Brief Definitive Report

EXPRESSION OF A MURINE POLYCLONAL T CELL RECEPTOR MARKER CORRELATES WITH THE USE OF SPECIFIC MEMBERS OF THE $V_\beta 8$ GENE SEGMENT SUBFAMILY

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The mAbs KJ16-133 (1, 2) and F23.1 (3) are thought to bind a subset of murine T cell receptor β chain peptides that express variable region genes from the three-member $V_\beta 8$ subfamily (4, 5). These reagents recognize 10–40% of peripheral T cells in most inbred strains of mice (1–3), with the exception of the SJL, SWR, C57L, and C57BR mice, which have deleted this subfamily of V_β genes (4, 5). To directly relate $V_\beta 8$ utilization with reactivity to the KJ16-133 and F23.1 antibodies and to better characterize the fine specificity of these commonly used mAbs, we have examined a series of class I and II MHC-restricted T lymphocyte clones for expression of the KJ16-133 and F23.1 epitopes and for the V_β gene usage of the KJ16-133$^+$ and/or F23.1$^+$ clones. We find that both KJ16-133$^+$ and KJ16-133$^-$ clones are present among the panel of F23.1$^+$ clones. Furthermore, we find that F23.1$^+$ cells can express either the $V_\beta 8.1$, $V_\beta 8.2$, or $V_\beta 8.3$ gene segments, while KJ16-133$^+$ cells were seen to express only the $V_\beta 8.1$ or $V_\beta 8.2$ gene segments.

Materials and Methods

Cloned Cytotoxic T Lymphocytes. CTL clones were generated from splenocytes of CB6F1/J, BALB/c, or C57BL/6J mice immunized with the A/JAP/305/57 strain of influenza virus and were maintained according to methods detailed elsewhere (6).

Flow Cytometry. CTL clones were prepared for indirect fluorescence and analyzed on a FACS IV (Becton Dickinson & Co., Mountain View, CA) as described in detail elsewhere (7). The mAbs KJ16-133 and F23.1 were prepared from hybridomas obtained, respectively, from Dr. P. Marrack, National Jewish Hospital, Denver, CO and Dr. M. Bevan, Scripps Clinic and Research Foundation, La Jolla, CA.

Hybridization Probes. T cell receptor β gene probes specific for C_β, $C_\beta 1$ 3′ untranslated (UT), $C_\beta 2$ 3′ UT, and $V_\beta 8.2$ were subcloned from cDNA clones as described previously

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(5, 8). Oligonucleotide probes specific for the individual members of the $V_\beta 8$ subfamily were synthesized (complementary to sequence beginning at nucleotide 322 and ending at nucleotide 361 in clones TB12, TB2, and TB23, reference 9). The sequence of each probe is: $V_\beta 8.1$ 5′-AATATACAGCTGTCTGAGAAAGGGAAGCCAACTCCAGAAT-3′, $V_\beta 8.2$ 5′-AGTACACTGATGTCTGAGAGGGGGTAGCCAACTCCAGAAT-3′, and $V_\beta 8.3$ 5′-AGTACAAAGATGTCTGAGAGGGAGAAGCCAATTCCAGCAG-3′. Oligonucleotide probe specificity was tested by hybridization to cDNA clones that had previously been shown (Anderson, S. J., personal observation) to contain $V_\beta 8.1$, 8.2, and 8.3 by sequence analysis; each cDNA clone was digested with Eco RI, separated on 1% agarose gels, and transferred to nitrocellulose filters (10). Oligonucleotide probes were 5′ end-labeled with γ-[^{32}P]ATP using polynucleotide kinase (11) and filters were hybridized at 68°C in 0.9 M NaCl, 0.18 M Tris, pH 7.5, 0.012 M EDTA, 2X Denhardt's solution, and 20 μg/ml salmon sperm DNA. Filters were washed in 0.3 M NaCl, 0.03 M sodium citrate (2X SSC) at 68°C and they were autoradiographed.

Northern Blots. Total cellular RNA was prepared from 1–2 × 10^8 T cell clones by the guanidium isothiocyanate method (12). Total RNA (10–30 μg/lane) was separated on 1% agarose/formaldehyde gels and electroblotted onto Magnagraph nylon membranes (Micron Separations, Inc., Honeoye Falls, NY). Filters were hybridized to nick-translated probes (1–3 × 10^8 cpm/μg) in 0.75 M NaCl, 0.075 M sodium citrate (5X SCC), 5X Denhardt's solution, 50 μg/ml salmon sperm DNA, and 2.5% dextran sulphate at 68°C for >12 h (11). Filters were washed in 0.3 M NaCl, 0.03 M sodium citrate (2X SSC) at 68°C and autoradiographed. Filters were washed in 0.3 M NaCl, 0.03 sodium citrate (2X SSC) at 68°C and autoradiographed. Filters were hybridized to oligonucleotide probes as described above.

Results and Discussion

Characterization of T Cell Clones. 50 independently derived class I or class II MHC-restricted influenza virus-specific T lymphocyte clones were examined for reactivity with the KJ16-133 and F23.1 antibodies by flow cytometry. 25% of the clones were KJ16-133$^+$ and/or F23.1$^+$. Seven clones were chosen for further analysis. Clones 11-1, 14-13, 14-2, U12 and V6 react with both KJ16-133 and F23.1, while clones 40-3 and B1-11 react only with F23.1. Fluorescence profiles for the clone 11-1 and B1-11 are shown in Fig. 1, and antibody binding data for all the clones are summarized in Table I. Both KJ16-133$^+$,F23.1$^+$ and KJ16-133$^-$,F23.1$^+$ clones were found, but no KJ16-133$^+$ clones have yet been identified that are F23.1$^-$. Thus, these two anti-receptor antibodies do not define identical T lymphocyte populations, and the K16-133 antibody apears to react with a subset of F23.1$^+$ clones.

V_β Gene Usage. Northern blots of total cellular RNA from the seven T lymphocyte clones, The T cell lymphoma EL-4 (which is both KJ16-133$^-$ and F23.1$^-$), and the myeloma CBPC49 (13) were hybrizied against nick-translated probes specific for C_β, $C_\beta 1$, $C_\beta 2$, and $V_\beta 8$ (data not shown). None of these T cell specific probes hybridized to the myeloma RNA. RNA from EL-4, which expresses a $V_\beta 12$-$C_\beta 2$ species (Loh, D. Y., unpublished observation), hybridized to the $C_\beta 2$ and C_β probes but not to the $C_\beta 1$ and $V_\beta 8$ probes. Consistent with the view that KJ16-133 and F23.1 are $V_\beta 8$ subfamily-specific reagents, RNA from all seven T lymphocyte clones hybridized to the C_β and $V_\beta 8$ probes. Furthermore, RNA from clones 11-1, 14-2, 40-3, and B1-11 hybridized to a $C_\beta 1$ probe, while RNA from clones 14-13, U12, and V6 hybridized to $C_\beta 2$ (Table I). No obvious correlation is evident between $J_\beta 1/C_\beta 1$ or $J_\beta 2/C_\beta 2$ usage and F23.1 or KJ16-133 reactivity.

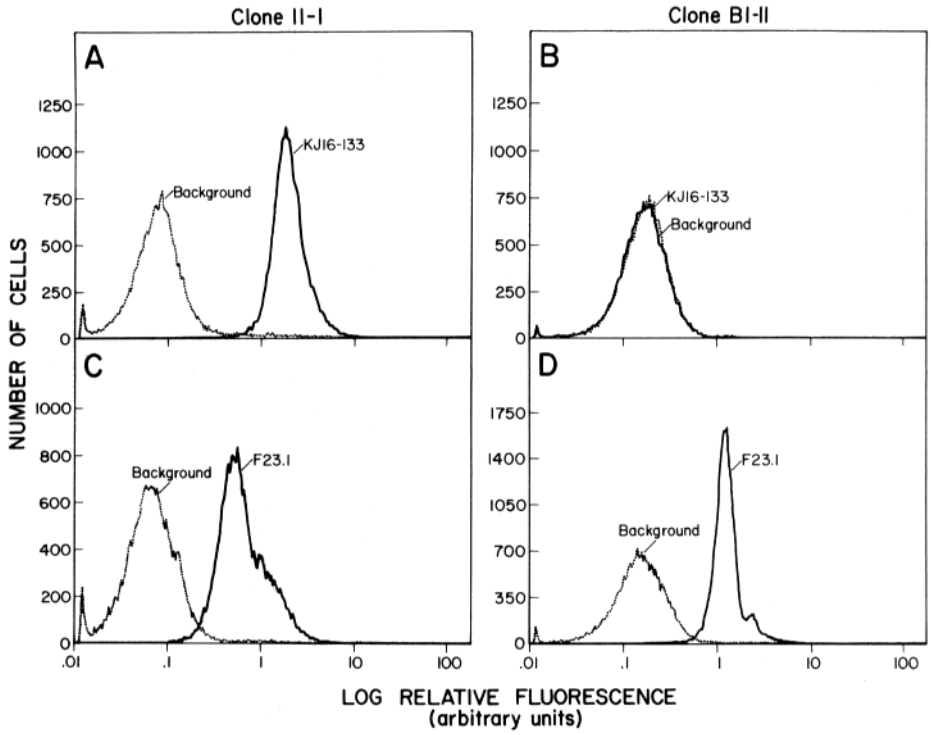

FIGURE 1. Differential recognition of two T lymphocyte clones by the mAbs KJ16-133 and F23.1. Clones 11-1 (*A* and *C*) and B1-11 (*B* and *D*) were examined by flow cytometry for expression of the T cell receptor determinants recognized by KJ16-133 and/or F23.1. Background flourescence is shown in each case.

TABLE 1

V_β Usage by a Panel of KJ16-133 and/or F23.1$^+$ T Cell Clones

T cell clone	MHC restriction	Antigen specificity*	Antibody Binding‡		V_β gene usage	J_β/C_β gene usage
			KJ16-133	F23.1		
11-1	K^d	A/JAP/57 (HA)	+	+	8.1	1
14-13	K^d	A/JAP/57 (NP)	+	+	8.1	2
C5	$A_\beta{}^b$	NP-Ova	+	+	8.1	2
14-2	K^d	A/JAP/57 (HA)	+	+	8.2	1
U12	$E_\beta{}^d$	A/JAP/57 (HA)	+	+	8.2	2
V6	$E_\beta{}^d$	A/JAP/57	+	+	8.2	2
2C	Class I allo	L^d	+	+	8.2	ND
40-3	K^d	A/JAP/57	−	+	8.3	1
B1.11	Class I H-2^b	A/JAP/57	−	+	8.3	1

* Abbreviations: HA, influenza virus hemagglutinin; NP, influenza virus nucleocapsid protein; NP-OVA, nitrophenol-OVA.
‡ Determined by flow cytometry.

To further characterize the β chain genes expressed by these T lymphocyte clones, synthetic oligonucleotide probes were synthesized that could distinguish between the V_β8.1, 8.2, and 8.3 genes by hybridization. In a control hybridization against known V_β8.1-, 8.2-, and 8.3- containing cDNA clones (Fig. 2), each probe

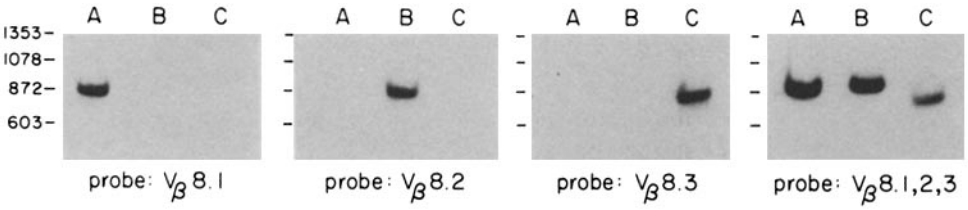

FIGURE 2. Oligonucleotide probe specificity controls. cDNA clones that had been previously identified by sequence analysis to contain $V_\beta 8.1$ (lane *A*), $V_\beta 8.2$ (lane *B*), and $V_\beta 8.3$ (lane *C*) gene segments were hybridized with the indicated oligonucleotide probes. Positions of marker DNA fragments are indicated in base pairs.

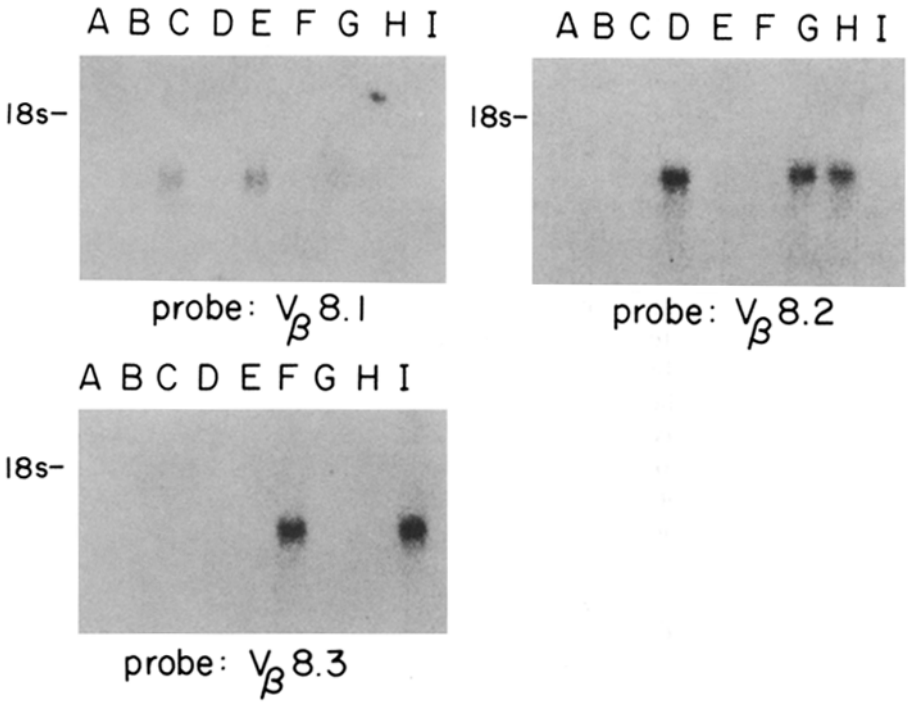

FIGURE 3. V_β gene usage in KJ16$^+$ and/or F23.1$^+$ T lymphocyte clones. Northern blots of total cellular RNA from myeloma CBPC29 (*A*), T lymphoma EL-4 (*B*), and T-cell clones 11-1 (*C*), 14-2 (*D*), 14-13 (*E*), B1-11 (*F*), U12 (*G*), V6 (*H*), and 40-3 (*I*) were hybridized with the indicated oligonucleotide probes. Position of the 18S ribosomal band is shown.

is clearly specific for only a single member of the $V_\beta 8$ subfamily. Northern blots were hybridized with the three oligonucleotide probes (Fig. 3). RNA from clones 11-1 and 14-13 hybridized to the $V_\beta 8.1$ probe, RNA from clones 14-2, U12, and V6 hybridized to the $V_\beta 8.2$ probe, and RNA from clones 40-3 and B1-11 hybridized to the $V_\beta 8.3$ probe. Thus, we find that the T lymphocyte clones that express $V_\beta 8.1$ or $V_\beta 8.2$ react with both KJ16-133 and F23.1, while the clones that express $V_\beta 8.3$ react only with F23.1.

Additional $V_\beta 8$-expressing T cell clones have been reported. The T_h clone C5 expresses $V_\beta 8.1$ (14) and the cytotoxic T lymphocyte clone 2C expresses $V_\beta 8.2$ (15); both clones react with both KJ16-133 and F23.1 (Freeman, G., P. Billings,

and J. Bluestone, personal communications). To date we have no conclusive evidence that expression of a $V_\beta 8$ subfamily gene can be dissociated from F23.1 reactivity. Since the antigenic epitopes detected by these two antibodies are not defined and any contribution of the α chain product to the formation of these epitopes is not understood, exceptions will possibly emerge. Notably, T cell clones may arise that are KJ16-133$^-$, F23.1$^-$ and still express a $V_\beta 8$ subfamily gene product. In addition, it is also possible that certain $V_\beta 8/J_\beta$ or $V_\beta 8/V_\alpha$ associations could lead to a KJ16-133$^+$,F23.1$^-$ phenotype in a $V_\beta 8$-expressing cell, or that certain non-$V_\beta 8/V_\alpha$ combinations might generate an epitope that can crossreact with the KJ16-133 and/or F23.1 antibodies. These predictions await experimental verification.

The KJ16-133 and F23.1 antibodies are the only murine nonclonotypic anti-V_β reagents reported. These antibodies were obtained by different immunization strategies in two different species (1, 3). F23.1 appears to recognize a determinant present on all three members of the $V_\beta 8$ subfamily, while KJ16-133 appears to recognize a determinant present on only two members of this subfamily. These antibodies, therefore, may recognize two distinct determinants. Since the F23.1 antibody interferes with KJ16-133 binding to T cell clones in an asymmetric fashion (Henkel, T. J., unpublished observation), these determinants may be sterically close to each other. Alternatively, the antibodies might recognize a single determinant that is polymorphic between members of the $V_\beta 8$ subfamily, with the observed differential binding patterns resulting from varying degrees of crossreactivity or affinity of each antibody for these polymorphic determinants. While we cannot predict what elements of $V_\beta 8$ peptides contribute to KJ16-133 and/or F23.1 reactivity, we do note that the predicted $V_\beta 8.1$ and $V_\beta 8.2$ amino acid sequences both contain a potential *N*-linked glycosylation site (residue 75, reference 9) that is not present in $V_\beta 8.3$ and that might contribute to KJ16-133 reacting with $V_\beta 8.1$ and $V_\beta 8.2$ but not $V_\beta 8.3$.

Summary

A series of murine T lymphocyte clones were examined for reactivity with the KJ16-133 and F23.1 mAbs. Clones that were KJ16-133$^+$,F23.1$^+$ and KJ16-133$^-$,F23.1$^+$ were identified, but no KJ16-133$^+$,F23.1$^-$ clones were observed. Within our panel of clones, therefore, the KJ16-133 antibody identifies a subset of F23.1$^+$ cells. All F23.1$^+$ clones examined express members of the $V_\beta 8$ subfamily of β chain variable region genes; clones expressing $V_\beta 8.1$ or $V_\beta 8.2$ reacted with both KJ16-133 and F23.1, while clones expressing $V_\beta 8.3$ reacted only with F23.1. Thus, the differential reactivity of the KJ16-133 and F23.1 antibodies with cloned T cells correlates with the V_β gene expression of each clone. Reactivity with these antibodies should therefore be of utility for predicting the V_β gene expression in some T cell clones.

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References

1. Haskins, K., C. Hannum, J. White, N. Roehm, R. Kubo, J. Kappler, and P. Marrack. 1984. The antigen-specific, major histocompatibility complex–restricted receptor on T cells. VI. An antibody to a receptor allotype. *J. Exp. Med.* 160:452.
2. Roehm, N., A. Carbone, E. Kushnir, B. Taylor, R. Riblet, P. Marrack, and J. Kappler. 1985. The major histocompatibility complex-restricted antigen receptor on T cells. The genetics of expression of an allotype. *J. Immunol.* 135:2176.
3. Staerz, U., H.-G. Rammensee, J. Benedetto, and M. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. *J. Immunol.* 134:3994.
4. Sim, G., and A. Augustin. 1985. V_β gene polymorphism and a major polyclonal T cell receptor idiotype. *Cell.* 42:89.
5. Behlke, M., H. Chou, K. Huppi, and D. Loh. 1986. Murine T-cell receptor mutants with deletions of β-chain variable region genes. *Proc. Natl. Acad. Sci. USA.* 83:767.
6. Braciale, T. J., M. E. Andrew, and V. L. Braciale. 1981. Heterogeneity and specificity of cloned lines of influenza-virus-specific cytotoxic T lymphocytes. *J. Exp. Med.* 153:910.
7. Braciale, T. J., T. J. Henkel, A. Lukacher, and V. L. Braciale. 1986. Fine specificity and antigen receptor expression among influenza virus specific cytolytic T lymphocyte clones. *J. Immunol.* 137:995.
8. Behlke, M., D. Spinella, H. Chou, W. Sha, D. Hartl, and D. Loh. 1985. T-cell receptor β-chain expression: dependence on relatively few variable region genes. *Science (Wash. DC).* 229:566.
9. Barth, R., B. Kim, N. Lan, T. Hunkapiller, N. Sobieck, A. Winoto, H. Gershenfeld, C. Okada, D. Hansburg, I. Weissman, and L. Hood. 1985. The murine T-cell receptor uses a limited repertoire of expressed V_β gene segments. *Nature (Lond.).* 316:517.
10. Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* 98:503.
11. Maniatis, T., E. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
12. Chirgwin, J., A. Przyblyla, R. MacDonald, and W. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294.
13. Azuma, T., L. Steiner, and H. Eisen. 1981. Identification of a third type of λ light chain in mouse immunoglobulins. *Proc. Natl. Acad. Sci. USA.* 78:569.
14. Patten, P., T. Yokota, J. Rothbard, Y. Chien, K. Arai, and M. Davis. 1984. Structure, expression, and divergence of T-cell receptor β-chain variable regions. *Nature (Lond.).* 312:40.
15. Garman, R., J.-L. Ko, C. Vulpe, and D. Raulet. 1986. T-cell receptor variable region gene usage in T-cell populations. *Proc. Natl. Acad. Sci. USA.* 83:3987.