T CELL RECEPTOR VARIABLE GENE USAGE IN A SPECIFIC CYTOTOXIC T CELL RESPONSE

Primary Structure of the Antigen-MHC Receptor of Four Hapten-specific Cyotoxic T Cell Clones

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Lymphocytes are capable of specifically recognizing foreign antigens and thus play an essential role in the immune system. B cells, a subset of lymphocytes, use immunoglobulins as cell surface receptors for foreign antigens (1). When B cells are stimulated by antigens, they differentiate to plasma cells and produce immunoglobulins in a soluble form. Taking advantage of plasma cell dyscrasias, the molecular basis of antigen recognition by immunoglobulins was studied earlier than that of the other subset of lymphocytes, the T cells (2, 3). However, during the last few years the structure of T cell receptor (TcR)[1] was identified and the genes encoding TcR were isolated (4–7).

The genomic organization of the genes for immunoglobulins (8, 9) and the TcR (10–15) are similar, and it is likely that these gene families originate from the same ancestral gene (16). Immunoglobulins are composed of two heavy- and light-chain disulfide-linked heterodimers, whereas one set of disulfide-linked α and β glycoproteins forms a functional T cell receptor (17, 18). These chains are assembled from noncontiguous variable (V); in the case of immunoglobulin heavy chain and the β chain of TcR, diversity (D); joining (J); and constant (C) gene segments. After somatic rearrangement, V, D, and J gene segments are joined and encode the variable region of the molecule. It has been shown that the variable regions of the immunoglobulin heavy chain (V_H) and the light chain (V_L) form the antigen-binding pocket that interacts with the foreign antigen (19). Considering the similarities at the genomic level of the immunoglobulin and the TcR, the variable region of the TcR should form the antigen recognition site.

However, two major differences between the structure and recognition of the B and T cell receptors exist. The assembly of the light and heavy chains of

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[1] *Abbreviations used in this paper:* AED, *N*-iodoacetyl-sulfonic-naphthyl-ethylene-diamine; TcR, T cell receptor for antigen.

immunoglobulins results in a divalent recognition structure. This ability to bind two identical epitopes appears to be important for antibody effector function. Furthermore, unlike B cells that recognize free antigen, T cells recognize the foreign antigens mostly in association with major histocompatibility complex (MHC) gene products on the cell surface. This phenomenon is known as MHC restriction (20).

In an attempt to gain further insight into the relationship between the TcR and the antigen-MHC interactions, the primary structure of the α and β chain genes of the TcR from four cytotoxic T cell lines that recognize the hapten *N*-iodoacetyl-sulfonic-naphthyl-ethylene-diamine (AED) associated with different MHC class I gene products were analyzed. Since the antigen in this system is a small molecule, we reasoned that it may be possible to define a common primary structure(s) in the α and/or β chain genes of TcR that could be important for antigen binding.

Materials and Methods

Cells. AED-specific CTL lines used in this study were characterized previously (21, 22). The T cell clones were grown in Iscove's modified Dulbecco's medium (Gibco Laboratories, Grand Island, NY) containing 10% heat-inactivated fetal calf serum, 10% rat concanavalin A–stimulated spleen cell supernatant, penicillin, streptomycin (100 IU/ml) and 5×10^{-5} M β-mercaptoethanol. The clones were restimulated weekly with AED-modified spleen cells. AED was purchased from Sigma Chemical Co., St. Louis, MO.

Cloning of cDNAs Encoding α or β Chains of TcR. Total cellular RNA was extracted from each cell line by guanidinium-isothiocyanate–CsCl-gradient method (23). Double-stranded cDNA was synthesized using 20 μg of total cellular RNA and an oligo-d$(T)_{12-18}$ primer (P. L. Biochemicals, Montreal, Canada) as previously described (24). cDNA libraries were constructed by adding synthetic Eco RI linkers (New England Biolabs, Beverly, MA) and cloning into the Eco RI site of λgt10 (25). These libraries were screened using ^{32}P nick-translated probes (Amersham, Oakville, Canada) for the constant region of the α (PlCα) or β (PL5) chains of the TcR (26). These plasmids were kindly provided by Dennis Loh (Washington University, St. Louis, MO). The λgt10 phage carrying α or β chain cDNA were isolated and the DNA was extracted from these clones.

DNA Sequencing. Clones with the longest cDNA inserts were chosen, purified through a 1% low-melting-point agarose gel (Bethesda Research Laboratories, Burlington, Canada), and cloned into the Eco RI site of M13mp19. DNA sequences were determined by dideoxy chain-terminating method using synthetic primers as previously described (27).

Results

CTL Specificities. To investigate the relationship between the primary structure of the TcR and a defined antigen-MHC combination, we have chosen a series of cytotoxic T cell lines that recognize AED-modified cells in the context of class I MHC gene products K^b or D^b. A summary of these four CTL lines and their specificities is presented in Table I. Both 5/10-20K and 8/10-2 are specific for AED-K^b, however, each clone recognizes a different determinant on the H-2K^b molecule (21). 5/10-20D and C9 originate from different mice, but both recognize the hapten in association with H-2D^b.

Primary Structures of the α and β Chain Genes of AED-specific CTLs. At least two α^+ cDNA clones from each cell line were sequenced. In all cell lines except 5/10-20K, both α^+ cDNA clones had the same V, J, and C sequences. One of the α^+ cDNA clones from 5/10-20K contained intron sequences. The other α^+

TABLE I

Usage of TcR Genes in Cytotoxic T Cells

CTL*	Strain	Specificity	α chain‡		β chains§		
			V	J	V	D	J
5/10-20K	B10	AED-K^b	V_α8.520K	J_α520K	V_β7	D_β1.1	J_β1.2
8/10-2	B10	AED-K^b	V_α3.810	J_α810	V_β8.2	D_β	J_β2.6
5/10-20D	B10	AED-D^b>	V_α1.520D	J_α810	V_β5.2	D_β1.1	J_β2.6
C9	B6	AED-D^b‖	V_α3.C9	J_αC9	V_β6	D_β	J_β1.1
2C	BALB.B	D^d	V_α3.HDS58	J_αHDS58	V_β7	D_β1.1	J_β2.6
3F9	BALB/c	D^b	V_α8.3F9	J_αTA19	V_β6		J_β1.1
BDF1.1.3	B6 × DBA/2	FL-D^d	V_α6.BDFL	J_αBDFLA1	V_β2	D_β1.1	J_β2.5
MDA	BALB/c	D^b	V_α5.MDA	J_αTA31	V_β8.3	D_β	J_β2.5

* Data of 2C, 3F9, BDFL1.13, and MDA are from the literature. References are: 2C (7, 31); 3F9 (40, 43); BDFL1.1.3 (45); and MDA (46).

‡ V_α nomenclature follows that of Arden et al. (30). Since each V_α family has 1–10 members (30), each V_α was classified according to the number of the family and the name of the cell line from which it originated. For example V_α8.520K and V_α8.3F9 are different members of the same V_α8 family. J_α gene segments were named according to the cell line from which it originated. J_αTA19 and J_α31 were reported by Arden et al. (30).

§ V_β nomenclature follows that of Barth et al. (32).

‖ Unpublished.

clone from 5/10-20K, and longer cDNA clones from the other three cell lines contained an initiation codon and maintained the correct open reading frame.

At least two β^+ cDNA clones were sequenced from 5/10-20K, 8/10-2, and C9. cDNA sequences of 8/10-2 and C9 included the initiation codon and were in the right translational reading frame. β^+ cDNA clones from 5/10-20K and 5/10-20D had the correct open reading frame. Since the mechanism of allelic exclusion is likely to be similar in TcR genes (28, 29), we concluded that these cDNA clones represent the functional genes (Fig. 1, *a* and *b*). The deduced amino acid sequences of these cDNA clones for the α and β chains are presented in Fig. 2, *a* and *b*, respectively.

Examination of the nucleotide sequences of the cDNA clones revealed several interesting findings. The variable region sequences of the α chain from each clone have not been published. However, these new V_α sequences have high homology to some of the reported V_α sequences. V_α sequences of 5/10-20K, 8/10-2, 5/10-20D, and C9 had 92% homology to TA61 (30), 97% to pHDS58 (7), 95% to TT11 (6), and 96% to pHDS58 in nucleotide sequences, respectively. The V_α used in these cell lines were grouped into known V_α gene families (30) and further subdivided according to the clone from which they originated (Table I). 8/10-2 and C9 use V_α gene segments that belong to the same family. These two V_α gene segments are 95% homologous in nucleotides to each other.

The four cell lines studied used three different J_α gene segments. All three J_α sequences have not been reported and were named according to the cell lines from which they originated (Table I). It is noteworthy that 8/10-2 and 5/10-20D use the same J_α gene segment.

DNA sequencing data of the β chain from these clones indicates that all four cell lines use different V_β gene segments. 5/10-20K, 8/10-2, and C9 use V_β gene

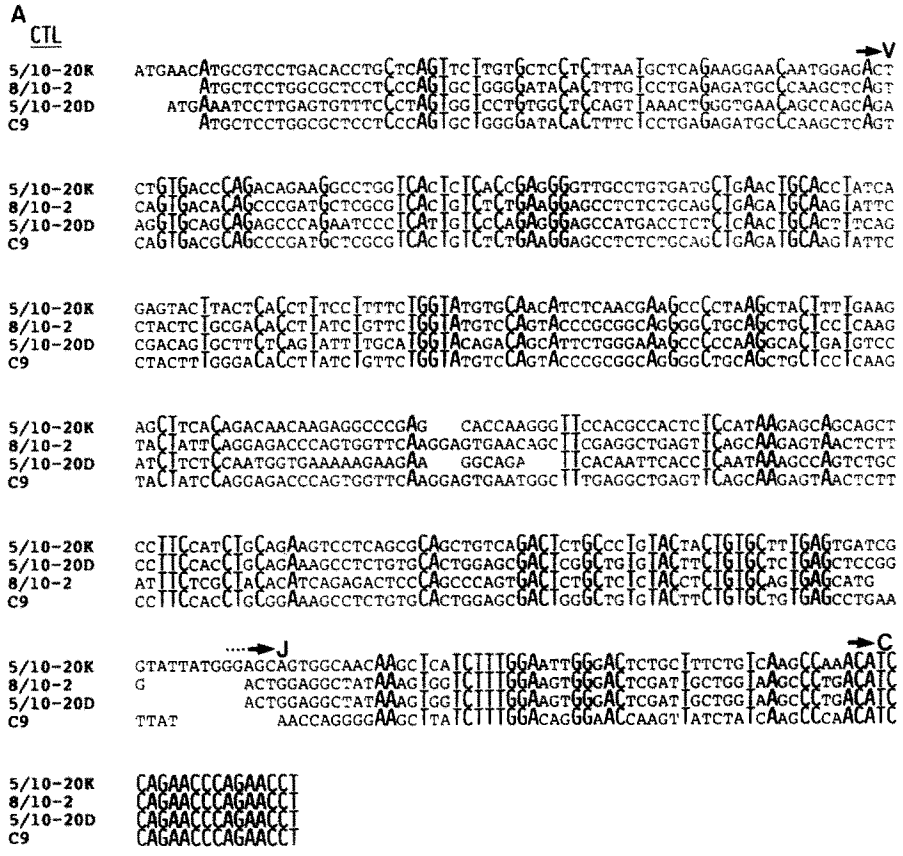
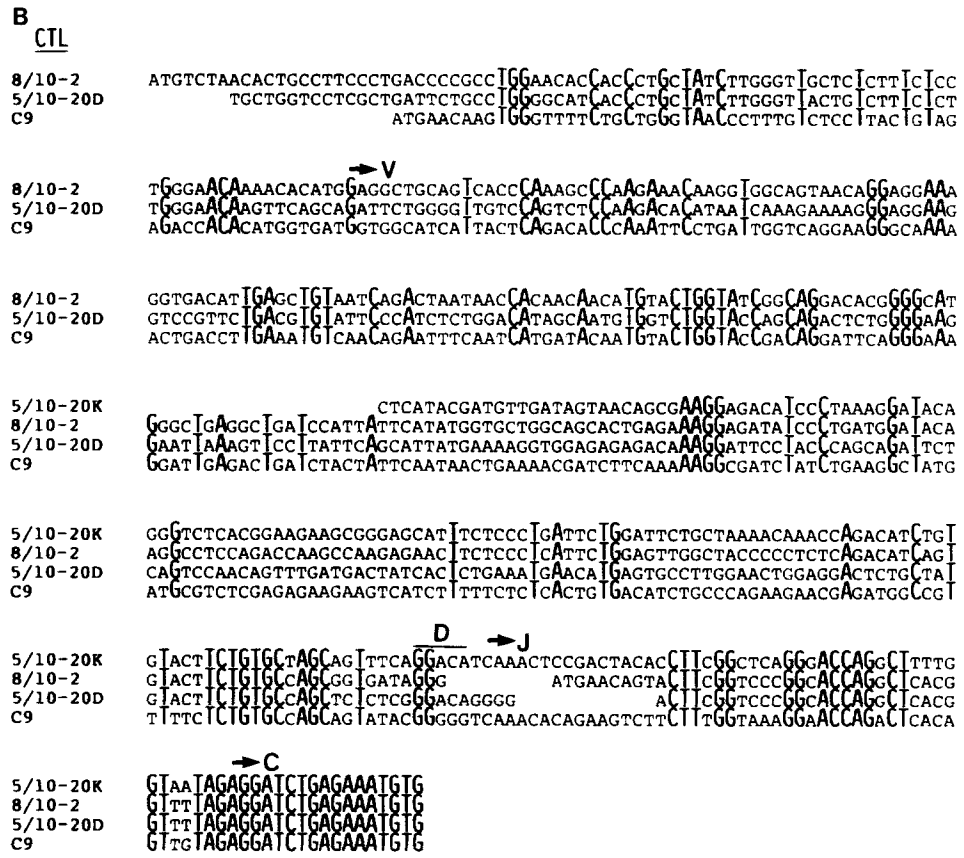


FIGURE 1. (*a*) Nucleotide sequences of the α chain from four AED-specific CTLs. V, J, and C regions are indicated. Homologies are indicated by large characters. Gaps are inserted to maximize similarity. (*b*) Nucleotide sequences of the β chain from four AED-specific CTLs.

segments that are 100% homologous to pHDS11 (31), TB2 (32), and $V_\beta 6$ (33, 34), respectively. Therefore, according to previous nomenclature (34, 35), these V_β gene segments were classified as $V_\beta 7$, $V_\beta 8.2$, and $V_\beta 6$ (Table I). Since these V_β sequences are identical to previously sequenced V_β cDNA clones, this strongly suggests that somatic mutation has not occurred in the generation of these AED-specific CTLs. This mechanism for generating diversity has been well described for the B cell response that has been allowed to mature in vivo (36). However, these AED-specific cloned lines were generated by priming the mouse once in vivo, followed by several repeated stimulations in vitro (21). 5/10-20D uses a V_β gene segment that is 85% homologous in nucleotides to $V_\beta 5.1$ (33, 34). The $V_\beta 5$ family has three members, only one of which has been sequenced (33, 35). It is obvious from the homology to $V_\beta 5.1$ that the V_β gene segment used in 5/10-20D should fall into the $V_\beta 5$ family, and therefore it was designated $V_\beta 5.2$ (Table I).

5/10-20K and 5/10-20D use diversity segments that are believed to originate



from $D_\beta 1.1$. Diversity-like regions of 8/10-2 and C9 are limited, and therefore it was difficult to determine which D locus was involved in the rearrangement, or whether these nucleotides represent *N*-region diversity.

The usage of J_β gene segments in these CTLs is interesting. Both 8/10-2 and 5/10-20D, which use the same J_α gene segment, also use the identical $J_\beta 2.6$ segment. The V, D, and J gene segments of both α and β chains of the TcR from these four CTL clones is summarized in Table I.

Comparison of the deduced amino acid sequences from the α and β chains of the four CTL clones only show homology in the framework regions. In the nonframework regions, not one amino acid residue was found to be conserved.

Discussion

In the immunoglobulin response, it appears that some cases preferentially use particular V regions in response to a certain antigen (36, 37). To seek a possible correlation between the primary structures of the TcR and the antigen-MHC specificity of the T cell clones, we have cloned and sequenced the α and β cDNAs from four CTLs specific for the hapten AED and restricted to class I determinants. Results indicate that a simple relationship between the antigen specificity and the variable gene segments of the α or β chains does not exist.

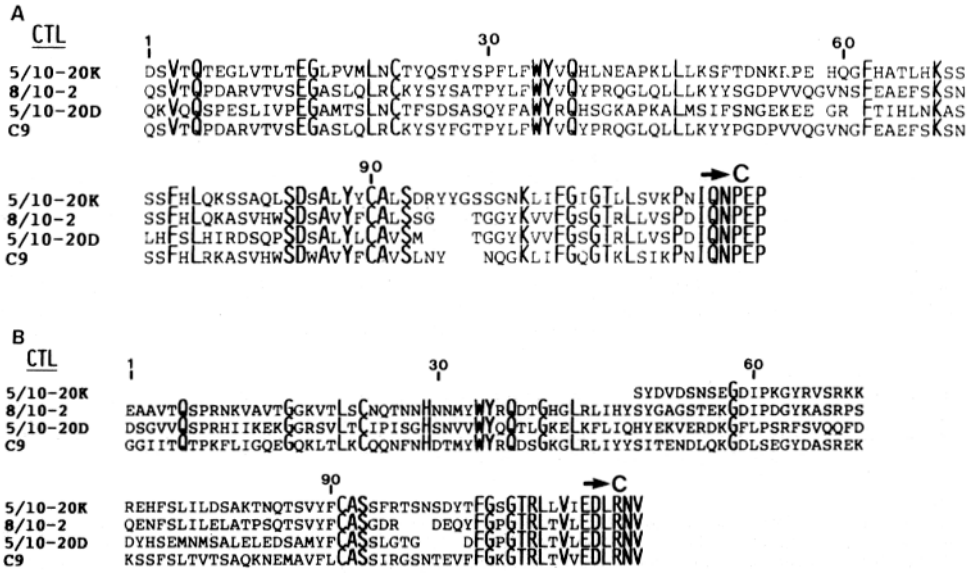


FIGURE 2. (*a*) Amino acid sequences of the α chain deduced from the DNA sequences listed in Fig. 1*a*. Amino acid numbering follows that of Arden et al. (30). (*b*) Amino acid sequences of the β chain deduced from the DNA sequences listed in Fig. 1*b*. Amino acid numbering follows that of Barth et al. (35).

Recently Fink et al. showed that, in certain pigeon cytochrome *c*–specific helper T cell clones, all specific for I-E^k, a very limited number of α and β chain variable gene segments were used (38). In this AED-MHC-restricted response, the use of many variable regions could be explained in several ways. It is possible that these CTLs recognize the hapten AED coupled to a specific protein on the cell surface. Therefore the CTL may interact with part of the cellular protein, or may recognize the conformation in which this AED molecule is presented on this particular protein. It is also possible that several different determinants on the hapten AED may be recognized. In addition, because these clones are restricted to different determinants on the H-2^b haplotype, it is reasonable to speculate that different V regions are required to recognize the combination of AED and MHC.

However, one notable correlation emerged after comparing the sequences derived from these four clones. 8/10-2 and 5/10-20D, which are restricted by different MHC antigens, use the same combination of J_α and J_β gene segments. It has been estimated that 50–100 J_α gene segments exist (30, 39), and in conjunction with the 12 functional J_β segments (10), over 1,000 combinations of J_α and J_β gene segments could potentially arise. Therefore it may be significant to have the identical combination of J_α and J_β gene segments expressed in randomly chosen T cell lines that recognize AED in association with the class I molecule. This combination may be important for recognition of a common determinant on AED, although the use of this J segment combination for the recognition of a shared epitope on the H-2K^b and H-2D^b molecule cannot be excluded.

Table I summarizes the gene segments used in the AED-MHC-restricted response along with the published data from cytotoxic T cell lines in which both α and β chain sequences were available at the time of submission of this report. As repeatedly described (40–42), there are examples of T cell clones with different specificities that use the same V_β. For example, 5/10-20K and 2C use the $V_\beta 7$ gene segment, and C9 and 3F9 use the $V_\beta 6$ gene segment. Furthermore, C9, which originates from B6 mice and is specific for AED-D^b, and 3F9, which originates from BALB/c mice and is alloreactive to D^b, use the same combination of $V_\beta 6$ and $J_\beta 1.1$. The β chain sequences from these two cell lines are different only at the junction between V_β and J_β. The α chains differ in these two clones (43). Because C9 does not crossreact with D^b alone, it is possible that this combination of V_β, J_β segments are involved in the recognition of class I D^b determinants, and the α chain or D region of the β chain allows C9 to react with AED and D^b. It is also interesting to note that V_β is capable of associating with different V_α family members, thus supporting combinational association as a mechanism to generate diversity in T cells.

Another example exists where two different helper T cell lines use the same combination of V_β and J_β gene segments (44). One helper cell line was lysozyme-specific and I-A^b-restricted, and the other was specific for cytochrome *c* in association with I-$E^{k/b}$. In this case it appears that a combination of similar gene segments can be used without any similarities in antigenic or restricting elements.

In general, it appears that identical gene segments can be used to construct a TcR that is specific for various epitopes. Our data shows that a variety of gene segments can be used in a MHC-restricted response to the particular hapten AED. Therefore, existing data indicates that there will be no simple rule between antigen or MHC specificity of a particular T cell, and the particular gene segments involved in the rearrangement of the TcR. Thus the combination between the α and β chains, the *N*-region diversity, and/or the alternate reading frames of the D segment contribute to the unique specificity that is characteristic of functional T cells.

Summary

The primary structure of the α and β chains of the T cell antigen receptor in four cytotoxic T cell clones specific for *N*-iodoacetyl-sulfonic-naphthyl-ethylene-diamine (AED)-haptenated target cells displaying a particular class I MHC molecule has been determined. Two of the T cell clones, 8/10-2 and 5/10-20K, recognize AED-modified targets in association with H-2K^b, while the other two clones 5/10-20D and C9 react with AED-modified cells in the context of H-2D^b. Comparison of the nucleotide sequences of both the α and β chain cDNAs and their deduced protein sequences indicates that a specific variable gene segment was not used to recognize the hapten and/or class I gene products. Furthermore, there does not appear to be any conserved amino acid residues used in the AED-specific response other than the framework amino acids. However, when the two clones 8/10-2 and 5/10-20D were compared, a striking similarity was seen in the J segments. These two clones that recognize AED in the context of different MHC epitopes used identical Jα ($J_\alpha 810$) and J_β ($J_\beta 2.6$) gene segments. C9, specific for AED-D^b, shared identical V_β ($V_\beta 6$) and J_β gene segments ($J_\beta 1.1$) as those of a

cytotoxic T cell that recogizes allogeneic targets expressing D^b. These data indicate that a simple rule governing the usage of the variable regions of either the α or β T cell receptor (TcR) genes in the recognition of antigen and MHC gene products cannot be formulated. However, subtle similarities can be detected in some situations between the primary structures of the TcR and the targets they recognize.

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