN6 $\gamma\delta$ TCR and $T22^b$ belong to this subset of $\gamma\delta$ TCR and class I molecules, respectively.

Although the effector function of $\gamma\delta$ T cells is not known, one might speculate that these cells initiate or regulate various defense reactions by lymphokine production or elimination of infected or stressed cells (Janeway et al. 1988, Bluestone & Matis 1989, Strominger 1989, Tonegawa et al. 1989, Born et al. 1990b). $\gamma\delta$ T cells that produce lymphokines such as IL-2 (Bank et al. 1986), IL-4 (Marušić-Galešić et al. 1988), interferon-γ (IFN-γ) (Bluestone et al. 1988) or granulocyte-macrophage colony-stimulating factor (GM-CSF) (Bluestone et al. 1988) have been reported, and $\gamma\delta$ cells that suppress B-cell responses were also identified (A. Bandeira and A. Coutinho, personal communication). Moreover, a variety of $\gamma\delta$ cells (e.g., a large portion of the i-IEL population) exhibit cytolytic activities (Bank et al. 1986, Moingeon et al. 1987, Bluestone et al. 1988, Goodman & Lefrancois 1988).

In conclusion, our data and data from others suggest that $\gamma\delta$ TCR and non-classical MHC class I molecules co-evolved for recognition of a conserved set of endogenous or mycobacterial antigens, and that this recognition initiates various defense reactions.

SUMMARY

We have studied the ligand specificity of a $\gamma\delta$ T-cell receptor (TCR) derived from a mouse T-cell hybridoma (KN6). KN6 cells reacted with syngeneic (C57BL/6) cells from various origins (splenocytes, thymocytes, peritoneal exudate cells, etc.) and cells from many different mouse strains. KN6 reactivity against cells from a panel of congenic and recombinant mouse strains demonstrated that the ligand recognized by KN6 is controlled by an MHC-linked gene that most probably maps in the TL region. We cloned this gene and formally proved that it does map in the TL region. This gene turned out to be a novel class I gene (designated *$T22^b$*) belonging to a hitherto unidentified cluster of TL region genes in strain

C57BL/6. This gene was expressed in many different tissues and cell types. We also examined the tissue expression of several other TL genes. One of these, the structural gene ($T3^b$) encoding the thymus leukemia (TL) antigen from C57BL/6 mice, was specifically expressed in the epithelium of the small intestine. Since the intestinal epithelium of the mouse is known to be the homing site for a subset of $\gamma\delta$ T cells (i-IEL) bearing diverse TCR with V_γ rearranged γ chains, we propose that the $T3^b$ gene product is part of the ligand recognized by some of the i-IEL. Our data support the idea that $\gamma\delta$ T cells might be specific for non-classical class I or class I-like molecules and suggest that $\gamma\delta$ TCR and non-classical MHC co-evolved for the recognition of a conserved set of endogenous or foreign peptides.

ACKNOWLEDGMENTS

We thank Sang Hsu for excellent technical help and Elly Basel for secretarial assistance. We also want to thank Don C. Wiley, Werner Haas, and Charles A. Janeway Jr. for useful comments. L.V.K. is supported by the Cancer Research Institute, M.W. is supported by the Howard Hughes Medical Institute, and Y.I. is supported by Kao Co. This work was supported in part by grants from the Howard Hughes Medical Institute, the National Institutes of Health (NIH) (CA28900 and AI17879 to S.T. and AI14349 to D.B.M.), the American Cancer Society, and Ajinomoto Co., Ltd.

REFERENCES

Asarnow, D. M., Goodman, T., LeFrancois, L. & Allison, J. P. (1989) Distinct antigen receptor repertoires of two murine epithelium-associated T cells. *Nature* **341**, 60.

Asarnow, D. M., Kuziel, W. A., Bonyhadi, M., Tigelaar, R. E., Tucker, P. W. & Allison, J. P. (1988) Limited diversity of $\gamma\delta$ antigen receptor genes of Thy-1$^+$ dendritic epidermal cells. *Cell* **55**, 837.

Ashwell, J. D., Cunningham, R. E., Noguchi, P. D. & Hernandez, D. (1987) Cell growth cycle block of T cell hybridomas upon activation with antigen. *J. Exp. Med.* **165**, 173.

Augustin, A., Kubo, R. T. & Sim, G.-K. (1989) Resident pulmonary lymphocytes expressing the γ/δ T-cell receptor. *Nature* **340**, 239.

Bank, I., DePinho, R. A., Brenner, M. B., Cassimeris, J., Alt, F. W. & Chess, L. (1986) A functional T3 molecule associated with a novel heterodimer on the surface of immature human thymocytes. *Nature* **322**, 129.

Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987a) Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* **329**, 506.

Bjorkman, P. J., Saper, M. A., Samraoui, B., Bennett, W. S., Strominger, J. L. & Wiley, D. C. (1987b) The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature* **329**, 512.

Bluestone, J. A., Cron, R. Q., Cotterman, M., Houlden, B. A. & Matis, L. A. (1988)

Structure and specificity of T cell receptor γ/δ on major histocompatibility complex antigen-specific CD3⁺, CD4⁻, CD8⁻ T lymphocytes. *J. Exp. Med.* **168**, 1899.

Bluestone, J. A. & Matis, L. A. (1989) TCRγδ cells – minor redundant T cell subset or specialized immune system component? *J. Immunol.* **142**, 1785.

Bluestone, J. A., Pardoll, D., Sharrow, S. O. & Fowlkes, B. J. (1987) Characterization of murine thymocytes with CD3-associated T-cell receptor structures. *Nature* **326**, 82.

Bonneville, M., Ishida, I., Itohara, S., Verbeek, S., Berns, A., Kanagawa, O., Haas, W. & Tonegawa, S. (1990) Self-tolerance to transgenic γδ T cells by intrathymic inactivation. *Nature* **344**, 163.

Bonneville, M., Ito, K., Krecko, E. G., Itohara, S., Kappes, D., Ishida, I., Kanagawa, O., Janeway, C. A. Jr., Murphy, D. B. & Tonegawa, S. (1989) Recognition of a self major histocompatibility complex TL region product by γδ T cell-receptors. *Proc. Natl. Acad. Sci. USA* **86**, 5928.

Bonneville, M., Janeway, C. A. Jr., Ito, K., Haser, W., Ishida, I., Nakanishi, N. & Tonegawa, S. (1988) Intestinal intraepithelial lymphocytes are a distinct set of γδ T cells. *Nature* **336**, 479.

Born, W., Hall, L., Dallas, A., Boymel, J., Shinnick, T., Young, D., Brennan, P. & O'Brien, R. (1990a) Recognition of a peptide antigen by heat shock-reactive γδ T lymphocytes. *Science* **249**, 67.

Born, W., Happ, M. P., Dallas, A., Reardon, C., Kubo, R., Shinnick, T., Brennan, P. & O'Brien, R. (1990b) Recognition of heat shock proteins and γδ cell function. *Immunol. Today* **11**, 40.

Borst, J., Van Dongen, J. J. M., Bolhuis, R. L. H., Peters, P. J., Hafler, D. A., De Vries, E. & Van de Griend, R. J. (1988) Distinct molecular forms of human T cell receptor γ/δ detected on viable T cells by a monoclonal antibody. *J. Exp. Med.* **167**, 1625.

Boyse, E. A., Old, L. J. & Stockert, E. (1965) The TL (thymus-leukemia) antigen. In: *Immunopathology Fourth International Symposium*, ed. Graber, P. & Miescher, P. A., p. 23. Schwabe, Basel, Switzerland.

Bradbury, A., Belt, K. T., Neri, T. M., Milstein, C. & Calabi, F. (1988) Mouse CD1 is distinct from and co-exists with TL in the same thymus. *EMBO J.* **7**, 3081.

Brenner, M. B., McLean, J., Dialynas, D. P., Strominger, J. L., Smith, J. A., Owen, F. L., Seidman, J. G., Ip, S., Rosen, F. & Krangel, M. S. (1986) Identification of a putative second T-cell receptor. *Nature* **322**, 145.

Brorson, K. A., Hunt, S. W. III, Hunkapiller, T., Sun, Y. H., Cheroutre, H., Nickerson, D. A. & Hood, L. (1989a) Comparison of exon 5 sequences from 35 class I genes of the BALB/c mouse. *J. Exp. Med.* **170**, 1837.

Brorson, K. A., Richards, S., Hunt, S. W. III, Cheroutre, H., Fischer Lindahl, K. & Hood, L. (1989b) Analysis of a new class I gene mapping to the *Hmt* region of the mouse. *Immunogenetics* **30**, 273.

Brown, G. D., Choi, Y., Egan, G. & Meruelo, D. (1988) Extension of the H-2 TL^b molecular map. Isolation and characterization of *T13*, *T14*, and *T15* from the C57BL/6 mouse. *Immunogenetics* **27**, 239.

Chen, Y.-T., Obata, Y., Stockert, E. & Old, L. J. (1985) Thymus-leukemia (TL) antigens of the mouse. Analysis of TL mRNA and TL cDNA from TL⁺ and TL⁻ strains. *J. Exp. Med.* **162**, 1134.

Chen, Y.-T., Obata, Y., Stockert, E., Takahashi, T. & Old, L. J. (1987) *Tla*-region genes and their products. *Immunol. Res.* **6**, 30.

Chorney, M. J., Mashimo, H., Bushkin, Y. & Nathenson, S. G. (1989) Characterization of the thymus leukemia (TL) product encoded by the BALB/c $T3^c$ gene by DNA-mediated gene transfer. Comparison to the $T13^c$ product and BALB/c leukemia TL. *J. Immunol.* **143**, 3762.

Chou, P. Y. & Fasman, G. D. (1978) Empirical predictions of protein conformation. *Ann. Rev. Biochem.* **47**, 251.

Cochet, M., Casrouge, A., Dumont, A.-M., Transy, C., Baleux, F., Maloy, W. L., Coligan, J. E., Cazenave, P.-A. & Kourilsky, P. (1989) A new cell surface molecule closely related to mouse class I transplantation antigens. *Eur. J. Immunol.* **19**, 1927.

Cron, R. Q., Koning, F., Maloy, W. L., Pardoll, D., Coligan, J. E. & Bluestone, J. A. (1988) Peripheral murine $CD3^+$, $CD4^-$, $CD8^-$ T lymphocytes express novel T cell receptor $\gamma\delta$ structures. *J. Immunol.* **141**, 1074.

Davis, M. M. & Bjorkman, P. J. (1988) T-cell antigen receptor genes and T-cell recognition. *Nature* **334**, 395.

Devlin, J. J., Lew, A. M., Flavell, R. A. & Coligan, J. E. (1985) Secretion of a soluble class I molecule encoded by the Q10 gene of the C57BL/10 mouse. *EMBO J.* **4**, 369.

Elliott, J. F., Rock, E. P., Patten, P. A., Davis, M. M. & Chien, Y. (1988) The adult T-cell receptor δ-chain is diverse and distinct from that of fetal thymocytes. *Nature* **331**, 627.

Fisch, P., Malkovsky, M., Braakman, E., Sturm, E., Bolhuis, R. L. H., Prieve, A., Sosman, J. A., Lam, V. A. & Sondel, P. M. (1990) γ/δ T cell clones and natural killer cell clones mediate distinct patterns of non-major histocompatibility complex-restricted cytolysis. *J. Exp. Med.* **171**, 1567.

Fischer Lindahl, K., Hermel, E., Loveland, B. E., Richards, S., Wang, C.-R. & Yonekawa, H. (1989) Molecular definition of a mitochondrially encoded mouse minor histocompatibility antigen. *Cold Spring Harbor Symp. Quant. Biol.* **49**, 563.

Fischer Lindahl, K. & Langhorne, J. (1981) Medial histocompatibility antigens. *Scand. J. Immunol.* **14**, 643.

Fisher, D. A., Hunt, S. W. III & Hood, L. (1985) Structure of a gene encoding a murine thymus leukemia antigen, and organization of *Tla* genes in the BALB/c mouse. *J. Exp. Med.* **162**, 528.

Fisher, D. A., Pecht, M. & Hood, L. (1989) DNA sequence of a class I pseudogene from the *Tla* region of the murine MHC: recombination at a B2 Alu repetitive sequence. *J. Mol. Evol.* **28**, 306.

Flaherty, L., Elliott, E., Tine, J. A., Walsh, A. C. & Waters, J. B. (1990) Immunogenetics of the Q and TL regions of the mouse. *CRC Crit. Rev. Immunol.* **10**, 131.

Garman, R. D., Doherty, P. J. & Raulet, D. H. (1986) Diversity, rearrangement, and expression of murine T cell gamma genes. *Cell* **45**, 733.

Garrett, T. P. J., Saper, M. A., Bjorkman, P. J., Strominger, J. L. & Wiley, D. C. (1989) Specificity pockets for the side chains of peptide antigens in HLA-Aw68. *Nature* **342**, 692.

Goodman, T. & Lefrancois, L. (1988) Expression of the γ-δ T-cell receptor on intestinal $CD8^+$ intraepithelial lymphocytes. *Nature* **333**, 855.

Haregewoin, A., Soman, G., Hom, R. C. & Finberg, R. W. (1989) Human $\gamma\delta^+$ T cells respond to mycobacterial heat-shock protein. *Nature* **340**, 309.

Hayday, A. C., Saito, H., Gillies, S. D., Kranz, D. M., Tanigawa, G., Eisen, H. N. & Tonegawa, S. (1985) Structure, organization, and somatic rearrangement of T cell gamma genes. *Cell* **40**, 259.

Heilig, J. S. & Tonegawa, S. (1986) Diversity of murine gamma genes and expression in fetal and adult T lymphocytes. *Nature* **322**, 836.

Heilig, J. S. & Tonegawa, S. (1987) T-cell γ gene is allelically but not isotypically excluded and is not required in known functional T-cell subsets. *Proc. Natl. Acad. Sci. USA* **84**, 8070.

Holoshitz, J., Koning, F., Coligan, J. E., De Bruyn, J. & Strober, S. (1989) Isolation of $CD4^-$ $CD8^-$ mycobacteria-reactive T lymphocyte clones from rheumatoid arthritis synovial fluid. *Nature* **339**, 226.

Houlden, B. A., Matis, L. A., Cron, R. Q., Widacki, S. M., Brown, G. D., Pampeno, C.,

Meruelo, D. & Bluestone, J. A. (1989) A TCR γδ cell recognizing a novel TL-encoded gene product. *Cold Spring Harbor Symp. Quant. Biol.* **49**, 45.

Ishida, I., Verbeek, S., Bonneville, M., Itohara, S., Berns, A. & Tonegawa, S. (1990) T-cell receptor γδ and γ transgenic mice suggest a role of a γ gene silencer in the generation of αβ T cells. *Proc. Natl. Acad. Sci. USA* **87**, 3067.

Ito, K., Bonneville, M., Takagaki, Y., Nakanishi, N., Kanagawa, O., Krecko, E. G. & Tonegawa, S. (1989) Different γδ T-cell receptors are expressed on thymocytes at different stages of development. *Proc. Natl. Acad. Sci. USA* **86**, 631.

Ito, K., Van Kaer, L., Bonneville, M., Hsu, S., Murphy, D. B. & Tonegawa, S. (1990) Recognition of the product of a novel MHC TL region gene (27^b) by a mouse γδ T cell receptor. *Cell* **62**, 549.

Itohara, S., Farr, A. G., Lafaille, J. J., Bonneville, M., Takagaki, Y., Haas, W. & Tonegawa, S. (1990) Homing of a γδ thymocyte subset with homogeneous T-cell receptors to mucosal epithelia. *Nature* **343**, 754.

Itohara, S., Nakanishi, N., Kanagawa, O., Kubo, R. & Tonegawa, S. (1989) Monoclonal antibodies specific to native murine T-cell receptor γδ: analysis of γδ T cells during thymic ontogeny in peripheral lymphoid organs. *Proc. Natl. Acad. Sci. USA* **86**, 5094.

Janeway, C. A. Jr., Jones, B. & Hayday, A. (1988) Specificity and function of T cells bearing γδ receptors. *Immunol. Today* **9**, 73.

Janis, E. M., Kaufmann, S. H. E., Schwartz, R. H. & Pardoll, D. M. (1989) Activation of γδ T cells in the primary immune response to *Mycobacterium tuberculosis*. *Science* **244**, 713.

Klein, J., Benoist, C., David, C. S., Demant, P., Fischer Lindahl, K., Flaherty, L., Flavell, R. A., Hämmerling, U., Hood, L. E., Hunt, S. W. III, Jones, P. P., Kourilsky, P., McDevitt, H. O., Meruelo, D., Murphy, D. B., Nathenson, S. G., Sachs, D. H., Steinmetz, M., Tonegawa, S., Wakeland, E. K. & Weiss, E. H. (1990) Revised nomenclature of mouse *H-2* genes. *Immunogenetics* **32**, 147.

Klein, J., Juretič, A., Baxevanis, C. N. & Nagy, Z. A. (1981) The traditional and a new version of the mouse *H-2* complex. *Nature* **291**, 455.

Koller, B. H., Marrack, P., Kappler, J. W. & Smithies, O. (1990) Normal development of mice deficient in β_2M, MHC class I proteins, and $CD8^+$ T cells. *Science* **248**, 1227.

Koning, F., Stingl, G., Yokoyama, W. M., Yamada, H., Maloy, W. L., Tschachler, E., Shevach, E. M. & Coligan, J. E. (1987) Identification of a T3-associated γδ T cell receptor on Thy-1^+ dendritic epidermal cell lines. *Science* **236**, 834.

Kuziel, W. A., Takashima, A., Bonyhadi, M., Bergstresser, P. R., Allison, J. P., Tigelaar, R. E. & Tucker, P. W. (1987) Regulation of T-cell receptor γ-chain RNA expression in murine Thy-1^+ dendritic epidermal cells. *Nature* **328**, 263.

Lefrancois, L., LeCorre, R., Mayo, J., Bluestone, J. A. & Goodman, T. (1990) Extrathymic selection of TCR $\gamma\delta^+$ T cells by class II major histocompatibility complex antigens. *Cell* **63**, 333.

Lew, A. M., Pardoll, D. M., Maloy, W. L., Fowlkes, B. J., Kruisbeek, A., Cheng, S.-F., Germain, R. N., Bluestone, J. A., Schwartz, R. H. & Coligan, J. E. (1986) Characterization of T cell receptor gamma chain expression in a subset of murine thymocytes. *Science* **234**, 1401.

Maher, L. J. III & Dove, W. F. (1984) Overt expression of *H-2* serotypes on EC cells in not necessary for host rejection. *Immunogenetics* **19**, 343.

Marušić-Galešić, S., Pardoll, D. M., Saito, T., Leo, O., Fowlkes, B. J., Coligan, J., Germain, R. N., Schwartz, R. H. & Kruisbeek, A. M. (1988) Activation properties of T cell receptor-γδ hybridomas expressing diversity in both γ- and δ-chains. *J. Immunol.* **140**, 411.

Matis, L. A., Cron, R. & Bluestone, J. A. (1987) Major histocompatibility complex-linked specificity of γδ receptor-bearing T lymphocytes. *Nature* **330**, 262.

Michaelson, J., Flaherty, L., Bushkin, Y. & Yudkowitz, H. (1981) Further biochemical data on Qa-2. *Immunogenetics* **14**, 129.

Modlin, R. L., Pirmez, C., Hofman, F. M., Torigian, V., Uyemura, K., Rea, T. H., Bloom, B. R. & Brenner, M. B. (1989) Lymphocytes bearing antigen-specific $\gamma\delta$ T-cell receptors accumulate in human infectious disease lesions. *Nature* **339**, 544.

Moingeon, P., Jitsukawa, S., Faure, F., Troalen, F., Triebel, F., Graziani, M., Forestier, F., Bellet, D., Bohuon, C. & Hercend, T. (1987) A γ-chain complex forms a functional receptor on cloned human lymphocytes with natural killer-like activity. *Nature* **325**, 723.

Morello, D., Daniel, F., Baldacci, P., Cayre, Y., Gachelin, G. & Kourilsky, P. (1982) Absence of significant H-2 and β_2-microglobulin mRNA expression by mouse embryonal carcinoma cells. *Nature* **296**, 260.

Murphy, D. B. & Homer, R. J. (1987) A Q region product as a CTL restriction element? In: *H-2 antigens: Genes, molecules, function*, ed. David, C. S., p. 359. Plenum Press, New York, USA.

Nakanishi, N., Maeda, K., Ito, K., Heller, M. & Tonegawa, S. (1987) Tγ protein is expressed on murine fetal thymocytes as a disulphide-linked heterodimer. *Nature* **325**, 720.

O'Brien, R. L., Happ, M. P., Dallas, A., Palmer, E., Kubo, R. & Born, W. K. (1989) Stimulation of a major subset of lymphocytes expressing T cell receptor $\gamma\delta$ by an antigen derived from Mycobacterium tuberculosis. *Cell* **57**, 667.

Old, L. J., Boyse, E. A. & Stockert, E. (1963) Antigenic properties of experimental leukemias. I. Serological studies *in vitro* with spontaneous and radiation-induced leukemias. *J. Natl. Cancer. Inst.* **31**, 977.

Old, L. J. & Stockert, E. (1977) Immunogenetics of cell surface antigens of mouse leukemia. *Ann. Rev. Genet.* **11**, 127.

Ostrand-Rosenberg, S., Nickerson, D. A., Clements, V. K., Garcia, E. P., Lamouse-Smith, E., Hood, L. & Stroynowski, I. (1989) Embryonal carcinoma cells express Qa and Tla class I genes of the major histocompatibility complex. *Proc. Natl. Acad. Sci. USA* **86**, 5084.

Oudshoorn-Snoek, M. & Demant, P. (1990) Identification and expression of the *Tla* region gene *T11*^{b} and its Qa-like product. *J. Immunol.* **145**, 1270.

Parham, P., Lomen, C. E., Lawlor, D. A., Ways, J. P., Holmes, N., Coppin, H. L., Salter, R. D., Wan, A. M. & Ennis, P. D. (1988) Nature of polymorphism in HLA-A, -B, and -C molecules. *Proc. Natl. Acad. Sci. USA* **85**, 4005.

Pelkonen, J., Traunecker, A. & Karjalainen, K. (1987) A new mouse TCR V_γ gene that shows remarkable evolutionary conservation. *EMBO J.* **6**, 1941.

Porcelli, S., Brenner, M. B., Greenstein, J. L., Balk, S. P., Terhorst, C. & Bleicher, P. A. (1989) Recognition of cluster of differentiation 1 antigens by human CD4$^-$ CD8$^-$ cytolytic T lymphocytes. *Nature* **341**, 447.

Saito, H., Kranz, D. M., Takagaki, Y., Hayday, A. C., Eisen, H. N. & Tonegawa, S. (1984) Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. *Nature* **309**, 757.

Salter, R. D., Benjamin, R. J., Wesley, P. K., Buxton, S. E., Garret, T. P. J., Clayberger, C., Krensky, A. M., Norment, A. M., Littman, D. R. & Parham, P. (1990) A binding site for the T-cell co-receptor CD8 on the α_3 domain of HLA-A2. *Nature* **345**, 41.

Shen, F.-W., Chorney, M. J. & Boyse, E. A. (1982) Further polymorphism in the *Tla* locus defined by monoclonal TL antibodies. *Immunogenetics* **15**, 573.

Simister, N. E. & Mostov, K. E. (1989) An Fc receptor structurally related to MHC class I antigens. *Nature* **337**, 184.

Singer, D. S., Hare, J., Golding, H., Flaherty, L. & Rudikoff, S. (1988) Characterization of a new subfamily of class I genes in the *H-2* complex of the mouse. *Immunogenetics* **28**, 13.

Stanton, T. H., Carbon, S. & Maynard, M. (1981) Recognition of alternate alleles and mapping of the *Qa1* locus. *J. Immunol.* **127**, 1640.

Steinmetz, M., Frelinger, J. G., Fisher, D., Hunkapiller, T., Pereira, D., Weissman, S. M., Uehara, H., Nathenson, S. & Hood, L. (1981) Three cDNA clones encoding mouse transplantation antigens: Homology to immunoglobulin genes. *Cell* **24**, 125.

Stern, P. L., Beresford, N., Bell, S. M., Thompson, S., Jones, K. & Mellor, A. (1986) Murine embryonal carcinoma cells express class I MHC-like antigens. *J. Immunogenetics* **13**, 133.

Strominger, J. L. (1989) The γδ T cell receptor and class Ib MHC-related proteins: Enigmatic molecules of immune recognition. *Cell* **57**, 895.

Stroynowski, I. (1990) Molecules related to class-I major histocompatibility complex antigens. *Ann. Rev. Immunol.* **8**, 501.

Terhorst, C., van Agthoven, A., LeClair, K., Snow, P., Reinherz, E. & Schlossman, S. (1981) Biochemical studies of the human thymocyte cell-surface antigens T6, T9 and T10. *Cell* **23**, 771.

Tonegawa, S. (1983) Somatic generation of antibody diversity. *Nature* **302**, 575.

Tonegawa, S., Berns, A., Bonneville, M., Farr, A., Ishida, I., Ito, K., Itohara, S., Janeway, C. A. Jr., Kanagawa, O., Katsuki, M., Kubo, R., Lafaille, J., Mombaerts, P., Murphy, D., Nakanishi, N., Takagaki, Y., Van Kaer, L. & Verbeek, S. (1989) Diversity, development, ligands and probable functions of γδ T cells. *Cold Spring Harbor Symp. Quant. Biol.* **49**, 31.

Townsend, A. & Bodmer, H. (1989) Antigen recognition by class I-restricted T lymphocytes. *Ann. Rev. Immunol.* **7**, 601.

Transy, C., Russel Nach, S., David-Watine, B., Cochet, M., Hunt, S. W. III, Hood, L. E. & Kourilsky, P. (1987) A low polymorphic mouse H-2 class I gene from the *Tla* complex is expressed in a broad variety of cell types. *J. Exp. Med.* **166**, 341.

Traunecker, A., Oliveri, F., Allen, N. & Karjalainen, K. (1986) Normal T cell development is possible without "functional" γ chain genes. *EMBO J.* **5**, 1589.

Vidović, D., Roglić, M., McKune, K., Guerder, S., MacKay, C. & Dembić, Z. (1989) Qa-1 restricted recognition of foreign antigen by a γδ T-cell hybridoma. *Nature* **340**, 646.

Watts, S., Wheeler, C., Morse, R. & Goodenow, R. S. (1989) Amino acid comparison of the class I antigens of mouse major histocompatibility complex. *Immunogenetics* **30**, 390.

Weiss, E. H., Golden, L., Fahrner, K., Mellor, A. L., Devlin, J. J., Bullman, H., Tiddens, H., Bud, H. & Flavell, R. A. (1984) Organization and evolution of the class I gene family in the major histocompatibility complex of the C57BL/10 mouse. *Nature* **310**, 650.

Yokoyama, K., Stockert, E., Old, L. J. & Nathenson, S. G. (1982) Structural evidence that the small subunit found associated with the TL antigen is β_2 microglobulin. *Immunogenetics* **15**, 543.

Zijlstra, M., Bix, M., Simister, N. E., Loring, J. M., Raulet, D. H. & Jaenisch, R. (1990) β2-Microglobulin deficient mice lack $CD4^-8^+$ cytolytic T cells. *Nature* **344**, 742.