Junctional Sequences Influence the Specificity of γ/δ T Cell Receptors

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Summary

T lymphocytes bearing the γ/δ T cell receptor (TCR-γ/δ) express a limited number of germline variable gene segments, generating receptor sequence diversity primarily through junctional mechanisms. To examine the role of V(D)J junctional sequences in antigen recognition by TCR-γ/δ, we derived an alloreactive murine TCR-γ/δ^+ T cell line, LKD1, specific for the I-A^d class II major histocompatibility complex (MHC) molecule, and compared its receptor with that expressed by a previously characterized class II MHC alloreactive T cell line, LBK5, specific for I-$E^{k,b,s}$ Ia molecules. Both LKD1 and LBK5 express receptors encoded by rearranged Vγ1.2Jγ2 and Vδ5Dδ2Jδ1 gene elements, differing in sequence only in the V(D)J junctional regions of the γ and δ genes. These results demonstrate that junctionally encoded sequences corresponding to the putative third complementarity determining region can influence the antigen specificity of TCR-γ/δ.

There is currently only limited information as to the structural basis of antigen recognition by the TCR-γ/δ. Analyses of expressed TCR γ and δ genes have shown that although there is quite limited expression of distinct germline variable (V) and joining (J) elements, there is extensive junctional sequence diversity generated by inexact V(D)J joining and the addition of N region nucleotides (1–5). This is especially true for TCR-δ, wherein many genes are encoded by VδDδ1Dδ2Jδ rearrangements, allowing for the unprecedented generation of diversity at three recombination sites rather than two (2). Thus, junctional diversity accounts for most of the sequence diversity generated in the TCR-γ/δ repertoire. One implication of this observation is that V(D)J junctionally encoded sequences should be important for antigen recognition by TCR-γ/δ. This region of the receptor, corresponding to the CDR3 of immunoglobulins, has a critical role in antigen recognition by TCR-α/β (6).

In this report, we show that two alloreactive TCR-γ/δ^+ T cell lines specific for distinct class II MHC molecules express receptors encoded by the same VJγ and VDJδ elements, differing in sequence only in the V(D)J junctions. These results directly demonstrate that sequences corresponding to the CDR3 can influence TCR-γ/δ specificity and presumably are important for antigen binding.

Materials and Methods

Animals. Inbred mice were purchased from The Jackson Laboratory (Bar Harbor, ME) or were bred in our own colony.

Cell Lines and Assays. Alloreactive $CD3^+CD4^-CD8^-$ TCR-γ/δ^+ T cell lines were derived as previously described (7) with one modification. After two cycles of in vitro stimulation with allogeneic APC, a single cycle of stimulation was performed with 10 μg/ml of a plate-bound mAb, UC7-13D5, specific for all TCR-γ/δ. Thereafter, the line was activated with allogeneic APC as before. $CD4^-CD8^-$ T cell populations were generated after treatment with CD4- and CD8-specific mAbs followed by complement-mediated lysis (7). The Ia^+ M12 B cell lymphoma and its Ia^- variant were provided by Dr. L. Glimcher (Harvard University, Boston, MA). Cell-mediated cytolysis assays were performed as described previously (7).

Monoclonal Antibodies and Antisera. The mAb 145-2C11, specific for CD3-ϵ, has been characterized (7). The Vγ1-specific antiserum has been reported previously (8). UC7-13D5 is a mAb specific for the TCR-γ/δ receptor expressed on all TCR-γ/δ cells (J. Bluestone, unpublished data). The I-A^d-specific mAb MK.D6 and the I-A^b-specific mAb Y-3P were generously provided by Dr. A. M. Kruisbeek (NCI, NIH).

Biochemical and Southern Analyses. These were performed as described (7). The Vδ5 (2) and Vγ1 (7) probes have been described previously.

DNA Sequencing. cDNA products encoding the productively

rearranged Vγ1.2 and Vδ5 genes of the LKD1 cell line were derived from high molecular weight cellular DNA by the PCR using Vγ1.2 and complementary Jγ2, and Vδ5 and complementary Jδ1 primers, respectively. The PCR products were cloned into the pGEM vector and sequenced by the dideoxy chain termination method.

Results and Discussion

An alloreactive T cell line was propagated from lymph node T cells of immunized B10.BR (H-2^k) mice by in vitro stimulation of $CD4^-CD8^-$ T cells with B10.D2 (H-2^d) splenic APC. A long-term line (LKD1) was derived and determined by flow microfluorometry to consist exclusively of TCR-γ/δ-expressing T cells (data not shown). The MHC-linked specificity of the LKD1 line was shown by demonstrating potent lysis of allogeneic B10.D2 targets relative to B10 (H-2^b) or syngeneic B10.BR target cells (Fig. 1 *A*). Lysis of D2.GD ($K^dI\text{-}A^dI\text{-}E^-D^b$) but not B10.A ($K^kI\text{-}A^kI\text{-}E^kD^d$) targets further mapped the specificity to K^d or I-A^d (Fig. 1 *B*), and I-A^d specificity was confirmed by virtue of the fact that Ia-expressing M12 (H-2^d) B cell lymphoma cells were killed but not Ia^- mutant M12 cells (Fig. 1 *C*). Inhibition of cytolysis by the I-A^d-specific mAb MK.D6 (Fig. 1 *C*) but not by the I-A^b-specific mAb Y-3P (data not shown) further confirmed the I-A^d specificity of this line.

The nature of the TCR expressed by the LKD1 cell line was examined and compared with that expressed by a previously characterized class II MHC-alloreactive γ/δ T cell line, LBK5, specific for I-$E^{k,b,s}$-encoded Ia molecules (9). Both lines expressed identically appearing CD3-associated heterodimers consisting of 31- and 45-kD proteins (Fig. 2 *A*). the LBK5 TCR is a Vγ1.2Jγ2/Vδ5Dδ2Jδ1-encoded heterodimer (9). A Vγ1-specific antiserum also precipitated the 31-kD protein expressed by LKD1 (Fig. 2 *A*), and Southern analysis showed that both LBK5 and LKD1 displayed a characteristic 16-kD Vγ1.2Jγ2 hybridizing EcoRI-rearranged band (Fig. 2 *B*) (7). Both lines also demonstrated identical rearrangements after hybridization of a Vδ5 probe to EcoRI-digested DNA (Fig. 2 *B*). The 9.6-kb Vδ5-hybridizing band represents the productive Vδ5Dδ2Jδ1 rearrangement (2, 9). Hybridization to Jδ probes revealed that there is only a single rearrangement of the TCR-δ locus in LKD1, confirming that this line expresses a Vδ5-encoded receptor protein (data not shown). Finally, analysis with additional TCR probes indicated that LKD1 was clonal (data not shown).

The functionally expressed Vγ1.2 Jγ2 and Vδ5Dδ2Jδ1 genes of LKD1 were cloned and their sequences examined in comparison with the corresponding LBK5 γ and δ receptor genes (Fig. 3). Because Southern analysis of LKD1 revealed both germline as well as rearranged Vγ1.2- and Vδ5-hybridizing bands (Fig. 2 *B*), the sequences generated by PCR necessarily represented the productive rearrangements. Extensive diversity created by inexact joining and N region nucleotide addition is present in the receptor genes of both clones. N region nucleotides encode distinct amino acids at the Vγ1.2 Jγ2 junctions of LKD1 and LBK5, and the LKD1 protein is smaller by a single amino acid as a result of the truncation of three Jγ2-encoded nucleotides during the V-J recombination (Fig. 3 *A*). The Vδ5Dδ2Jδ1 junctions of LKD1 and LBK5 are quite disparate (Fig. 3 *B*). As in the case of TCR-γ, the LKD1 δ gene encodes one less amino acid than its LBK5 counterpart, and there is little homology among the other junctionally encoded residues (Fig. 3 *B*). Both genes have N region additions at the VDδ and DJδ junctions, and the Dδ2 elements are encoded in distinct reading frames. Thus, these data are illustrative of the capacity of TCR-γ/δ for generating extensive junctional diversity.

Some analyses of TCR-γ/δ usage have suggested that the variable elements themselves mediate ligand recognition by TCR-γ/δ. For example, 28 of 28 murine γ/δ hybridomas specific for mycobacterial HSP-65 were found to express Vγ1.1Jγ4, despite significant junctional diversity shown by sequencing (10), and of these, 25 also expressed Vδ6 family-encoded proteins. Also, the selective pairing of individual TCR Vγ and Vδ segments, as well as the preferential expression

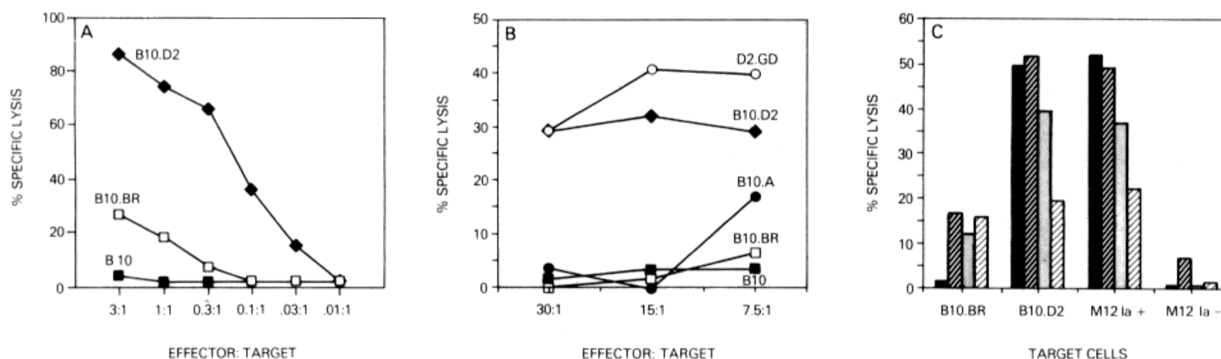

Figure 1. Class II MHC specificity of the B10.BR anti-B10.D2-alloreactive TCR-γ/δ^+ T cell line. Cytolytic activity of effector T cells was measured as referenced in Materials and Methods at various effector to target ratios on LPS-stimulated splenic target cells and on Ia^+ and Ia^- M12 (H-2^d) B lymphoma cells. (*A*) B10.D2 (K^d, I-A^d, I-E^d, D^d) (◆); B10.BR (K^k, I-A^k, I-E^k, D^k) (□); B10 (K^b, I-A^b, I-E^-, D^b) (■). (*B*) D2.GD (K^d, I-A^d, I-E^-, D^b) (○); B10.A (K^k, I-A^k, I-E^k, D^d) (●). D2.GD APC, like B10 APC, express no I-E-encoded Ia molecules. (*C*) Lysis of B10.BR and B10.D2 LPS splenic blasts, and Ia^+ vs. Ia^- M12 target cells in the presence of various concentrations of the I-A^d-specific mAb MK.D6 (0 μg/ml [■]; 1 μg/ml [▨]; 10 μg/ml [▩]; 100 μg/ml [▨]). SEs in all cases were <10% mean specific lysis.

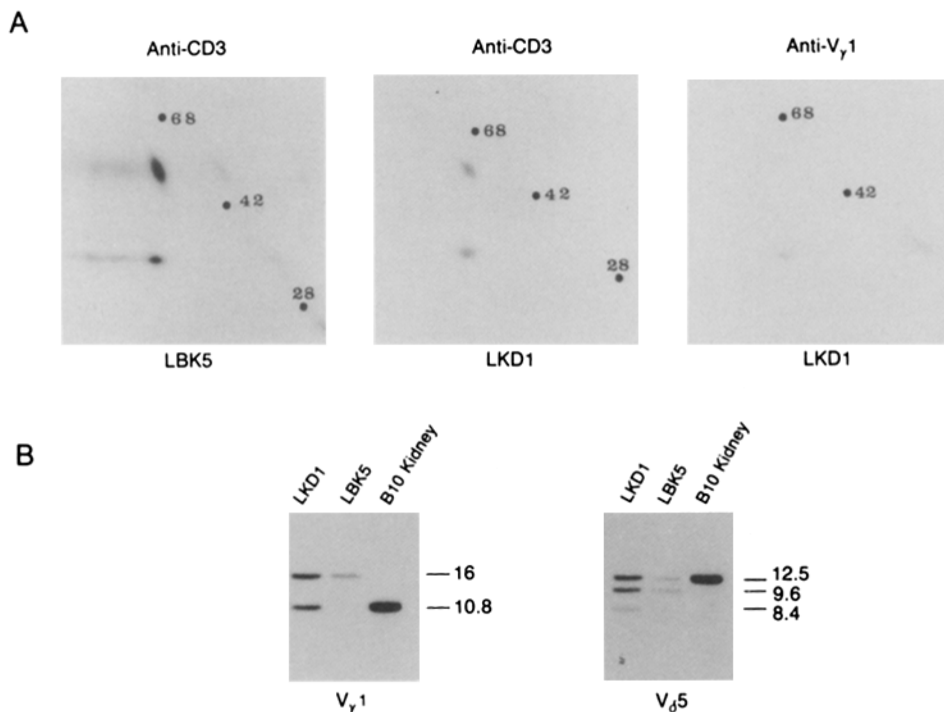

Figure 2. LKD1 and LBK5 express Vγ1.2Jγ2Cγ2- and Vδ5Dδ2Jδ1-encoded receptors. (*A*) Two-dimensional SDS-PAGE gels, run under nonreducing conditions along the horizontal axis followed by reducing conditions along the vertical axis. The molecular masses (kD) of protein standard markers are shown. Specific immunoprecipitations were performed with 145-2C11 (anti-CD3ε) or with an antiserum to Vγ1 (8) on lysates of surface-radiolabeled cells. (*B*) DNA blot analysis of the Vγ1 and Vδ5 rearrangements in LKD1 and LBK5. Probes were hybridized as described (7) to EcoRI-digested DNA. The sizes of the hybridizing bands (kb) are shown. The 8.4-kb Vδ5 hybridizing band also hybridized to an upstream Dδ2 probe and represents the reciprocal restriction fragment created by the Vδ5Dδ2 inversional joining event, containing the 3′ Vδ5 and 5′ Dδ2 signal recombination sequences.

A

	$V_\gamma 1.2$	N	$J_\gamma 2$
	W M	R	G S
LKD1	TGGATGA	GGG	GCTCG
	W M	K	Y S S
LBK5	TGGATGA	AAT	ATAGCTCG

B

	Vδ5	N1	Dδ2	N2	Jδ1
	G	I	S E G Y	S	A T D K
LKD1	GGG	AT	ATCGGAGGGATAC	TCAG	CTACCGACAAA
	G Y	I P	I G G I R	E G	K
LBK5	GGGTAT	ATCCCG	ATCGGAGGGATACGAG	AGGGC	AAA

Figure 3. DNA sequences including the junctional sequences of the functionally expressed TCR γ (*A*) and δ (*B*) genes of the LKD1 (I-A^d-specific) and LBK5 (I-$E^{k,b,s}$-specific) T cell lines. The V, D, and J segments are indicated as are the nucleotides representing N region additions. The protein sequences are shown above the nucleotide sequences, represented by the single-letter code.

of particular VγVδ heterodimers in distinct anatomic sites (1, 11, 12), have been taken as possible evidence for direct antigen selection of TCR-γ/δ variable gene elements. Because both class II MHC alloreactive γ/δ T cell lines we have described express Vγ1.2/Vδ5 heterodimers, it would be of interest to determine whether other Ia-specific murine TCR-γ/δ^+ T cells also express the same pair of variable segments.

On the other hand, striking evidence for selection of γ/δ junctional sequences has also been observed, such as the invariant Vγ3Jγ1 and Vδ1Jδ2 rearrangements of murine DEC cells (11), the Vγ4Jγ1 receptors in other epithelia (12), and the invariant Vδ5Dδ2Jδ1 rearrangement (BID) of pulmonary γ/δ^+ T lymphocytes in BALB/c mice (13). There is direct evidence for selection of this Vδ5 BID junctional rearrangement in that it is not detected in B6 mice but is readily expressed in (BALB/c × B6)F_1 mice. Also, Born et al. (14) have suggested that junctional sequences may influence the fine specificity of recognition of HSP-65-derived peptides by TCR-γ/δ^+ hybridomas.

The data in this report demonstrate that junctionally encoded sequences can influence antigen recognition by TCR-γ/δ^+. Further studies will use site-directed mutational analysis to examine which residues within the TCR γ and δ V(D)J junctions are critical for determining receptor specificity.

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