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T-cell antigen receptors with identical variable regions but different diversity and joining region gene segments have distinct specificities but cross-reactive idiotypes

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ABSTRACT **The T-cell antigen receptor α-chain genes of an alloreactive, H-2D^b-specific cytotoxic T-cell clone (3F9) are described. This study and our work on the 3F9 β-chain genes reveal that the variable region gene segments for the α and β chains expressed in 3F9 are identical to the ones used by a chicken erythrocyte-specific, I-A^b-restricted helper T-cell clone (LB2). These two clones differ, however, in the diversity and joining portions of the α and β chains of their T-cell receptor molecules. The analysis of 3F9 and LB2 with monoclonal antibodies specific for the 3F9 T-cell receptor shows that these two T-cell clones share the same idiotype; however, 3F9 and LB2 do not exhibit any antigen and/or major histocompatibility complex cross-reactivity. This suggests that the diversity and joining regions of the T-cell receptor may play a key role in antigen and/or major histocompatibility complex recognition.**

For several years, one of the key questions in immunology has been: what is/are the structure(s) of the T-cell antigen receptor that enables T cells to recognize cell-bound foreign antigenic determinants together with self-transplantation antigens coded by the major histocompatibility gene complex (MHC) (restricted T cells) or to recognize foreign transplantation antigens (alloreactive T cells). The T-cell receptor, or part of it, has now been identified as a heterodimeric glycosylated protein consisting of disulfide-linked α and β chains each with a relative molecular mass of 40–50 kDa (1–4). These chains are organized similarly to immunoglobulin molecules, i.e., they consist of an amino-terminal variable and a carboxyl-terminal constant region and are encoded by noncontiguous gene segments that are rearranged during B- or T-cell differentiation to form a functional transcription unit (5–13). A third class of genes, called γ, has also been identified at the mRNA level. The γ genes possess many properties in common with the α- and β-chain genes, which indicates that they are a third class of T-cell receptor chain genes (14–18). However, the role of the γ-chain in T-cell antigen recognition is still unknown.

We have reported (19, 20) that the β-chain variable region (V_β) gene used by an alloreactive cytotoxic T cell (3F9) is the same as that of an antigen-specific, class II-restricted helper T cell (LB2-1). Here we report on the isolation and sequence determination of the α-chain gene of the alloreactive cytotoxic T-cell clone 3F9. Surprisingly, the α-chain variable region (V_α) gene expressed in 3F9 is also identical to the V_α gene expressed in the helper T-cell clone LB2-1 (21). Although 3F9 and LB2-1 share an idiotype on their T-cell receptor, they do not exhibit any immunological cross-reactivity.

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MATERIALS AND METHODS

T-Cell Clones. 3F9 is an alloreactive cytotoxic T-cell clone from BALB/c (H-2^d) mice and is specific for the D^b allele of the murine MHC region (22). 3A2 is a subclone of 3F9. LB2-1 is an I-A^b-restricted, chicken erythrocyte-specific helper T-cell clone from C57BL/6 (H-2^b) mice (23, 24). The other control T-cell lines used in the fluorescence-activated cell sorter analyses are also described (23, 24).

cDNA Library Construction and Screening. The construction of the λgt11-3F9 library was described (19). The screening of this library for cross-hybridizing clones was performed with a ^{32}P-labeled human α-chain cDNA clone (a gift of Tak W. Mak) by standard procedures (25). Thirty α-positive clones out of 2×10^5 recombinant phages were isolated, and the DNA sequence of the longest insert was determined using the Maxam and Gilbert procedure (26).

Proliferation Assays. 3A2 cells (1×10^4 cells) were stimulated with 1×10^6 γ-irradiated (2000 rad; 1 rad = 1.000×10^{-2} J/kg) spleen cells of the indicated H-2 (K, I, D) haplotype in 0.2 ml of fetal serum supplemented Iscove's modified Dulbecco's medium (IMDM) containing 10% (vol/vol) rat Con A supernatant (22). LB2-1 cells (2×10^4 cells) were stimulated with 1×10^6 γ-irradiated (2000 rad) spleen cells in flat-bottomed wells of microtiter plates with 0.2 ml of complete IMDM without rat Con A supernatant in the presence or absence of 0.04% chicken erythrocytes (23, 24). After 48 hr of incubation in an atmosphere of 95% air/5% CO_2 at 37°C, 0.025 ml of complete medium containing 2 μCi of [^{3}H]thymidine (1 Ci = 37 GBq) was added per well. Uptake of radioactivity was measured after 16 hr of incubation. Measured values are means of triplicate determinations, standard deviations were smaller than 10%.

RESULTS

Nucleotide Sequence of 3F9 α Chain Gene. A cDNA library of 3F9 in λgt11 was used to screen for α-chain sequence bearing clones. Thirty such clones were isolated and analyzed by restriction mapping and shown to represent an unusual type of transcript. The nucleotide sequence corresponding to the V_α region of the longest α-chain cDNA clone (3F9-α7) is shown in Fig. 1. The V_α sequence of 3F9 is with one exception identical to the corresponding V_α part of LB2-1 (21). This single-nucleotide substitution does not change the corresponding amino acid sequence and could be due to a somatic mutation, to a strain difference between BALB/c

Abbreviations: D, diversity; J, joining; V, variable; D_α, J_α, and V_α are D, J, and V regions of the α chain of the T-cell antigen receptor, respectively; D_β, J_β, and V_β are D, J, and V regions of the β chain of the T-cell antigen receptor, respectively; MHC, major histocompatibility complex; Ag/MHC, a neoantigen formed by antigen and MHC molecules.

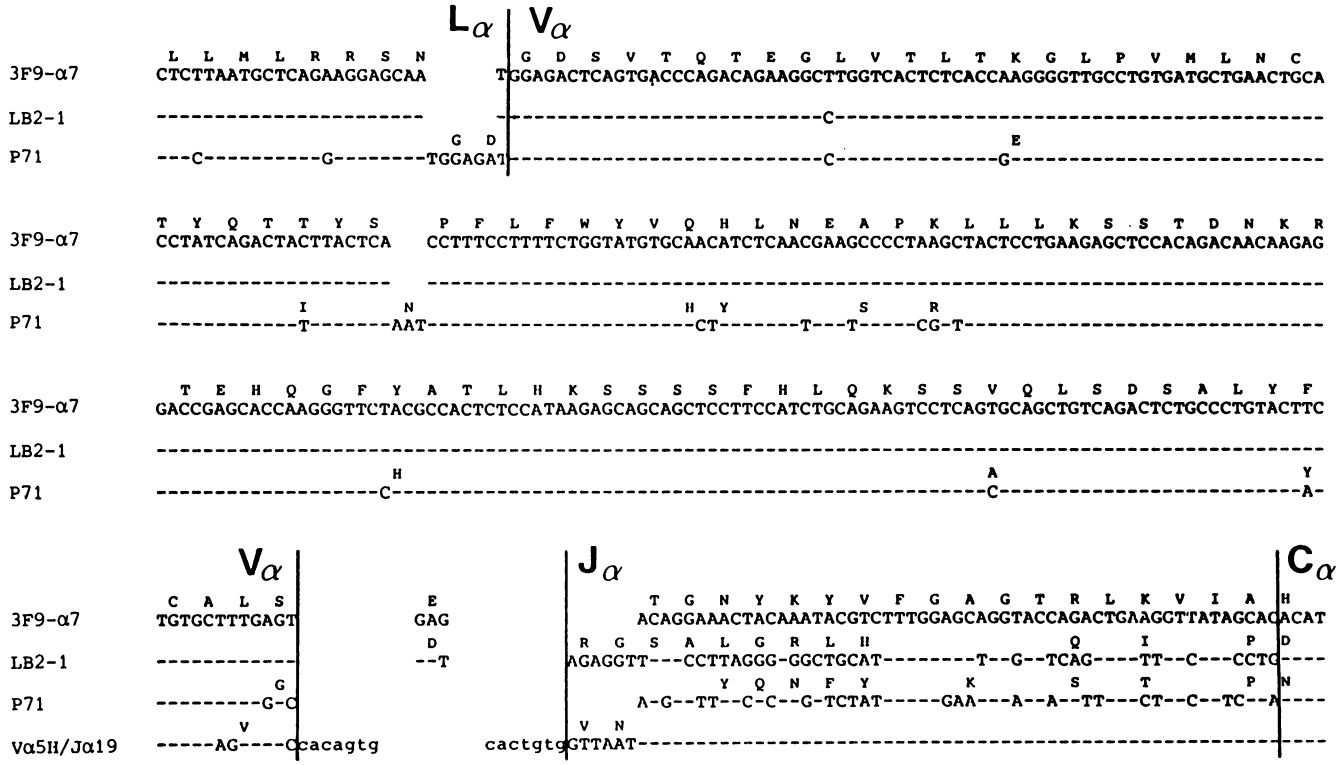

FIG. 1. V_α gene sequences: the V_α-J_α nucleotide sequence of 3F9-α7 is compared with the V_α-J_α sequence of LB2-1 and the genomic V_α 5H and J_α 19 sequences. The beginning of the V_α gene segment with its leader sequence and the J_α and C_α regions are indicated by vertical lines. Identical nucleotides are shown by dashes and the conserved heptamers at the end of the germ-line V_α 5H and at the beginning of the J_α 19 are indicated in small letters.

(3F9) and C57BL/6 (LB2-1), or to a cloning artifact. 3F9 and LB2-1 α-cDNA clones differ, however, in the α-chain joining region (J_α) and at the point of V_α-J_α joining. The 3F9 α chain bears the genomic sequence corresponding to J_α 19; LB2-1 carries a different J_α segment that has not yet been identified at the genomic level (12).

The 3F9 and LB2-1 α-cDNA clones bear three nucleotides between the corresponding V_α and J_α gene segments that cannot be accounted for by germ-line sequences. The triplet GAG encoding glutamic acid in 3F9 corresponds to GAT encoding aspartic acid in LB2-1 (21). These triplets could be part of a not-yet identified α-chain diversity region (D_α) gene segment or the result of a terminal deoxynucleotidyl-transferase activity during V_α-J_α rearrangement, a mechanism that has been postulated to be important for the generation of antibody diversity during B-cell development (27).

Additional comparisons with published sequences for α-chain genes revealed that the D^b-specific alloreactive cytotoxic T-cell hybridoma P71 (28) bears a V_α gene segment that is closely homologous to the one expressed in 3F9 (and LB2-1) but rearranged to a different J_α gene segment (21) (Fig. 1). Therefore, it would be of interest to compare the 3F9 and P71 β chains to determine similarities that might be involved in D^b recognition.

Amino Acid Comparison of the α and β Chains of 3F9 and LB2. The comparison of the amino acid sequences of the α and β chains of 3F9 and LB2-1 is shown in Fig. 2. The β chains of these two T-cell clones differ mainly in a continuous stretch of seven amino acids consisting of four "deletions" and three nonconservative substitutions spanning the diversity region of the β-chain (D_β) and the beginning of the joining region of the β chain (J_β). Comparison of the J_α region reveals as many nonconservative amino acid changes as in the J_β region, however, they are scattered throughout the J_α sequence. It is also noteworthy that the α and β chains from LB2-1 bear longer D-J regions of two and four amino acids, respectively, than do the corresponding regions in 3F9. The 3F9 and LB2-1 V_α and V_β regions differ only by three point mutations: two in the case of the V_β gene (Ala/Thr and Leu/Ile) and a silent one in the V_α chain gene. Although an influence of these changes on antigen and/or MHC recognition is possible, we consider this as rather unlikely because one substitution is conservative (Leu/Ile) and the other one does not lead to any change in charge or size (Ala/Thr).

Idiotype Analysis of 3F9 and LB2-1. The monoclonal antibodies 44-22-1 and 46-6B5 (22), specific for the 3F9 T-cell receptor, were tested on LB2-1, 3A2 (a subclone of 3F9), and two chicken erythrocyte-specific T-cell clones that share fine specificity (GK15-1) or MHC restriction (LB19-1) with LB2-1. The data in Table 1 indicate that 3A2 and LB2-1 share the same idiotype, which, therefore, might be located in the variable domains and not in the D-J regions of the T-cell receptor molecule. The idiotype was not detected on the two T-cell clones that differed from LB2-1 either in fine specificity or MHC restriction.

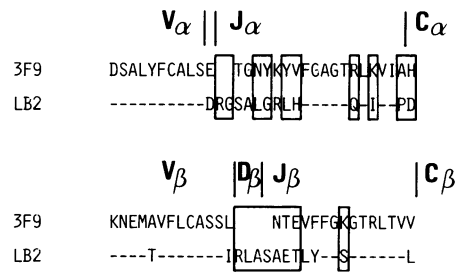

FIG. 2. Amino acid sequence and comparison of 3F9 and LB2-1 α and β chains. The amino acid sequences encoded by the 3′ end of the *V* gene segments and the *D-J* regions are shown in the single letter amino acid code. Nonconservative amino acid changes and "deletions" are indicated in boxes.

Table 1. Binding of anti-idiotype antibodies to T-cell lines

T-cell line	Specificity	Restriction	Antibody binding, arbitrary units			
			44-22-1	46-6B5	J-2A	J-5C
3A2	$H2D^b$	—	144	42	15	24
LB2-1	CRBC-1	$H2I^b$	123	30	7	6
LB19-1	CRBC-2	$H2I^b$	9	10	9	10
GK15-1	CRBC-1	$H21^k$	4	4	4	4

The binding of anti-idiotype [44-22-1 (IgG) and 46-6B5 (IgM)] and control antibodies [J-2A (IgG) and J-5C (IgM)] to T-cell lines was tested by flow cytometry, using fluorescein-labeled goat anti-mouse immunoglobulin as a second-stage antibody. Results are expressed as the median of the fluorescence intensity, in arbitrary units. The specificities CRBC-1 and CRBC-2 refer to the ability to respond to all chicken erythrocytes (CRBC) in the test panel (CRBC-2) or all CRBCs except those of the B^{13}/B^{13} MHC genotype (CRBC-1) (23). MHC restriction specificities have been determined (24).

It is interesting that an additional cytotoxic T-cell hybridoma P71 specific for D^b (28) also binds to the same antiidiotypic antibodies as 3F9 and LB2-1 (Z. Eshhar, personal communication). However, since the β-chain gene of P71 has not yet been cloned, it is difficult to correlate the presence of the 3F9 idiotype with a particular V_α–V_β combination or with a single V_α or V_β gene segment.

Antigen and/or MHC Specificity of 3F9 and LB2-1. To further analyze the antigen specificity and/or MHC restriction of the T-cell clones 3F9 and LB2-1, these clones were compared with respect to their proliferative responses. Fig. 3 summarizes proliferation assays of 3A2 (a subclone of 3F9) and LB2-1 T-cell clones with different combinations of stimulator cells in the presence or absence of chicken erythrocytes. 3A2 proliferated only with stimulators bearing $H\text{-}2D^b$ molecules and did not proliferate with stimulators expressing the $H\text{-}2K^bI^bD^d$ haplotype, independent of whether the stimulators were pulsed or not with chicken erythrocytes. LB2-1 proliferated when cultured with stimulators of the $H\text{-}2K^bI^bD^d$ haplotype only if chicken erythrocytes were present. These results indicate that the immunological specificities of 3A2 (i.e., 3F9) and of LB2-1 are different.

DISCUSSION

In this study we show that the two T-cell clones 3F9 and LB2-1, which have completely different antigen and MHC specificities as well as different effector functions, use identical V_α and V_β gene segments but different D-J segments for their respective antigen receptors. If we assume that both antigen and MHC recognition occurs via a single T-cell receptor molecule, it follows that, at least in this particular case, the D-J regions of the T-cell receptor are able to confer antigen and MHC specificity.

The open and crucial question concerns the contribution to antigen and MHC specificity of the variable portion of the 3F9 and LB2-1 T-cell receptor molecules. Studies on the α- and β-chain genes of cytochrome *c*-specific, MHC-restricted helper T-cell (29) and hapten-specific, MHC-restricted cytotoxic (A. Iwamoto, P. S. Ohashi, C. L. Walker, H. Pircher, F.R., H.H. & T.W. Mak, unpublished results) T-cell clones seem to indicate that the binding sites responsible for antigen or MHC recognition are not located on only one of the two chains of the T-cell receptor heterodimer. Structural analyses have led to the hypothesis that the three-dimensional structure of the T-cell receptor heterodimer could be similar to the Fab portions of the immunoglobulin light and heavy chains, where only one antigen binding site could be identified (30). In the absence of firm evidence for a second additional specific receptor on T cells, these considerations favor the hypothesis that there is a single recognition site for the neoantigen, formed by the antigen and MHC molecules (hereafter referred to as Ag/MHC), within a single receptor structure. In this context, two possible explanations of the roles of the variable portions of the T-cell receptor in mediating Ag/MHC specificity could be proposed.

First, the D-J-segments could influence the three-dimensional conformation of the variable domains. In this case the single recognition site of 3F9 and LB2-1 T-cell receptors could be located in the variable domains, which assumes different conformations depending upon the associated D-J regions.

The second model proposes that the variable domains have conformations that do not depend on the D-J regions used. In this case the variable regions would contribute to the formation of Ag/MHC binding sites that are unable to provide the necessary affinity that is high enough to activate effector T cells. Sufficiently high specific Ag/MHC affinity would then be contributed by the relatively small D-J regions. If this model is correct, the variable region segments could select the Ag/MHC by forming pockets for selected but differing Ag/MHC complexes. The D-J regions would be located in these binding sites in such a way that they would be responsible for a particular fine specificity.

The fact that two monoclonal antibodies specific for the T-cell receptor of 3F9 also react with the T-cell receptor of LB2-1 indicates that these receptor molecules share antigenic

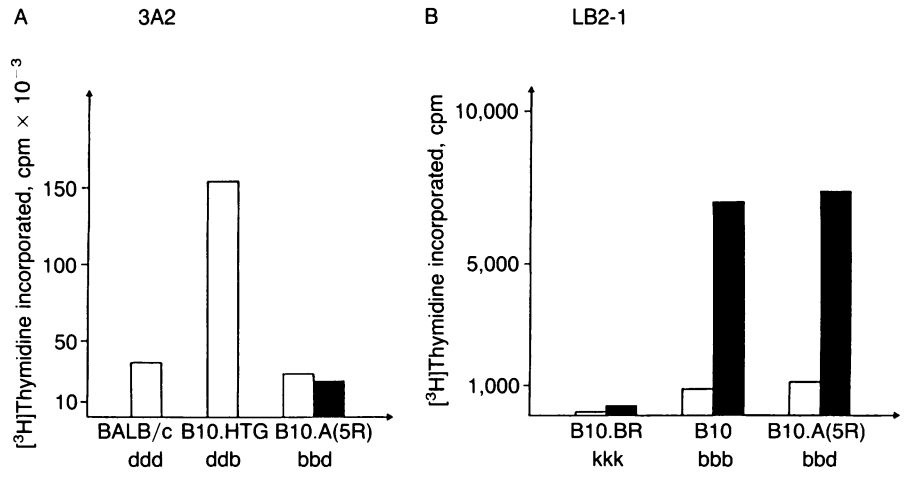

FIG. 3. Cross-reactivity analysis of the alloreactive cytotoxic T-cell clone 3A2 (a subclone of 3F9) (*A*) and the helper T-cell clone LB2-1 (*B*) by *in vitro* restimulation. Clones were stimulated with spleen cells of the indicated H-2 (K, I, D) haplotype in the presence (solid bars) or absence (open bars) of chicken erythrocytes, and [^{3}H]thymidine incorporation in activated cells was measured.

determinants in the variable region domains. Therefore, it seems possible that the T-cell receptor variable regions of 3F9 and LB2-1 could have similar three-dimensional structures. In this context it is interesting that KJ16-like monoclonal antibodies (31) exist that are capable of detecting a particular V_β sequence, independently of the associated V_α region or of the joined D-J regions. These data support the second model.

Independently of the mechanism of interaction between Ag/MHC complexes and T-cell receptor molecules, we suggest that the role of the variable segments of the T-cell receptor could be of lower importance than the D-J regions for Ag/MHC recognition. The limited contribution of the T-cell receptor variable domains to antigen recognition could represent a major difference between T and B cells. This could explain some differences between T-cell receptor and immunoglobulin gene segment organization and the respective mechanisms to increase antigen receptor diversity. Evident differences between T-cell receptor and immunoglobulin genes include the different sizes of the repertoires of *V* vs *D* and *J* gene segments. It seems that the *D* and *J* gene segment repertoires of the T-cell receptor are larger than that of the *V* gene segments, whereas for the immunoglobulin genes the opposite is true. Moreover, the *D* gene segments of the β chain of the T-cell receptor are facultatively rearranged and possess three open reading frames, and the recombination site between *D* and *J* genes is less defined as compared to immunoglobulin heavy chain genes. These mechanisms that increase the diversity at the *V-D-J* junctions of the T-cell receptors have not been observed in immunoglobulins.

Furthermore, somatic mutation in the variable regions, which is a very important mechanism to generate antibody diversity, has not been reported in T-cell receptor genes. A possible reason for this might be the requirement that T cells create receptor diversity that avoids autoreactivity.

All these observations and considerations would imply different roles for variable vs diversity and joining regions in antigen recognition by T and B cells.

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