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Recognition of a self major histocompatibility complex TL region product by $\gamma\delta$ T-cell receptors

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ABSTRACT **Ligand specificity of a murine $\gamma\delta$ T-cell receptor-expressing hybridoma (KN6) derived from adult thymocytes has been analyzed in detail. The molecule recognized by the KN6 $\gamma\delta$ T-cell receptor is expressed on syngeneic cells of various sources (peritoneal macrophages, thymocytes, spleen cells, and Abelson murine leukemia virus-transformed cell lines) and on transformed cells arrested at an early stage of development (e.g., PCC3 embryonal carcinoma cells). Linkage of the gene coding for the KN6 ligand to the major histocompatibility complex genes could be demonstrated by testing KN6 hybridoma reactivity to cells from congenic strains that differ only at H-2. In addition, analysis of recombinant strains indicates that the gene controlling the KN6 ligand is located in or distal to the TL region. Involvement of the KN6 $\gamma\delta$ T-cell receptor in this recognition process could be directly demonstrated by transferring the KN6 TL specificity after introduction of the productively rearranged KN6 γ and δ genes into an $\alpha\beta$ T-cell clone or into the germ line in transgenic mice. These observations raise the possibility that at least some $\gamma\delta$ cells regulate hemopoietic cell maturation and activation.**

T cells participating in most immune responses bear a receptor specific for antigen fragments bound to autologous major histocompatibility complex (MHC) class I and II molecules. These cells express T-cell receptors (TCRs) comprised of variable α and β chains associated with the invariable CD3 molecules and either CD4 or CD8 molecules, which influence T-cell activation by antigens bound to class II or class I molecules, respectively (1). A subset of T lymphocytes has been identified that expresses surface CD3-associated heterodimers composed of γ and δ chains (2–5). Lymphocytes bearing $\gamma\delta$ TCRs, representing 0.5–10% of $CD3^+$ thymocytes or splenocytes, often lack CD4 and CD8 molecules (3, 6–9). Unlike well-characterized T cells bearing $\alpha\beta$ TCRs, the function and ligand specificity of $\gamma\delta$ TCR T cells remains poorly understood. However, the preferential distribution of $\gamma\delta$ TCR T cells in epithelial tissues, such as skin (10, 11) or gut epithelium (12, 13), suggests a role as regulators of epithelial cell differentiation and/or as a first line of defense against epithelial cell infection (14). In addition, because of their early appearance during thymic ontogeny (5, 7, 8), these lymphocytes have also been thought to act as regulators of lymphoid cell differentiation and activation.

To investigate $\gamma\delta$ T-cell function, we have produced a set of $\gamma\delta$ hybridomas derived from thymocytes isolated at various stages of development (15). The ligand specificity of one of these hybridomas is analyzed in detail in the present report. The data will show that the $\gamma\delta$ TCR from this hybridoma recognizes an autologous cell surface MHC molecule that is distinct from the conventional class I and class II products usually recognized by $\alpha\beta$ TCRs. The tissue distribution of this ligand raises the possibility that at least some $\gamma\delta$ cells interact with hemopoietic cells of both T-cell and non-T-cell lineages and regulate their differentiation and/or activation.

MATERIALS AND METHODS

Animals. Mouse strains utilized in this study are listed in Table 1 and were obtained from our breeding colony or were purchased from The Jackson Laboratory. Lorraine Flaherty (The Wadsworth Center, Albany) kindly provided the Boy sublines. Transgenic animals carrying the functionally rearranged γ and δ chain genes from hybridoma KN6 were constructed after microinjection of DNA into the pronucleus of fertilized eggs (unpublished data).

Cells. T-cell hybridomas were obtained after fusion of BW5147 thymoma cells with C57BL/6 $CD4^-$ $CD8^-$ thymocytes (15). Features of their $\gamma\delta$ TCRs (γ and δ genes rearrangement and expression) have been described (15). A-20 cells are B-lymphoma cells of BALB/c origin; the Fc receptor-negative variant of this line has been described (17). Abelson murine leukemia virus-transformed T-cell line 2052C was kindly provided by Rex Risser (University of Wisconsin, Madison). The PCC3 embryonal carcinoma cell line, of strain 129 origin, was obtained from Suzanne Rosenberg (University of Maryland, Baltimore) (18). The $\alpha\beta$ T-cell clone D10 recognizes conalbumin in the context of class II $I\text{-}A^k$ molecules (19). D10 cells were transfected using the electroporation technique (20) with KN6 δ TCR genomic DNA pδ23/15 inserted into pII-15 vector (unpublished data). D10 cells transfected with KN6 δ TCRs were supertransfected, using the DEAE-dextran technique as modified by Grosschedl and Baltimore (21), with KN6 γ TCR cDNA inserted in the CDM8 cDNA expression vector containing enhancer and promoter sequences from the Moloney leukemia virus long terminal repeat. One week after transfection, cells were positively selected by using monoclonal antibody (mAb) 3A10 (anti-δ) conjugated to magnetic beads according to procedures recommended by the manufacturer (Dynal, Oslo).

Antibodies. mAb 2C11 is directed against the ε subunit of the CD3 complex (22) and was kindly provided by J. A. Bluestone (University of Chicago). mAb H57 (23) that reacts with all murine $\alpha\beta$ T cells was a kind gift of R. Kubo (University of Colorado, Denver). mAbs 3A10, 8D6, and 5C10 were generated in this laboratory and are directed against a constant region of the TCR δ chain, $V_{\gamma}4/V_{\delta}5$

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Abbreviations: TCR, T-cell receptor; MHC, major histocompatibility complex; mAb, monoclonal antibody; Vγ4, $V_{\delta}5$, etc., variable regions of γ, δ, etc. chains, respectively; IL, interleukin.

Table 1. Ligand recognized by KN6 is controlled by MHC genes telomeric to the Q region

Strain	Region*				Phenotype	% proliferation
	K	D	Q	TL		
A.BY/SnJ	b	b	b	b	+	−75
C57BL/6J(B6)	b	b	b	b	+	−76 ± 12
C57BL/10SnJ(B10)	b	b	b	b	+	−89 ± 6
A.CA/Sn	f	f	f	f	±?	−37 ± 19
B10.M/Sn	f	f	f	f	±?	−36 ± 29
B10.D2/nSnJ	d	d	d	d	−	+30 ± 17
B10.BR/SgSnJ	k	k	k	k	−	−5 ± 12
P/J	p	p	p	p	−	+31 ± 27
B10.G/Sg	q	q	q	q	−	+8 ± 6
B10.RIII(71NS)/SnJ	r	r	r	r	−	+11 ± 2
B10.S/Sg	s	s	s	s	−	+8 ± 23
A/Boy	a	a	a	a	−	+4 ± 8
B6/Boy	b	b	b	b	+	−85 ± 4
A-*Tla*^{b}/Boy	a	a	a	b	+	−66 ± 14
B6-*Tla*^{a}/Boy	b	b	b	a	−	−2 ± 13

Other positive strains include BALB.B/Li, B10.A(2R)SgSnJ, B10.A(R149)-*Tla*^{b}/Mrp, B10.A(R410)-*Tla*^{b}/Mrp, B10.P(13R)/Sg, B10.SM(70NS)/Sn, and TBR2. Other minus strains include AKR/J, A/WySnJ, BALB/c, B6-*H-2*^{k}/Boy, B6.K1/Fla, B6.K2/Fla, B10.A/SgSnJ, B10.PL(73NS)/Sn, B10(R297)-*Tla*^{a}/Mrp, B10(R310)-*Tla*^{a}/Mrp, C3H/HeJ, and MA.My/J. +, Strong inhibition; ±?, possible intermediate inhibition (results variable and not consistent); −, no inhibition. For % proliferation, data shown are with a single clone, KN6-7. All experiments were done at least twice, except with A.BY, which also tested positive with the original KN6 line.
*Haplotype origin of region.

containing TCRs (where $V_\gamma4$ and $V_\delta5$ are γ and δ chain variable regions), and unique epitopes of the $\gamma\delta$ TCR from hybridoma KN6, respectively (9).

Proliferation Assays. All experiments were carried out in triplicate.

TCR crosslinking. Hybridoma culture medium was Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal calf serum and 2 mM L-glutamine. Hybridoma cells (2×10^3 cells per well) were cultured for 3 days in the presence of anti-CD3 mAb or anti-CD8 mAb (final dilution of hybridoma supernatant, 1:4) and/or irradiated (6000 rads; 1 rad = 0.01 Gy) A-20 B lymphoma cells (2.5×10^4 cells per well). Plates were pulsed-labeled for 6 hr with [*methyl*-^{3}H]thymidine (NEN) (1 μCi per well; 1 Ci = 37 GBq) and harvested. Percent of growth inhibition was calculated as follows: [(cpm from hybridoma with stimuli)/(cpm from hybridoma without stimulus) − 1] × 100.

Cocultures. Hybridoma cells were cultured for 3 days in the presence of irradiated spleen cells or thymocytes (1500 rads; 10^5 cells per well), peritoneal macrophages (1500 rads; 5×10^4 cells per well), Abelson-transformed cells (6000 rads; 5×10^4 cells per well), or PCC3 teratocarcinoma cells (10,000 rads; 10^4 cells per well) and were pulse-labeled for 6 hr with [^{3}H]thymidine.

Reversal of growth inhibition. Hybridoma cells were preincubated for 30 min with mAb prior to addition of irradiated stimulator cells and cultured for 3 days.

D10 proliferation and lymphokine production assay. Approximately 5×10^4 responder cells were cultured with irradiated spleen cells (1500 rads; 10^5 cells per well) in 200 μl of EHAA medium supplemented with 10% fetal calf serum, 5×10^{-5} M 2-mercaptoethanol, and 2 mM L-glutamine (19). Proliferation was assayed after 3 days of culture and is expressed as percentage of relative response (RR): %RR = (cpm of responder cells with stimulator cells)/(cpm of responder cells alone). For interleukin (IL) 2/IL-4 production assay, 100 μl of culture supernatant was removed after 2 days of culture and tested for its ability to trigger proliferation of IL-2/IL-4-dependent HT-2 cells, as described (19).

Culture of $\gamma\delta$ cells from transgenic animals. Approximately 5×10^4 spleen cells or thymocytes from $\gamma\delta$ KN6 transgenic animals were cultured with irradiated spleen cells (1500 rads; 10^5 cells per well) in RPMI 1640 medium/10% fetal calf serum/2 mM L-glutamine/10% (vol/vol) supernatant from Con A-stimulated spleen cells. Proliferation was assayed after 5 days of culture and cells were stained with mAbs 3A10 and 5C10 (biotin-conjugates or uncoupled mAb) after 8 days of culture.

RESULTS

KN6 Hybridoma Growth Is Inhibited by Syngeneic But Not Allogeneic Cells. Hybridomas expressing a variety of $\gamma\delta$ TCRs were generated after fusion of C57BL/6 thymocytes with the BW5147 thymoma (15). Before analyzing the ligand specificity of these hybridomas, the cells were assayed for their ability to respond when their TCRs were crosslinked. Crosslinking the $\alpha\beta$ TCR, which usually promotes growth of normal T cells, results in growth inhibition of T-cell hybridomas (24). To test whether this also occurred with $\gamma\delta$ T cells, hybridomas were cultured in the presence of the CD3 mAb 2C11 (17) and their receptors were crosslinked by incubation with Fc receptor-positive A-20 B-lymphoma cells. Under these culture conditions, [^{3}H]thymidine uptake by the CD3-positive hybridomas listed in Table 2 was strongly inhibited, whereas proliferation of BW5147 thymoma cells or of CD3-negative variants of a $\gamma\delta$ hybridoma (KN6-19 and KN6-2) was not affected (data not shown). Thus the growth of $\gamma\delta$ hybridomas can be inhibited when their TCRs are crosslinked.

Using the growth inhibition assay, $\gamma\delta$ hybridomas were then screened for their reactivity with stimulator cells from various tissue and strains. Although crosslinking with CD3 mAb inhibited growth of these cells, only hybridoma KN6 significantly responded to syngeneic (C57BL/6) but not allogeneic [BALB/c (Table 2), CBA/J, and AKR/J (data not shown)] spleen cells. The response to syngeneic spleen cells was observed in three CD3-positive subclones of the KN6 hybridoma (KN6-7, KN6-12, and KN6-10) (Table 2). KN6 hybridoma growth was inhibited efficiently by thymocytes, peritoneal macrophages, splenocytes, cells from the Abelson-transformed B6 T-cell line 2052C, and PCC3 embryonal

Table 2. Proliferation of $\gamma\delta$ hybridomas cultured with BALB/c or C57BL/6 spleen cells

Hybridoma	TCR status	% proliferation	
		BALB/c	C57BL/6
BW	—	−15	+5
152	$V_\gamma5/V_\delta1$	+8	−3
129	$V_\gamma5/V_\delta1$	+50	+43
98	$V_\gamma6/V_\delta1$	−10	−8
56	$V_\gamma6/V_\delta1$	−12	−5
106	$V_\gamma7/V_\delta5$	+37	+30
1	$V_\gamma4/V_\delta7$	+48	+42
28	$V_\gamma4/V_\delta5$	+27	+16
102	$V_\gamma4/V_\delta5$	+60	+64
108	$V_\gamma4/V_\delta7$	+38	+23
159	$V_\gamma4/V_\delta5$	+18	+35
KN6-7	$V_\gamma4/V_\delta5$	+30	**−62**
KN6-12	$V_\gamma4/V_\delta5$	−4	**−42**
KN6-10	$V_\gamma4/V_\delta5$	+48	**−47**
KN6-19	—	+48	+35
KN6-2	—	+5	+15

[^{3}H]Thymidine uptake of $\gamma\delta$ hybridomas was assayed after 3 days of culture and percent proliferation was calculated. Stimulation of hybridoma growth in these experiments does not seem to involve $\gamma\delta$ TCR since it occurred with both $\gamma\delta$ TCR-positive and -negative cells (e.g., KN6-19). Boldface numbers are significant ($P < 0.05$, Student's t test) growth inhibition.

Table 3. Hybridoma KN6 proliferation in the presence of stimulator cells of H-2^b origin

	% proliferation				
Responder	Thymus	Spleen	PM	2052C	PCC3
KN6-7	−80	−48	−62	−78	−95
KN6-10	−48	−38	−25	NT	−81
KN6-12	−52	−35	−62	−65	−90
KN6-19 (−)	+13	+62	+27	+10	+1
KN6-2 (−)	+4	NT	+18	NT	+4

Three $CD3^+$ subclones (KN6-7, KN6-10, and KN6-12) and two $CD3^-$ variants (KN6-19 and KN6-2) of hybridoma KN6 were cultured with Abelson-transformed cells 2052C, freshly isolated C57BL/6 thymocytes, spleen cells, peritoneal macrophages (PM), or PCC3 embryonal carcinoma cells. Their proliferation was assayed after 3 day in culture. NT, not tested.

carcinoma cells (Table 3). The fact that growth of TCR-negative variants of KN6 (KN6-19 and KN6-2) was not affected by syngeneic cells (Tables 2 and 3) provides evidence that growth inhibition was mediated by an interaction involving the TCR.

KN6 Growth Inhibition Induced by Syngeneic Cells Is Specifically Reversed by Antibodies Directed Against $\gamma\delta$ TCR. Several antibodies directed against KN6 $\gamma\delta$ TCR were generated after immunization of Syrian hamsters with purified KN6 $\gamma\delta$ TCR–anti-CD3 immune complexes (9). Three of these mAbs (3A10, 8D6, and 5C10) were extensively characterized. mAb 3A10 is directed against a conserved region of the TCR δ chain and, hence, recognizes all $\gamma\delta$ T lymphocytes (9). In contrast, mAbs 8D6 and 5C10 recognize $\gamma\delta$ subsets: 8D6 detects $V_\gamma4$- and $V_\delta5$-encoded TCRs and mAb 5C10 reacts only with the KN6 hybridoma (9).

To directly demonstrate that growth inhibition of KN6 by syngeneic cells was mediated through the KN6 $\gamma\delta$ TCR, we prevented growth inhibition with mAbs 3A10, 8D6, or 5C10 in a soluble form. Proliferation of hybridoma KN6 was strongly inhibited by the Abelson cell line 2052C or by syngeneic thymocytes (Fig. 1). Addition of anti-δ (3A10), anti-V4/V5 (8D6), or anti-KN6 (5C10) mAbs at the onset of culture caused a dose-dependent reversal of growth inhibition, whereas addition of an irrelevant antibody (L3T4, an anti-mouse CD4) had no effect (Fig. 1). It should be noted that mAb 3A10 was a much less efficient blocker of growth inhibition than mAbs 8D6 and 5C10. These differences may reflect differences in the distance between the epitopes recognized by the mAbs and the ligand-binding site, with

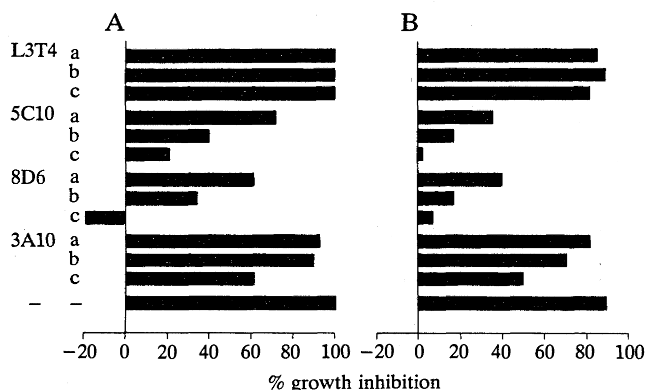

FIG. 1. Reversal of hybridoma KN6 growth inhibition by antibodies directed against KN6 $\gamma\delta$ TCR. Hybridoma cells were cultured with irradiated 2052C Abelson-transformed cells (*A*) or C57BL/6 thymocytes (*B*) alone (−) or in the presence of anti-$\gamma\delta$ (3A10), anti-V4V5(8D6), anti-KN6(5C10), or anti-CD4 (RL172-4, labeled L3T4) hybridoma culture supernatant. Final dilutions of hybridoma culture supernatant are as follows. Bars: a, 1:64; b, 1:16; c, 1:4.

epitopes 8D6 and 5C10 being much closer to the ligand-binding site than epitope 3A10.

Polymorphic Gene Mapping in the MHC Telomeric to the Q Region Controls Expression of the KN6 Ligand. The observation that hybridoma KN6 specifically responds to syngeneic (C57BL/6) but not to allogeneic (BALB/c) cells (Table 2) suggests that the gene controlling the KN6 ligand is polymorphic. To confirm this and to map the gene, hybridoma KN6 was screened for reactivity with a panel of spleen cells from various strains (Table 1). Linkage to the MHC was demonstrated by results with congenic strains that differ only at the H-2 locus. Thus, KN6 cell proliferation was strongly inhibited by cells from $H\text{-}2^b$ strains, partially inhibited by cells from $H\text{-}2^f$ strains, and not affected by cells from $H\text{-}2^d$, $H\text{-}2^k$, $H\text{-}2^p$, or $H\text{-}2^s$ mice. Most important, analysis of recombinant strains shows that the gene controlling the KN6 ligand is located in or distal to the TL region. This conclusion is based on the observation that the hybridoma responds to B6/Boy ($K^bD^bQa^bTla^b$) and A.Tla^b/Boy cells ($K^kD^dQa^d/TL^b$), but not to A/Boy ($K^kD^dQa^dTL^d$) or B6-Tla^a/Boy ($K^bD^bQa^b/TL^d$) cells (Table 1).

Hybridoma KN6 Reacts with PCC3 Embryonal Carcinoma Cells That Do Not Express Class I H-2K, H-2D, and H-2L or class II I-A and I-E Molecules. To confirm that KN6 cells recognized a determinant distinct from classical MHC class I and class II molecules, we analyzed their reactivity with embryonic transformed cell lines arrested at a very early stage of development (25). These cells usually do not express detectable amounts of the class I H-2K, -D, or -L or class II I-A or I-E MHC molecules but do express to some extent some Qa and TL MHC gene products on their surface (25, 26). Growth of hybridoma KN6 was strongly inhibited by one of these embryonal carcinoma cell lines, PCC3 (18) (Table 3). This PCC3-induced growth inhibition was restricted to CD3-positive subclones of hybridoma KN6 (KN6-7, KN6-12, and KN6-10) (Table 3) and could be blocked by mAb directed against KN6 $\gamma\delta$ TCR (data not shown). This indicates that the inhibition of proliferation was probably due to interaction between KN6 $\gamma\delta$ TCR and a ligand borne by PCC3 cells.

KN6 Specificity Generated by Transferred γ and δ Genes. The genes coding for KN6 γ and δ chains have been cloned (unpublished data) and transfected into D10, a T-cell clone that expresses $\alpha\beta$ TCRs and recognizes class II I-A^k molecules plus the foreign antigen conalbumin (19). Unlike parental D10 cells or D10 cells transfected with the KN6 δ gene (D10δ), D10 cells transfected with both KN6 γ and δ genes (D10$\gamma\delta$) expressed significant levels of $\gamma\delta$ TCR, although to a much lesser extent than $\alpha\beta$ TCRs (Fig. 2*A*). B10.A(2R)/SnJ (K^kD^d/Qa^bTL^b) and A.Tla^b/Boy ($K^kD^dQa^d/TL^b$) spleen cells stimulated proliferation and lymphokine production by D10$\gamma\delta$ but not by D10δ (Fig. 2*B*). The TL region specificity of the transferred response was confirmed by the observation that none of the transfectants responded to A/Boy ($K^kD^dQa^dTL^d$) or B10.A/SgSnJ ($K^kD^dQa^dTL^d$) cells (Fig. 2*B*).

In addition, transgenic mice carrying the functionally rearranged KN6 γ and δ genes have been constructed by microinjection of the cloned DNA into the pronucleus of fertilized eggs (unpublished data). In these animals, ≈2% of thymocytes and 5% of splenocytes are $\gamma\delta$ TCR-positive and most of them carry on their surface the transgene-encoded $\gamma\delta$ TCR (Fig. 3*C* and data not shown). Thymocytes and splenocytes from $H\text{-}2^{k/d}$ KN6 transgenic mice proliferated when they were cultured with irradiated A-Tla^b spleen cells but not with control A/Boy spleen cells (Fig. 3*A*). Proliferation was mostly due to the selective activation and growth of T cells bearing the KN6 $\gamma\delta$ TCR. After 8 days in culture, ≈80% of the total cells (Fig. 3*E*) and >95% of CD3-positive blasts (data not shown) bore the KN6 $\gamma\delta$ TCR. Uncultured cells or cells cultured with control A/Boy splenocytes contained <4% of total cells as KN6 $\gamma\delta$ TCR-bearing cells (Fig. 3 *C* and

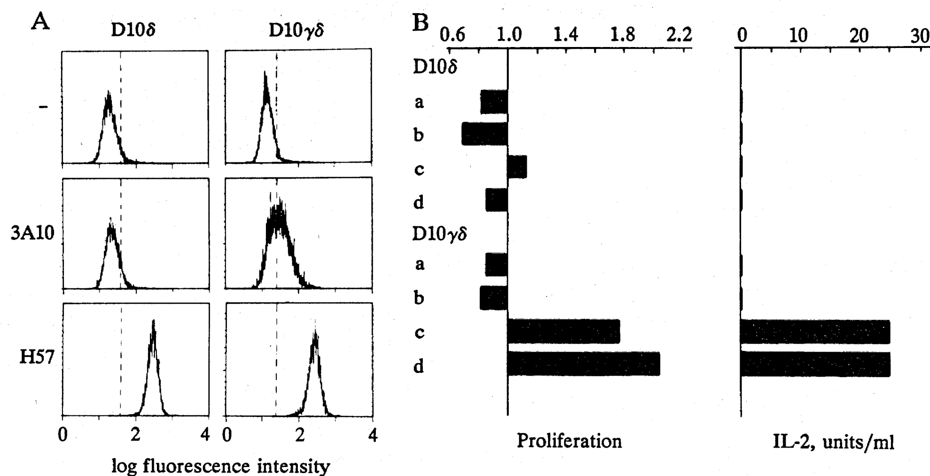

FIG. 2. Transfer of KN6 reactivity after transfection of KN6 γ and δ genes into D10 cells. (*A*) D10 $\alpha\beta$ T-cell clone was transfected with the KN6 δ gene (D10δ) or with the KN6 γ and δ genes (D10$\gamma\delta$). After selection, transfectants were stained with mAb 3A10 (anti-C_δ) or mAb H57 (anti-$\alpha\beta$ TCR) or − (irrelevant antibody) and analyzed with a fluorescence-activated cell sorter. (*B*) Proliferation of D10δ and D10$\gamma\delta$ cells was assayed after 3 days of culture with irradiated B10.A/SgSnJ ($K^kD^dQa^dTL^d$) (bars a), A/Boy ($K^kD^dQa^dTL^d$) (bars b), B10.A(2R)SgSnJ (K^kD^d/Qa^bTL^b) (bars c), and A-*Tla*^{b}/Boy ($K^kD^dQa^d/TL^b$) (bars d) spleen cells and data are expressed as percentage of relative response. Culture supernatant from these cultures was harvested after 2 days and IL-2 was measured by an HT-2 cell proliferation assay.

D). The amplification index of the KN6 splenic or thymic cell populations [(total number of KN6$^+$ cells after culture)/(total number of KN6$^+$ cells before culture)] after 8 days in culture with A-*Tla*^{b} or A/Boy spleen cells is shown on Fig. 3*B*. Thus these observations indicate that the KN6 specificity for syngeneic TL-region-associated MHC gene products can be transferred by introduction of the functionally rearranged KN6 γ and δ chain genes into a T-cell clone or into the germ line in transgenic mice.

DISCUSSION

In the present study, we describe the recognition by a $\gamma\delta$ TCR of a molecule expressed on syngeneic cells. The ligand recognized by the KN6 $\gamma\delta$ TCR is expressed on cells of various hemopoietic lineages—namely peritoneal macrophages, thymocytes, and Abelson-transformed cell lines (Tables 2 and 3). Linkage of the KN6 ligand to the MHC was demonstrated after testing KN6 reactivity with congenic strains that differ only at the *H-2* locus. However, the fact that KN6 cells strongly react with embryonal carcinoma cell lines (such as PCC3) suggested that KN6 cells recognized a ligand distinct from MHC K- and D-region class I or I-region class II molecules, since PCC3 cells do not express detectable levels of these molecules (25, 26). Indeed, based on data with several recombinant strains, the MHC-linked gene controlling the KN6 ligand maps in or distal to the TL region. To date, at least three cell surface class I molecules are known to be controlled by the TL region. These include Tla molecules (M_r, 45,000), which are expressed on thymocytes and leukemia cells, and the ubiquitously expressed Qa-1 (M_r, 48,000) and Hmt molecules (27–29). Since the KN6 ligand is expressed on cells outside the thymus in TL$^-$ *H-2*^{b} strains, the classically defined Tla molecules do not appear to be involved. Based on strain distribution patterns, the KN6 ligand can also be distinguished from known specificities on Qa-1 and Hmt molecules. However, we cannot rule out the possibility that a new specificity on one of these latter molecules is being recognized. Other possible candidates include products of other class I genes (*T1–T15* and *-37* in *H-2*^{b} mice) (29, 30), products of closely linked class I-like

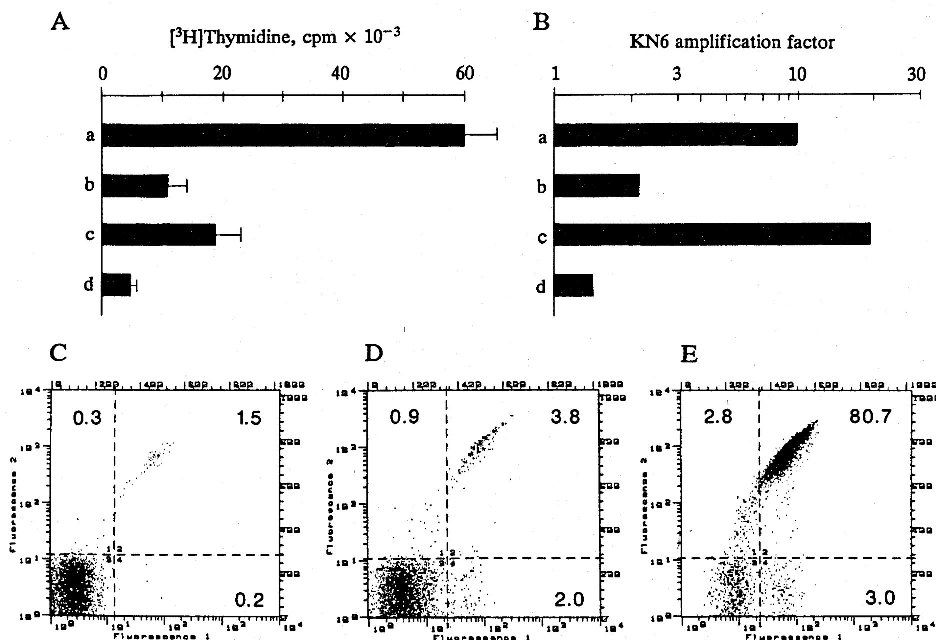

FIG. 3. Reactivity of splenic and thymic $\gamma\delta$ cells from $\gamma\delta$ KN6 transgenic animals with A/Boy and A-T*la*^{b}/Boy cells. (*A*) Proliferation of spleen cells (bars a and b) or thymocytes (bars c and d) from $\gamma\delta$ KN6 transgenic animals (*H-2*$^{k/d}$ heterozygotes) was assayed after 5 days of culture with irradiated A.*Tla*^{b} ($K^kD^dQa^d/TL^b$) (bars a and c) or A ($K^kD^dQa^dTL^d$) (bars b and d) spleen cells. (*B*) Cells were cultured as in *A* and stained before and after culture with mAb 5C10 (anti-$\gamma\delta$ KN6). KN6 amplification factor was calculated by the formula [(total number of KN6$^+$ cells after culture)/(total number of KN6$^+$ cells before culture)]. (*C–E*) Thymocytes from a $\gamma\delta$ KN6 transgenic animal were stained with mAb 5C10 (anti-KN6) (fluorescence 2) and mAb 3A10 (anti-δ) (fluorescence 1) before culture (*C*) or after 8 days culture with A (*D*) or A-*Tla*^{b} (*E*) spleen cells and analyzed with a fluorescence-activated cell sorter.

genes (e.g., *Mbl*) (31) or unrelated loci (e.g., *Tlevl*) (32), and TL region controlled cell surface molecules of M_r 53,000 (33), M_r 60,000 (34), or M_r 55,000–75,000 (35).

$\gamma\delta$ T-cell lines reactive with allogeneic K- and D-region class I molecules and with the I-region class II molecules have been described (36, 37). In one study (37), possible control of the class I ligand by a TL region gene was inferred based on correlation with Tla serological typing. These observations suggested that $\gamma\delta$ cells may not be different from $\alpha\beta$ T cells with respect to ligand specificity and could recognize antigen-derived peptides presented by H-2 molecules. However, these studies dealt only with those $\gamma\delta$ T-cell lines that were derived after strong allogeneic stimulation. It is therefore possible that the specificities observed are not physiologically representative ones, reflecting instead a crossreactivity.

Although the data presented here provide clear evidence that a polymorphic structure mapping in or to the right of the TL region is the ligand for the KN6 $\gamma\delta$ TCR, they do not address the possible involvement of an antigen-derived peptide as a part of the ligand. If the TL-encoded molecule is a class I-like molecule, which seems most likely, the structural similarities between $\alpha\beta$ and $\gamma\delta$ TCR, on the one hand, and between K- or D-region and TL-region class I molecules, on the other, suggest that a peptide is part of the KN6 ligand. Since hybridoma KN6 is responsive to autologous cells from a number of sources, the putative peptide would appear to be derived from a self protein rather than from a foreign antigen. In this regard, it is interesting that recent studies suggest that at least some $\gamma\delta$ T cells are reactive with bacterial proteins with a high degree of homology to mammalian heat shock proteins. Possibly, peptides derived from the host's own stress-induced proteins bound to TL-region-encoded class I molecules are recognized by some $\gamma\delta$ T cells, such as hybridoma KN6 (14).

How general is the autoreactivity represented by hybridoma KN6 among $\gamma\delta$ TCR-bearing thymocytes? Although proliferation of most of the >30 thymocyte-derived $\gamma\delta$ hybridomas studied in this work was inhibited when their TCRs were crosslinked by CD3 mAb, only one (i.e., KN6) could be shown to react specifically with autologous cells. It is possible that during the fusion procedure, we are selecting against such autoreactive cells. Indeed, the growth of autoreactive hybridomas should be inhibited by the hybridoma cells themselves, since they express TCRs and are of the correct genotype to express the ligands. KN6 cells may have escaped this selection process because of down-regulation of ligand expression on their surface. Another possible explanation of the relatively low frequency of autoreactive $\gamma\delta$ hybridomas is that autoreactive $\gamma\delta$ thymocytes are negatively selected in the thymus and hybridoma KN6 is derived from a rare autoreactive thymocyte yet to be selected against.

Finally, the occurrence of autoreactive thymocytes capable of recognizing differentation molecules on cells of distinct hemopoietic lineage raises the interesting possibility that these cells regulate hemopoietic cell maturation or activation. Since $\gamma\delta$ cell lines frequently exhibit cytotoxic activity (7, 16, 37), one can imagine that autoreactive $\gamma\delta$ cells would actively eliminate cells at specific stages of differentiation (e.g., unselected or negatively selected $\alpha\beta$ thymocytes) or cells expressing abnormal levels of such ligands (e.g., after viral infection and/or cell transformation). The analysis of the physiology of $\gamma\delta$ T cells in animals carrying functionally rearranged KN6 γ and δ chain genes in their germ line (i.e., transgenic mice) should help resolve this issue.

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