

MULTIPLE CLASS I AND CLASS II MAJOR
HISTOCOMPATIBILITY COMPLEX ALLOSPECIFICITIES ARE
GENERATED WITH T CELL RECEPTOR VARIABLE (V)
DOMAINS CREATED BY A SINGLE $Ti\beta$ V GENE FAMILY

BY ORESTE ACUTO, REBECCA E. HUSSEY, AND ELLIS L. REINHERZ

*From the Division of Tumor Immunology, Dana-Farber Cancer Institute and the Departments
of Medicine and Pathology, Harvard Medical School, 44 Binney Street,
Boston, Massachusetts 02115*

Variability among individual T cell receptor $Ti\alpha$ and $Ti\beta$ subunits results from germline diversity of variable, diversity, and joining (V, D, and J)-like elements that encode them and from combinatorial events linking these discontinuous gene segments during ontogeny (1–3). To examine the rules governing $Ti\beta$ V gene usage in formation of T cell antigen/major histocompatibility complex (MHC) receptors of individual T cell subpopulations, we developed and characterized a monoclonal antibody directed against a specific epitope on products of one prototypic $Ti\beta$ gene family, REX (4). The REX V gene products are expressed on 2% of unprimed peripheral T cells of all human beings and are not restricted to either $T4^+$ or $T8^+$ subpopulations. Southern analysis, isoelectric focusing, and two-dimensional peptide mapping of a wide range of T cell clones indicated that each REX V gene could form a linkage to different $Ti\beta$ D and/or J segments. Moreover, the $Ti\alpha$ chains of such clones were distinct. This implies that $Ti\beta$ V gene usage is not restricted to functionally or phenotypically defined subsets and that there is presumably little restriction on mechanisms generating combinatorial, junctional, or chain association-mediated diversity.

Materials and Methods

Derivation of Lymphoid Populations and T Cell Clones. Mononuclear cells were rosetted with sheep erythrocytes, and the E^+ fraction was incubated with anti- Ti_{3A} monoclonal antibody (mAb) (100 μ l of a 1:250 dilution per 10^6 T cells) for 30 min at room temperature. Subsequently, anti- Ti_{3A} -reactive cells (Ti_{3A}^+) were separated from anti- Ti_{3A} -nonreactive cells (Ti_{3A}^-) by indirect rosetting with ox erythrocytes (5). The anti- Ti_{3A}^+ cells obtained (~70% Ti_{3A}^+) were then stimulated with the irradiated (5,000 rad) B lymphoblastoid line Laz 509 at a T cell/Laz 509 ratio of 1:1 in RPMI 1640 containing 5,000-rad-irradiated feeder cells (0.5×10^6 autologous whole mononuclear cells/ml). After 1 wk in vitro culture at 37°C, activated cells were reacted with anti- Ti_{3A} mAb, stained with goat anti-mouse F(ab')₂ fluorescein isothiocyanate (FITC), and sorted on an Epics V cell sorter (Coulter Electronics, Inc., Hialeah, FL) using the autoclone method (4). For this purpose,

This work was supported by grants AI 22211, AI 19807, AI 21226, and CA 40134 from the National Institutes of Health. Address reprint requests to O. Acuto.

TABLE I
Specificity Analysis of Ti_{3A}^+ Cytotoxic T Lymphocyte Clones

Clone	Phenotype	Target cell lysed								Allospecificity	
		L509	L156	L503	L467	L468	L471	L475	S002		
AA2	$T3^+T4^-T8^+$	+	-	-	-	-	-	-	-	II	SB _x
AA6	$T3^+T4^-T8^+$	+	-	-	-	-	-	-	ND	I	NA
AA8	$T3^+T4^-T8^-$	+	-	-	-	-	-	-	-	II	Dr7p or 4p
AA9	$T3^+T4^-T8^+$	+	-	-	-	-	-	-	-	II	Dr7p or 4p
AA13	$T3^+T4^-T8^+$	+	-	-	-	-	-	-	+	I	HLA-B13
AA14	$T3^+T4^-T8^+$	+	-	+	-	-	+	+	ND	I	HLA-A2 (sub)
AA18	$T3^+T4^-T8^-$	+	-	-	-	-	-	-	-	II	Dr7p or 4p
CP4	$T3^+T4^-T8^-$	+	-	-	+	-	+	-	ND	II	SB _x
CP6	$T3^+T4^-T8^+$	+	-	-	-	+	-	-	-	I	HLA-A25
CP10	$T3^+T4^-T8^+$	+	+	+	+	+	-	-	ND	I	NA

Clones AA and CP were derived from two separate donors. MHC genotypes for individual B lymphoblastoid lines are as follows. Laz 509: A2, A25, B13, Bw38, DR4, DR7, Laz 156: A2, A3, B7, B40, DR2, DR4; Laz 503: A2, B27, DR6; Laz 467: A3, A11, B15, Bw35, DR1; Laz 468: A25, B18, DR2; Laz 471: A2, Bw44, DR7; Laz 475: A2, A9, Bw44, B40, DR5; and cell SEL002: A1, A2, B13, B40, Bw4, Bw6, DR2, DR7, DRw53. Donor AA is A3, B35, Bw6, Cw4, DR2, DR4, DRw53, Dqw1, Dqw3 and donor CP is A2, B14, B44, Bw4, Bw6, DR4, DR7, DRw53, Dqw2, Dqw3. Antibody blocking studies were performed as described in Materials and Methods and in the legend to Fig. 1. I, MHC class I; II, MHC class II. (-) $\leq 5\%$ specific lysis; (+) $>30\%$ specific lysis, at a 10:1 E/T ratio. Allospecificity assignment is based on the present panel analysis and the testing of 10 additional B lymphoblastoid lines. SB_x and SB, signify unknown presumptive SB specificities; Dr7p or Dr4p denotes private Dr specificities, and HLA-A2 (sub), a subset of HLA-A2 molecules. NA, not assignable. ND, not done.

cells were cloned directly into 96-well microtiter plates (Rochester Scientific Co., Rochester, NY) at 1 cell per well, into which were placed 50,000 autologous irradiated feeders per well, 20,000 irradiated Laz 509 per well, and 10% (vol/vol) IL-2.

Functional T Cell Assays and mAb. The cytotoxicity assay was a standard 4 h killing system using ^{51}Cr -labeled B lymphoblastoid cell lines in which effector/target ratios were 40:1, 20:1, 10:1, and 1:1 (6). For antibody blocking studies, a 1:250 dilution of hybridoma ascites containing anti-T8, anti-T4, anti-BT3.4, anti-BT2.9, anti-PTF29, anti-W6/32, or anti- Ti_{3A} was used (6-9). Anti- Ti_{3A} was recently shown (4) to identify an epitope on the V gene product of members of the REX V gene family.

Results and Discussion

To examine the potential diversity of specificities among T lymphocyte antigen/MHC receptors using a single $Ti\beta$ V gene set, we characterized allospecificities of cytotoxic T lymphocyte (CTL) clones expressing a product of the REX $Ti\beta$ V gene family. Resting peripheral blood T lymphocytes were first identified and separated with mAb anti- Ti_{3A} , directed at an epitope on the products of the REX V gene family (~2% of total T lymphocytes) (4) and then stimulated in vitro with the allogeneic B lymphoblastoid line, Laz 509. The alloreactive Ti_{3A}^+ T lymphocytes were cloned by micromanipulation and the specificity of clones for the stimulating Laz 509 line verified in mixed lymphocyte culture and/or by cell-mediated lympholysis. We obtained 27 anti- Ti_{3A} -reactive, Laz 509-allo-specific clones derived from three unrelated donors. 10 of these 27 clones manifested cytotoxic effector function and are the subject of the present report.

To estimate the number of specificities encoded by the alloreactive Ti_{3A}^+ CTL clones, we assembled a panel of Epstein-Barr virus (EBV)-transformed B lymphoblastoid target cells expressing known class I and class II MHC specificities and determined the pattern of killing by individual clones. As shown in Table I, even without examining the serologically defined alldeterminants of the individual target cells, a minimum of six distinct clonal specificities was defined. For example, clones AA2, AA6, AA8, and AA9, AA18 were selectively cytotoxic

for the B lymphoblastoid line Laz 509, but for none of the other seven target cells in this panel. In contrast, the other CTL clones killed Laz 509 and one or more different target cells. Thus, AA13 killed cell SEL002; AA14 killed Laz 503, Laz 471, and Laz 475; CP4 killed Laz 467 and Laz 471; CP6 killed Laz 468; and CP10 killed Laz 156, Laz 503, Laz 467, and Laz 468.

The class of the target cell MHC molecules recognized by individual CTL clones was examined by antibody blocking studies. For this purpose, Laz 509 was preincubated in parallel with either the W6/32 mAb directed at a framework determinant on the HLA α chain of A, B, and C loci or mAb directed against class II MHC gene products. The latter included antibody PTF29 directed against a monomorphic epitope on DR molecules, the mAb BT2.9 directed against a monomorphic region of both DR and SB gene products, and the mAb BT3.4 directed against a polymorphic DC epitope (related to DC1) expressed by Laz 509 (7–9). Such analysis of putative class II specificities with these anti-Ia antibodies provisionally allows for localization of specificities to a gene product of one of the three MHC class II subloci.

A composite of the antibody blocking data is provided in Table I and the representative experimental data that resulted in the assignments is depicted in Fig. 1. For example (Fig. 1A), it is evident that the T4⁺ clone AA18 is directed against a class II MHC molecule. The anti-HLA antibody failed to block specific cytotoxicity whereas the anti-DR-specific mAb PTF29 inhibited specific killing by 91% and mAb BT2.9 inhibited killing by 41%. Note that these effects cannot be nonspecific because, unlike the other anti-class II antibodies, the anti-DC1 antibody BT3.4 did not block killing function. On the other hand, the T8⁺ clone AA6 was blocked in an altogether different pattern, consistent with a class I clonal specificity (Fig. 1B). In this case, anti-HLA antibody inhibited cytotoxicity by 87% while all three of the anti-Ia antibodies blocked cytotoxicity by <6%. Fig. 1C shows yet another different pattern, with the T8⁺ clone AA9. Because this clone is inhibited by anti-BT2.9 and anti-PTF29 antibody and not by anti-HLA, it has been assigned a class II specificity. Nevertheless, the inhibition is distinct from the class II-specific T_{H3}⁺ clone AA18, in that AA9 was equally well inhibited by BT2.9 and PTF29. These results suggest that, while both clones recognize epitopes on DR molecules, the epitopes are distinct from one another. The collective data (Table I) indicate that five CTL clones are directed at class II and five CTL clones at class I MHC gene products.

To relate the specificity of individual T cell clones resulting in lysis of select target cells to known serologically defined histocompatibility types, we examined the class I and II MHC type of each target cell in the panel. Furthermore, the panel analysis shown in Table I was extended to 10 more B lymphoblastoid lines of known genotype (not shown). Specificities in Table I (right-hand column) were assigned on the basis of individual B lymphoblastoid lines sharing a known MHC allele to serve as targets for a given clone. Results were always consistent with the class of MHC target molecule defined by the mAb blocking studies in Fig. 1. While there was often no precise correlation between serologically defined HLA specificities and T cell clone allospecificities, this was not unexpected given the possibility that there are differences in epitopes recognized by antibodies and T cell receptors and the monoclonality of the T cell populations vs. the polyclonality of alloantibodies used. Thus, clones AA6 and CP10 could not be assigned to

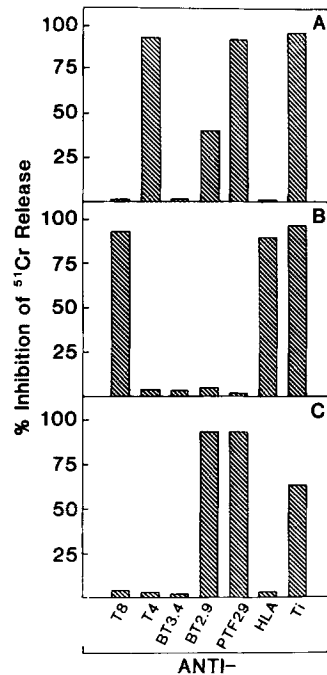


FIGURE 1. Inhibition of lytic function of various Ti_{3A}^+ CTL clones by mAb directed at T cell surface structures or MHC gene products. Percent inhibition of Laz 509 killing is noted on the ordinate for the $T4^+$ clone AA18 (A); the $T8^+$ clone AA6 (B); and the $T8^+$ clone AA9 (C). ^{51}Cr release from Laz 509 was as follows: maximal release, 1,966 cpm; spontaneous release, 215 cpm; and AA18-induced release, 950 cpm in the absence of antibodies. Results are representative of similar values obtained for clones in B and C (E/T ratio, 10:1).

known class I determinants, and clone AA14 recognized only a subset of HLA-A2 molecules. Nevertheless, this analysis indicated that clone AA13 is directed at HLA-B13, and clone CP6 at HLA-A25. In addition, three clones, AA8, AA9, and AA18, appear to be directed at private DR7 or DR4 determinants expressed only on the Laz 509 line. These three clones are distinct from one another, however, since the restriction pattern of REX V genes in Southern analysis was different in each (not shown). Whether this indicates that several different antigen/MHC receptor molecules may give rise to the same private specificity or that such T cell receptor molecules view different private epitopes on the DR7 or DR4 molecules of Laz 509, is not presently known. Even in the absence of SB typing, it is likely that T cell clones AA2 and CT4 recognized distinct SB determinants as judged by the ability of BT2.9 mAb to inhibit killing of these clones better than PTF29 (83–96% vs. 21–55%) and, in the case of CP4, to kill homozygous DR typing cells lacking related DR alleles. The present data imply that the REX $Ti \beta$ V genes can be used to generate both class I and II specificities, and that multiple MHC specificities can be generated against each of these MHC gene products.

It was previously suggested (10) that the antigen binding structure for nominal antigen and polymorphic MHC is the Ti glycoprotein and that T8 and T4 function as ancillary recognition elements which bind to invariant epitopes of class I or II molecules, respectively. One prediction from this model is that the

T3-Ti complexes of T4⁺ and T8⁺ T lymphocytes are fundamentally similar. Class II and class I MHC preferences of T4⁺ and T8⁺ clones are consequences of the ability of T4⁺ clones to bind class II and nominal antigen "X" on the stimulating cell more efficiently than T8⁺ clones (and vice versa for class I-nominal antigen X molecules) during the primary immune response. On the other hand, clones with intrinsically high affinity T3-Ti complexes that viewed class II-X or class I-X directly would not use the ancillary T4 and T8 molecules.

The experimental results herein with the T8⁺ clones AA6 and AA9 are consistent with this prediction. Thus, whereas the T8⁺ MHC class I-specific clone AA6 was inhibited by anti-T8 and not anti-T4 antibody, the MHC class II-specific T8⁺ clone AA9 was inhibited neither by anti-T8 nor anti-T4 antibody. Moreover, the antigen/MHC receptor antibody anti-Ti_{3A} virtually abrogated the cytotoxic effector function of clone AA6 but only inhibited the CTL effector function of AA9 by 65%. Titration analysis (not shown) indicates clearly that AA6 is inhibited using approximately one order of magnitude less anti-Ti_{3A} antibody. These results suggest that the T3-Ti complex of AA6 is of lower affinity than that of AA9, even though both employ a REX Ti β V gene. Consequently, the former requires the T8 ancillary recognition structure.

If both class II and I specificities can be generated from T cell clones by using a single set of highly homologous Ti β V genes, what is the basis upon which unique Ti domains encoding multiple and distinct specificities are generated? This question must be addressed in light of the lack of evidence for somatic mutation in T cell receptor V genes and a growing body of data indicating that the number of germline V genes encoding the Ti β subunit is small relative to the number of IgH V genes (4). It is likely, therefore, that combinatorial diversity created by association of different gene segments during active V gene formation within the Ti β subunit (including at least 2 possible D segments and 12 possible J segments, 6 Jβ1, and 6 Jβ2 segments) is in large part responsible for receptor repertoire generation. The fact that recombination sequences allow for direct V-J joining as well as V to multiple D and J joining, uniquely in T cells, further supports this view (3). In addition, junctional diversity would be created by variations in the joining sites of these gene segments. Moreover, extensive diversity would be created by combinatorial and junctional mechanisms in Ti α subunits as well. The latter subunit appears to be encoded by a substantially larger number of V and J gene segments (L. Hood, personal communication). Finally, association-mediated diversity resulting from assembly of different Ti α and β V region polypeptides would amplify diversity.

In conclusion, the present data show that a single set of highly related Ti β V genes can give rise to multiple T lymphocyte receptors with class I as well as class II specificities. The sequences of V β cDNAs isolated from several murine T cell clones of different specificities are also consistent with the present findings (11, 12). While not enough is known about the structural basis of T cell receptor antigen recognition or fine specificities of a large number of T cell clones using a prototypic Ti β V gene, it is tempting to speculate that at least certain different allospecificities may be created by junctional diversification alone.

Summary

10 alloreactive cytotoxic T lymphocytes using REX Ti β variable region (V)

gene segments in formation of their antigen/major histocompatibility complex (MHC) T3-Ti receptor were selected, cloned, and characterized in an effort to examine the extent of receptor diversity created by this one V gene family. Multiple and distinct class II as well as class I allospecificities were generated from the formation of different Ti β V domains. Five allospecificities were directed at various class I epitopes whereas the other five were directed at class II MHC gene products. The following conclusions were drawn: (a) Ti β V genes do not segregate into those that encode class I and those that encode class II allospecificities; and (b) there is no restriction on the Ti β V gene pool available to T4⁺ vs. T8⁺ T lymphocytes.

We thank Dr. Charles B. Carpenter for his assistance in HLA typing and Dr. Giorgio Corte for his helpful suggestions and provision of anti-Ia monoclonal antibodies.

Received for publication 24 June 1985 and in revised form 29 July 1985.

References

1. Meuer, S. C., K. A. Fitzgerald, R. E. Hussey, J. C. Hodgdon, S. F. Schlossman, and E. L. Reinherz. 1983. Clonotypic structures involved in antigen specific human T cell function: relationship to the T3 molecular complex. *J. Exp. Med.* 157:705.
2. Haskins, K., R. Kubo, J. White, M. Pigeon, J. Kappler, and P. Marrack. 1983. The major histocompatibility complex-restricted antigen receptor on T cells. I. Isolation with a monoclonal antibody. *J. Exp. Med.* 157:1149.
3. Davis, M. M., Y. Chien, N. R. J. Gascoigne, and S. M. Hedrick. 1984. A murine T cell receptor gene complex: isolation, structure and rearrangement. *Immunol. Rev.* 81:235.
4. Acuto, O., T. J. Campen, H. D. Royer, R. E. Hussey, C. B. Poole, and E. L. Reinherz. 1985. Molecular analysis of T cell receptor variable region (V) gene expression: evidence that a single Ti β V gene family can be used in formation of V domains on phenotypically and functionally diverse T cell population. *J. Exp. Med.* 161:1326.
5. Royer, H. D., O. Acuto, M. Fabbi, R. Tizard, K. Ramachandran, J. E. Smart, and E. L. Reinherz. 1984. Genes encoding the Ti β subunit of the antigen/MHC receptor undergo rearrangement during intrathymic ontogeny prior to surface T3-Ti expression. *Cell.* 39:261.
6. Meuer, S. C., S. F. Schlossman, and E. L. Reinherz. 1982. Clonal analysis of human cytotoxic T lymphocytes: T4⁺ and T8⁺ effector T cells recognize products of different major histocompatibility complex regions. *Proc. Natl. Acad. Sci. USA.* 79:4395.
7. Tosi, R., N. Tanigaki, R. Accolla, R. Sorrentino, and G. Corte. 1981. Binding of one monoclonal antibody to human Ia molecules can be enhanced by a second monoclonal antibody. *Eur. J. Immunol.* 11:721.
8. Corte, G., F. Calabi, G. Damiani, A. Bargellisi, R. Tosi, and R. Sorrentino. 1981. Human Ia molecules carrying DC1 determinants differ in both alpha and beta subunits from Ia molecules carrying DR determinants. *Nature (Lond.)* 292:357.
9. Accolla, R. S., G. Corte, and R. Tosi. 1984. Classification of anti-class II monoclonal antibodies based on epitope and molecular assignment. *Dis. Markers.* 2:39.
10. Reinherz, E. L., S. C. Meuer, and S. F. Schlossman. 1983. The delineation of antigen receptors on human T lymphocytes. *Immunol. Today.* 4:5.
11. Gorman, J., K. Mirard, N. Shastri, T. Hunkapiller, D. Hansburg, E. Sarcarz and L. Hood. 1985. Rearranged β T cell receptor genes in a helper T cell clone specific for lysozymes: no correlation between V β gene segments and antigen specificity or MHC restrictions. *Cell.* 40:859.
12. Rupp, F., H. Acha-Orbea, H. Hengartner, R. Zinkernagel, and R. Joho. 1985. Identical V β T cell receptor genes used in alloreactive cytotoxic and antigen plus I-A-specific helper T cells. *Nature (Lond.)* 315:425.