Title
Positive selection determines T cell receptor V beta 14 gene usage by CD8+ T cells.

Journal
Journal of Experimental Medicine, 170(1)

ISSN
0022-1007

Authors
Liao, NS
Maltzman, J
Raulet, DH

Publication Date
1989-07-01

DOI
10.1084/jem.170.1.135

Peer reviewed

POSITIVE SELECTION DETERMINES T CELL RECEPTOR Vβ14 GENE USAGE BY CD8+ T CELLS

By NAN-SHIH LIAO, JONATHAN MALTZMAN, and DAVID H. RAULET

From the Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Mature T cells recognize fragments of antigens bound to cell surface molecules encoded by the polymorphic genes of the MHC. There is increasing evidence that during T cell development within the thymus, the T cell specificity repertoire is shaped by both positive and negative selection processes involving MHC molecules. Self-MHC-reactive T cells are negatively selected (clonally deleted) during T cell development, as first revealed by studies of particular TCR Vβ regions that confer defined autospecificities on T cell receptors: T cells expressing these Vβ regions are specifically eliminated intrathymically (1–3). Recent studies with transgenic mice expressing male-specific or alloantigen-specific transgenic TCRs have dramatically confirmed and extended these observations (4, 5). In addition, accumulating evidence indicates that positive intrathymic selection events allow the maturation of only those T cells that are capable of reacting with foreign peptides bound to *self*-MHC molecules. Classic studies to this effect (6–8) involved bone marrow chimeras and thymus grafting experiments, and showed that MHC molecules expressed by radioresistant thymic cells, presumably thymic epithelial cells (9), determine the restriction specificity of developing T cells. Some authors raised concerns about these studies, however, due to the possibility that artifactual suppressive effects in chimeras could account for the failure to detect T cells restricted to nonthymic MHC molecules (10, 11). More recently, elegant studies of transgenic mice expressing defined TCRs have provided strong evidence that maturation of T cells requires allele-specific interactions between the TCR and MHC molecules in the thymus (5, 12). Moreover, these studies suggest that the interactions of a TCR with either class I or class II MHC molecules determine the ultimate phenotype (CD8 or CD4) of developing T cells. Evidence of MHC-dependent skewing of TCRs in normal, nontransgenic, nonchimeric mice would serve to complement both the earlier chimera experiments and recent transgenic experiments by showing that positive selection occurs in unmanipulated mice.

In the present study we provide evidence that the frequency of T cells expressing a particular Vβ-region (Vβ14) is regulated in a positive fashion by specific MHC genes. These data provide evidence from normal mice that positive selection is important in shaping the repertoire, and that the germline sequence of a Vβ gene can be important in influencing the positive selection process.

This work was supported by National Institutes of Health grant CA-28900 to D. H. Raulet and by a Cancer Center Core Grant (P. A. Sharp, prinicipal investigator), and by an investigator award to D. H. Raulet from the Cancer Research Institute/Frances and Edwin L. Cummings Memorial Fund, and by the Ajinomoto Corp. N.-S. Liao is supported by a fellowship from the American Cancer Society, Massachusetts Division.

Materials and Methods

Mice. BALB.B., BALB.K and (BALB.B × BALB.K)F_1 mice were bred at the Center for Cancer Research, MIT. All other strains were purchased from The Jackson Laboratories, Bar Harbor, ME.

T Cell Lines and Hybrids. J6.19 is a B10.A-derived helper T cell clone specific for ovalbumin plus IA^k (13) that has been previously shown to express a TCR β chain encoded by a Vβ14-Dβ1.1-Jβ2.3-Cβ2 rearranged gene (14). The cells were generously supplied by Gerry Nau and Dr. Frank Fitch of the University of Chicago (Chicago, IL). Four T cell hybrids that express Vβ14 (15BBM21, 17BBM159, 4BR145, and 18BBM61) were generously provided by Drs. Jerome Bill and Ed Palmer, National Jewish Center, Denver, CO. All four cell lines were typed by those authors by hybridization with V-specific probes. The 15BBM21 and 17BBM159 cell lines express Vβ14 transcripts and no other donor Vβ transcripts, and the Vβ14 transcripts were shown by sequencing to be productive. The 4BR145 and 18BBM61 cell lines expressed both Vβ14 and Vβ8 transcripts, but our analysis (in Table I) indicates that Vβ8 chains are not expressed at the cell surface. The 2B4 (15, 16) and 2Q23-34 (17) cell lines were generously provided by Drs. Samelson (National Institutes of Health, Bethesda, MD) and Kappler and Marrack (National Jewish Center, Denver, CO), respectively. The 42G8 and 42H11 (18) T hybrids were provided by Dr. Brigitte Huber (Tufts University Medical School, Boston, MA); DAAP1 was generated by Dr. D. Wegman and provided to us by B. Huber.

Production of Vβ14 mAb. Male Fisher rats were immunized four times intraperitoneally with 2-wk intervals with 10^7 live J6.19 cloned T cells. The rats were rested for 1 mo, boosted intravenously with 10^7 live J6.19 cells, and 3 d later spleen cells were fused with the myeloma P3X63.AG8, using PEG 4000 and a ratio of five spleen cells/myeloma cells (19). Culture supernatants of growing hybridomas were each tested by their capacity to stimulate IL-2 production by two independent Vβ14-expressing T hybrids (15BBM21 and 17BBM159) and failure to stimulate a Vβ6-expressing T hybrid (DAAP1). For these tests, plastic microwells (No. 3596 microtiter plates; Costar, Cambridge, MA) were coated with purified goat anti-rat Ig antibodies (0.5 μg/ml antibody in 100 μl PBS overnight at 4°C). After thorough washing 200 μl of each hybridoma culture supernatant was added to the wells for 3 h at 37°C. The plates were again washed thoroughly, and 10^5 T hybridoma cells were added to each well in 200 μl of RPMI 1640 medium supplemented with 10% FCS, 50 μM 2-ME, 0.02% glutamine, and antibiotics. After 24 h incubation at 37°C, cell-free culture supernatants were collected and tested for IL-2 content using the IL-2 indicator cell line CTLL-2-15H as described (20). Of three initial hybridoma culture supernatants that stimulated the Vβ14⁺ T hybrids but not the Vβ6⁺ hybrid, one (14-2) maintained activity after two recloning efforts. 14-2 is an IgM/κ by the criteria that it reacts well with specific FITC-conjugated goat anti-rat μ (Southern Biotechnology Associates, Birmingham, AL) and mouse anti-rat κ (Bioproducts for Science, Indianapolis, IN) second reagents in cell surface staining analysis. It reacts relatively poorly with goat anti-rat IgG (mouse-serum absorbed; Kirkegaard & Perry Laboratories, Gaithersburg, MD) second reagents.

Other mAbs. Anti-CD3 (145-2C11, reference 21) anti-Vβ6 (RR4-7) and anti-Vβ8 (F23.1, reference 22) hybridomas were generously provided by Drs. Bluestone (University of Chicago, Chicago, IL), Kanagawa (Lilly Research Foundation, La Jolla, CA), and Bevan (Scripps Clinic and Research Foundation, La Jolla, CA), respectively. J1J (anti-Thy-1, reference 23) and GK1.5 (anti-CD4, reference 24) were generously provided by Drs. Jonathan Sprent (Scripps Clinic and Research Foundation, La Jolla, CA) and Frank Fitch (University of Chicago, Chicago, IL), respectively. The anti-CD8 monoclonal (AD4 (15), reference 25) was provided by Dr. Paul Gottlieb (University of Texas, Austin, TX).

Radiation Bone Marrow Chimeras. Bone marrow cells were prepared from (BALB.B × BALB.K)F_1 mice and were depleted of T cells by two consecutive treatments with anti-Thy-1 (J1J), anti-CD4 (GK1.5), and anti-CD8 (AD4-15) mAbs plus complement. 10^7 bone marrow cells were injected into either BALB.B or BALB.K mice (14–20 wk old) that had been irradiated (900 rad from a ^{137}Cs source) 2 h earlier. Lymph node cells from chimeras were harvested for staining analysis 8–12 wk later. At that time samples of lymph node cells

from each chimera were typed in microcytotoxicity assays using anti-D^b or anti-D^k mAbs plus complement. In all cases the lymph node cells were >95% of donor origin.

Immunofluorescent Staining Analysis. Lymph node or thymus cell suspensions from individual mice were stained at 5×10^5 to 10^6 cells/50 μl sample for 20 min at 4°C in PBS/5% FCS/0.2% NaN_3. Between stains, cells were washed three times. After the final steps, cells were fixed in 1% formaldehyde/PBS. Cells were subjected to two-color analysis on an Epics C flow cytometer.

For two-color analysis of Vβ14$^+$ T cells vs. CD4 or CD8, cells were stained first with 14-2 culture supernatant, followed by FITC-goat anti-rat IgM (mouse serum adsorbed) (Southern Biotechnology Associates), followed by either PE-GK15 (CD4) or biotin-53.6.7 (CD8) (Becton Dickinson & Co., Mountain View, CA). For CD8 staining we followed with PE-streptavidin (Biomedia, Foster City, CA). In all experiments control staining with KJ16 (anti-Vβ8.1, 8.2) (revealed by FITC-goat anti-rat IgG; Kirkegaard & Perry Laboratories) were run in parallel. The differences observed with Vβ14 were not observed with KJ16. For staining of J11d$^-$ thymocytes, we also determined the proportion of CD3$^+$ cells in each subset by staining with 145-2C11 and FITC-goat anti-hamster IgG (Cappel Laboratories, Malvern, PA); in all cases >98% of the CD4$^+$ or CD8$^+$ cells were CD3$^+$. For determining the percentage of Vβ14$^+$ T cells in each subset, we compared staining with and without 14-2 antibody present. The background staining in the positive quadrant in the absence of 14-2 antibody was subtracted in each case.

Results and Discussion

Vβ14 Transcript Levels Are Regulated by MHC Genes. To assess whether usage of Vβ genes by CD4 and CD8 T cells is controlled by MHC genes, mRNA levels corresponding to different Vβ genes were determined in purified T cell subsets from MHC-congenic strains. We assumed that a difference in the frequency of cells expressing a given Vβ would be reflected in differences in the level of the corresponding transcript in the population. Total RNA samples prepared from purified CD4 and CD8 cells were analyzed by RNA blot hybridization or ribonuclease protection assays using Vβ-specific probes. Analysis with a Vβ14 probe revealed that Vβ14 transcripts are reproducibly three to fivefold more abundant in CD4 T cells of BALB.B (H-2^b) and BALB/c (H-2^d) strains than in CD8 T cells from these strains (data not shown). In contrast, Vβ14 transcript levels in CD4 and CD8 T cells of the BALB.K (H-2^k) strain are nearly equal, and comparable to the level in CD4 cells of BALB.B and BALB/c mice. These results suggest that Vβ14 usage is regulated by MHC genes, presumably as a consequence of thymic selection processes (see below).

A mAb Reactive with Vβ14. Because Vβ-transcript levels are an indirect measure of the frequency of Vβ-expressing cells in population, and are difficult to quantitate exactly, we produced an anti-Vβ14 mAb that can be used to enumerate Vβ14$^+$ T cells directly. Spleen cells from rats immunized several times with the Vβ14-expressing T helper cell clone J6.19 were used to generate hybridomas. Hybridoma supernatants were tested for their capacity to stimulate IL-2 production by two independent Vβ14-expressing T hybridomas. One mAb, called 14-2, stimulated both Vβ14-expressing T hybrids, but failed to stimulate a Vβ6-expressing T hybrid. After cloning, the 14-2 hybridoma antibody was tested further for specificity by indirect immunofluorescent staining of various cells, followed by analysis on a flow cytometer (Table I). The antibody stained four independent Vβ14-expressing T hybrids, but failed to stain five other T hybrids that use different Vβ regions. Furthermore, two-color fluorescence analysis of lymph node T cells with 14-2 and a Vβ8-specific mAb revealed

TABLE I

Reactivity of 14-2 Antibody with T Hybrids

Cell line‡	Vβ§	Vα‖	Mean fluorescence intensity*			
			14-2	Anti-Vβ6¶	Anti-Vβ8**	Anti-CD3‡‡
15BBM21	14	3	120/6.2	ND§§	ND	200/8.0
17BBM159	14	8	60/21	ND	ND	100/9.8
4BR145	14,8	3	200/10	ND	9/9	410/10
18BBM61	14,8	4	45/5.5	ND	5.2/5.3	310/8
DAAP1	6	?	5/4.8	100/9.5	ND	160/5.5
2B4	3	11.2	6.5/6.0	ND	ND	33/6.2
42G8	?	?	0.9/0.8	ND	ND	7/0.9
42H11	4	?	2.5/2.5	ND	ND	32/3.2
2Q23-34	17	?	2.7/3.0	ND	ND	10.2/2.5

* Staining intensity (arbitrary linear units) with each reagent plus a second reagent is compared with that of cells stained with second reagent only.

‡ See Materials and Methods.

§ V usage by the first four cell lines listed was determined by hybridization with V-specific probes (E. Palmer, personal communication). Although two of the cell lines express both Vβ14 and Vβ8 transcripts, our analysis here shows that they do not express Vβ8 at the cell surface, and are therefore apparently Vβ14⁺ cells.

‖ The Vα family, where known, is indicated.

¶ The Vβ6 specific hybridoma, RR4-7, was a gift of Dr. Osami Kanagawa.

** The F23.1 hybridoma, which detects all three Vβ8 family members, was used.

‡‡ The 145-2C11 monoclonal was used.

§§ Not determined.

that 14-2 expression and Vβ8 expression are mutually exclusive, properties consistent with a Vβ-specificity of the 14-2 antibody (data not shown). The 14-2 antibody stained ~8% of lymph node T cells (see below) and did not stain B cells (data not shown), again consistent with its assignment as a Vβ-specific antibody. We conclude that 14-2 is a Vβ14-specific antibody, useful for cell surface staining analyses.

Vβ Usage Differs between CD4 and CD8 Subsets and Is Controlled by MHC Genes. The frequencies of Vβ14⁺ CD4 and CD8 T cells for several inbred MHC congenic strains were determined by two-color immunofluorescence analysis (Figs. 1 and 2). In the BALB.K strain (H-2^k), 7.5% ± 0.5% of CD4 T cells and 8.4% ± 0.3% of CD8 T cells are Vβ14⁺. In the BALB.B (H-2^b) strain, 8.0% ± 1.0% of CD4 T cells but only 2.3% ± 0.4% of CD8 T cells express Vβ14 (Fig. 1). These results indicate that Vβ14 usage by CD8 T cells is regulated by MHC genes. This conclusion was confirmed by analysis of an independent MHC congenic set, B10.BR (H-2^k) and C57BL10/J (H-2^b) (Fig. 2).

MHC control of Vβ-usage could be the result of intrathymic selection pressures during T cell development or to postthymic events, such as selective expansion of Vβ14⁺ CD8 T cells by enviromental antigens. As one means to distinguish these possibilities we examined Vβ14 usage by thymocytes of mature phenotype, which were enriched by lysis of immature thymocytes with the J11d antibody plus complement (23). The results indicate that the differential usage of Vβ14 between CD8 T cells of B10.BR and B10 strains is already evident in the thymus: 8.1 ± 0.5% of B10.BR CD8⁺ CD4⁻ thymocytes but only 2.8 ± 0.2% of C57BL/10 CD8⁺CD4⁻ thymocytes express Vβ14. The frequencies of T cells expressing Vβ8.1 and Vβ8.2,

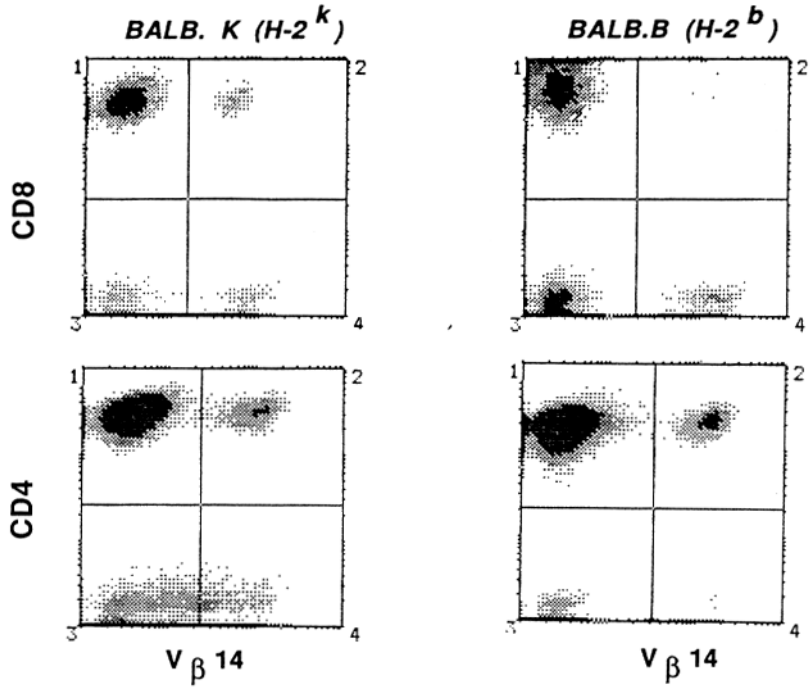

FIGURE 1. Two-color immunofluorescence analysis of Vβ14 vs. CD4 and CD8 in lymph node cells from BALB.K and BALB.B mice. In this experiment, 8.6% of BALB.K $CD8^+$ T cells and 8.3% of BALB.K $CD4^+$ T cells were $V\beta14^+$; 2.4% of BALB.B $CD8^+$ T cells; and 7.8% of BALB.B $CD4^+$ T cells were $V\beta14^+$.

detected with the KJ16 antibody, were similar between the $CD8^+$ thymocytes of the two strains (data not shown). These results suggest that the differential usage of Vβ14 is the result of intrathymic selection events.

CD8 T cells generally recognize antigens in the context of class I MHC molecules, so we reasoned that MHC class I genes are most likely to influence Vβ usage in CD8 T cells. In support of this notion, we found that the usage of Vβ14 was high in the B10.AKM strain but relatively low in the B10.MBR strain (Fig. 2). These strains differ only in that B10.AKM expresses K^k while B10.MBR expresses K^b. These results indicate that expression of the K^k class I molecules results in more frequent usage of Vβ14 by CD8 T cells. In contrast, the C3H-$H2^{02}$ strain, which expresses D^k but not K^k, shows infrequent usage of $V\beta14^+$ T cells among CD8 T cells. As a control, C3H (K^k, D^k) mice, like other H-2^k mice, show a high frequency of $V\beta14^+CD8^+$ T cells. In other experiments (not shown) we also found that H-2^d mice express a low (2.8%) frequency of $V\beta14^+CD8^+$ T cells. Taken together, these data indicate that expression of the K^k molecule leads to a high frequency of $V\beta14^+CD8^+$ T cells. The other class I molecules tested (D^k, K^b, D^b, K^d, D^d, L^d, D^q), in the absence of K^k, resulted in a low frequency of $V\beta14^+CD8^+$ T cells.

Both positive and negative intrathymic selection events are thought to influence the formation of the T cell repertoire. Thus, it was possible that $V\beta14^+$ T cells are eliminated intrathymically because they show strong reactivity with H-2^b molecules

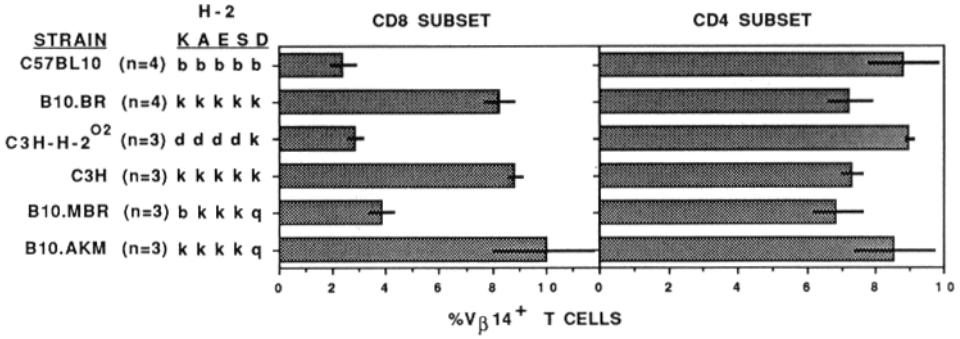

FIGURE 2. Frequency of Vβ14⁺ T cells in CD8 and CD4 subsets of lymph node cells from several mouse strains. Results are presented as the mean percent of CD8 or CD4 cells that are Vβ14⁺ ± SD. The number of animals tested (n) is indicated. Student's t-test calculations indicate that $p < 0.001$ for C57BL/10 vs. B10.BR CD8⁺ T cells and for C3H-H2^{02} vs. C3H CD8⁺ T cells. $p < 0.05$ for B10.MBR vs. B10.AKM CD8⁺ T cells.

or with another murine antigen associated with H-2^b molecules. Alternatively, Vβ14⁺ TCRs may interact with H-2^k MHC molecules in a manner that leads to preferential positive selection of T cells in H-2^k mice compared with H-2^b or H-2^d mice. Several arguments and experiments suggest that the latter mechanism, preferential positive selection of Vβ14⁺ T cells by H-2^k molecules, accounts for our findings. First, in all systems examined to date in which Vβ-expressing T cells undergo negative selection, they are eliminated efficiently from both the CD4 and CD8 subsets. In contrast, Vβ14 usage by CD4 T cells in H-2^b mice is similar to that in H-2^k mice (7–8%). Second, if H-2^b molecules cause clonal deletion of Vβ14⁺ CD8⁺ T cells, H-$2^{k/b}$ heterozygous mice should express Vβ14⁺ T cells infrequently among their CD8⁺ T cells. In fact, the frequency of Vβ14⁺ CD8⁺ T cells in the H-$2^{k/b}$ mice is 5.1%, intermediate between the H-2^b and H-2^k parents (Fig. 3). This result is consistent with positive selection of Vβ14⁺ T cells by H-2^k molecules, since it might be predicted that approximately one-half of the T cells in H-$2^{k/b}$ mice will be positively selected by H-2^k MHC molecules and half by H-2^b molecules; thus it might

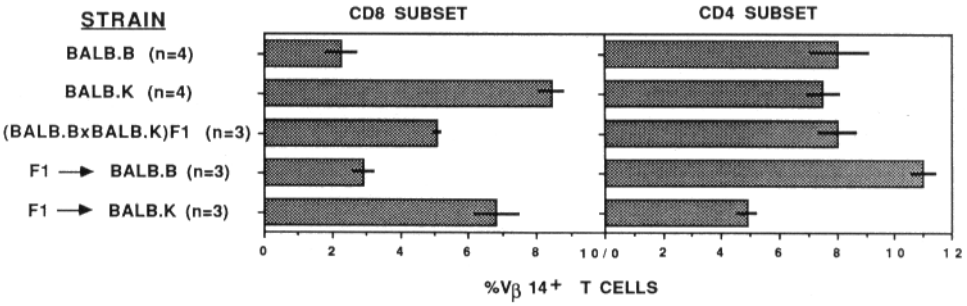

FIGURE 3. Frequency of Vβ14⁺ T cells in CD4 and CD8 subsets of (BALB.B × BALB.K)F_1 → parent radiation bone marrow chimeras. The number of animals tested (n) is indicated. Student's t test calculations indicate that $p < 0.001$ for BALB.B vs. BALB.K CD8⁺ T cells and $p < 0.002$ for F_1 → BALB.B vs. F_1 → BALB.K CD8⁺ T cells. Also, $p < 0.001$ for F_1 → BALB.B vs. F_1 → BALB.K CD4⁺ T cells.

be expected that the frequency of $V\beta14^+$ $CD8^+$ T cells in the heterozygote would correspond approximately to the average of the parents of the heterozygote.

To demonstrate directly that frequent usage of $V\beta14^+$ T cells in $H\text{-}2^k$ mice is due to positive selection by $H\text{-}2^k$ MHC molecules, we constructed radiation chimeras in which $H\text{-}2^{k/b}$ bone marrow cells were transferred to irradiated $H\text{-}2^b$ or $H\text{-}2^k$ parental mice. Previous studies have shown that while T cells from such mice are rendered tolerant to both parental H-2 molecules, the MHC restriction specificity is determined by the thymus of the irradiated host (6–8). The results indicate that $CD8^+$ T cells from $H\text{-}2^{k/b} \rightarrow H\text{-}2^k$ chimeras more frequently express $V\beta14$ than do $CD8^+$ T cells from $H\text{-}2^{k/b} \rightarrow H\text{-}2^b$ chimeras (Fig. 3). These results directly demonstrate that the differential frequency of $V\beta14^+$ $CD8^+$ T cells is due to the positive and not negative selection, since the T cells are expected to be tolerant of the same spectrum of antigens in both types of chimeras. It is noteworthy that the "skew" observed in the chimeras is slightly less pronounced than in the normal parental strains. It is possible that a few mature $H\text{-}2^{k/b}$ T cells in the bone marrow inoculum escaped out T cell depletion procedure, despite two consecutive anti-Thy-1 plus C treatments. Also noteworthy is that the frequency of $CD4^+$ $V\beta14^+$ T cells in the $H\text{-}2^{k/b} \rightarrow H\text{-}2^k$ chimeras is significantly depressed. The reason for the latter effect is under investigation.

Our results demonstrate that $CD8^+$ T cells expressing $V\beta14$ are positively selected in $H\text{-}2^k$ mice to a much greater extent than in $H\text{-}2^b$ mice. Significant effects of positive selection can therefore be observed in normal unmanipulated mice. A similar MHC-dependent "skew" of $V\beta6$ usage was recently reported by MacDonald and colleagues (26), but these authors did not generate chimeras to demonstrate directly that the observed skew was due to positive selection effects. Skewing of T cells expressing other $V\beta$s may also occur (Liao, N.-S., J. Maltzman, and D. H. Raulet, unpublished results). However, such skewing is not necessarily the rule, as earlier studies of several other $V\beta$s and $V\alpha$s showed little effect of MHC on their usage, and little difference in their usage between CD4 and CD8 subsets (27, 28).

In our experiments, although the usage of $V\beta14$ by CD8 T cells varies depending upon the MHC, the usage of $V\beta14$ by CD4 T cells in all strains examined is relatively high (~8%). Recent studies of TCR transgenic mice suggest that positive selection determines the phenotype (CD8 or CD4) of developing T cells depending on whether they interact with class I or class II MHC molecules, respectively (5, 11). Similarly, we interpret our results as reflecting a relative deficit in the interaction between $V\beta14^+$ TCR and the K^b and D^b (among other) class I molecules resulting in inefficient positive selection of $V\beta14^+$ thymocytes into the CD8 subset in $H\text{-}2^b$ mice. According to this line of reasoning $V\beta14^+$ TCR are better able to interact with the K^k class I molecule, and with class II molecules of both $H\text{-}2^k$ and $H\text{-}2^b$ haplotypes, accounting for the more efficient positive selection evident in the $H\text{-}2^k$ CD8 subset and the CD4 subset of both strains. This interpretation implies that the $V\beta14$ sequence, with significant independence of the $D\beta$, $J\beta$, and $V\alpha$-$J\alpha$ sequence, is important in determining the class I restriction specificity of the TCR. We prefer a model in which $V\beta14$ domains interact directly and effectively with K^k molecules but less effectively with the other class I molecules tested. This model fits with a recent proposal (29) that the first and second complementarity-determining regions (CDRs) of TCR subunits, which are encoded by the germline sequences of the $V\beta$ and $V\alpha$ genes, interact directly with MHC residues; the third CDR, created

in large part by V-D-J junctional diversity, may interact with antigenic peptides. However, since it is not known whether peptides bound to MHC molecules may be involved in positive selection, we cannot rule out the possibility that K^k molecules on thymic epithelial cells present specific peptides that are recognized by Vβ14-containing TCR, resulting in efficient positive selection of these cells. Further studies of MHC-mutant mice may help resolve this question.

Summary

We report here a mAb, 14-2, reactive with TCRs that include Vβ14. The frequency of Vβ14$^+$ T cells varies with CD4 and CD8 subset and is controlled by the H-2 genes. Thus CD8$^+$ T cells from H-2^b mice include ~2.3% Vβ14$^+$ T cells while CD8$^+$ T cells from mice expressing K^k include >8% Vβ14$^+$ T cells. In all strains examined, 7–8% of CD4$^+$ T cells express Vβ14. The frequenct usage of Vβ14 in CD8$^+$ T cells of K^k-expressing mice is a result of preferential positive selection of Vβ14$^+$ CD8$^+$ T cells, as demonstrated by analysis of radiation chimeras. These studies demonstrate that H-2-dependent positive selection occurs in unmanipulated mice. Furthermore, the results imply that positive selection, and possibly H-2 restriction, can be strongly influenced by a Vβ domain, with some independence from the β-junctional sequence and α chain.

We thank our colleagues for many useful cell lines and reagents, especially Dr. Frank Fitch for the J619 cell line and Dr. Ed Palmer for the Vβ14$^+$ T cell hybrids. We gratefully acknowledge Gerry Nau in Dr. Fitch's lab for growing the J619 cells. In addition we thank Dr. Osami Kanagawa for helpful advice on production of TCR-specific mAbs, and A. Stewart Conner for expert assistance with flow cytometry.

Received for publication 22 February 1989.

References

1. Kappler, J. W., N. Roehm, and P. Marrack. 1987. T cell tolerance by clonal elimination in the thymus. *Cell.* 49:273.
2. Kappler, J. W., U. Staerz, J. White, and P. C. Marrack. 1988. Self-tolerance eliminates T cells specific for Mls-modified products of the major histocompatibility complex. *Nature (Lond.).* 332:35.
3. MacDonald, H. R., R. Schneider, R. K. Lees, R. C. Howe, H. Acha-Orbea, H. Festenstein, R. M. Zinkernagel, and H. Hengartner. 1988. T-cell receptor Vβ use predicts reactivity and tolerance to *Mls^a*-encoded antigens. *Nature (Lond.).* 332:40.
4. Kisielow, P., H. Blüthmann, U. D. Staerz, M. Steinmetz, and H. von Boehmer. 1988. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4$^+$8$^+$ thymocytes. *Nature (Lond.).* 333:742.
5. Sha, W. C., C. A. Nelson, R. D. Newberry, D. M. Kranz, J. H. Russell, and D. Y. Loh. 1988. Positive and negative selection of an antigen receptor on T cells in transgenic mice. *Nature (Lond.).* 336:73.
6. Bevan, M. J. 1977. In a radiation chimera host H-2 antigens determine the immune-responsiveness of donor cytotoxic T cells. *Nature (Lond.).* 269:417.
7. Zinkernagel, R. M., G. Callahan, A. Althage, S. Cooper, P. A. Klein, and J. Klein. 1978. On the thymus in the differentiation of H-2 self recognition by T cells: evidence for dual recognition? *J. Exp. Med.* 147:882.
8. Fink, P., and M. J. Bevan. 1978. H-2 antigens of the thymus determine lymphocyte specificity. *J. Exp. Med.* 148:766.

9. Lo, D., and J. Sprent. 1986. Identity of cells that imprint H-2 restricted T-cell specificity in the thymus. *Nature (Lond.).* 319:672.
10. Matzinger, P. 1981. A one-receptor view of T-cell behaviour. *Nature (Lond.).* 292:497.
11. Smith, F., and J. F. A. P. Miller. 1980. Suppression of T cells specific for the non-thymic parental H-2 haplotype in thymus grafted chimeras. *J. Exp. Med.* 151:246.
12. Teh, H. S., P. Kisielow, B. Scott, H. Kishi, Y. Uematsu, H. Blüthmann, and H. von Boehmer. 1988. Thymic major histocompatbility complex antigens and the $\alpha\beta$ T-cell receptor determine the CD4/CD8 phenotype of T cells. *Nature (Lond.).* 335:229.
13. Wilde, D. B., M. B. Prystowsky, D. I. Beller, E. Goldwasser, J. N. Ihle, S. N. Vogel, and F. W. Fitch. 1984. Comparison of allogeneic and self-restricted stimulation of lymphokine production by dual-reactive cloned T cells. *J. Immunol.* 133:1992.
14. Malissen, M., C. McCoy, D. Blanc, J. Trucy, C. Devaux, A.-M. Schmitt-Verhulst, F. Fitch, L. Hood, and B. Malissen. 1986. Direct evidence for chromosomal inversion during T-cell receptor β-gene rearrangements. *Nature (Lond.).* 319:28.
15. Samelson, L., R. Germain, and R. H. Schwartz. 1983. Monoclonal antibodies against the antigen receptor on a cloned T-cell hybrid. *Proc. Natl. Acad. Sci. USA.* 80:6972.
16. Chien, Y., N. Gascoigne, J. Kavaler, N. Lee, and M. Davis. 1984. Somatic recombination in a murine T-cell receptor gene. *Nature (Lond.).* 309:322.
17. Kappler, J., T. Wade, J. White, E. Kushner, M. Blackman, J. Bill, N. Roehm, and P. Marrack. 1987. A T cell receptor Vβ segment that imparts reactivity to a class II major histocompatibility complex product. *Cell.* 49:263.
18. Sherman, D., P. Hochman, R. Dick, R. Tizard, K. Ramachandran, R. Flavell, and B. Huber. 1987. Molecular analysis of antigen recognition by insulin specific T cell hybridomas from B6 wild-type and bm12 mutant mice. *Mol. Cell. Biol.* 7:1865.
19. White-Scharf, M., and T. Imanishi-Kari. 1981. Characterization of the idiotype through the analysis of monoclonal Balb/c anti-NP antibodies. *Eur. J. Immunol.* 11:897.
20. Gillis, S., M. M. Ferm, W. Ou, and K. Smith. 1978. T cell growth factor: parameters of production and quantitative microassay for activity. *J. Immunol.* 120:2027.
21. Leo, O., M. Foo, D. Sachs, L. Samelson, and J. Bluestone. 1987. Identification of a monoclonal antibody specific for a murine T3 polypeptide. *Proc. Natl. Acad. Sci. USA.* 84:1374.
22. Staerz, U. D., H.-G. Rammensee, J. D. Benedetto, and M. J. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. *J. Immunol.* 134:3994.
23. Bruce, J., F. W. Symington, T. J. McKearn, and J. Sprent. 1981. A monoclonal antibody discriminating between subsets of T and B cells. *J. Immunol.* 127:2496.
24. Dialynas, D. P., D. B. Wilde, P. Marrack, A. Pierres, K. A. Wall, W. Havran, G. Otten, M. R. Loken, M. Pierres, J. Kappler, and F. W. Fitch. 1983. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: expression of L3T4a by functional T cell clones appears to correlate primarily with class II MHC antigen-reactivity. *Immunol. Rev.* 74:29.
25. Raulet, D. H., P. Gottlieb, and M. J. Bevan. 1982. Fractination of lymphocyte populations with monoclonal antibodies specific for Lyt-2.2 and Lyt-3.1. *J. Immunol.* 125:1136.
26. MacDonald, H. R., R. K. Lees, R. Schneider, R. M. Zinkernagel, and H. Hengartner. 1988. Positive selection of $CD4^+$ thymocytes controlled by MHC class II gene products. *Nature (Lond.).* 336:471.
27. Garman, R. D., J.-L. Ko, C. D. Vulpe, and D. H. Raulet. 1986. T cell receptor variable region gene usage in T cell populations. *Proc. Natl. Acad. Sci. USA.* 83:3987.
28. Roehm, N., L. Herron, J. Cambier, D. Diguisto, K. Haskins, J. Kappler, and P. Marrack. 1984. The major histocompatibility complex-restricted antigen receptor on T cells: distribution on thymus and peripheral T cells. *Cell.* 38:577-584.
29. Davis, M. M., and P. J. Bjorkman. 1988. T cell antigen genes and T cell recognition. *Nature (Lond.).* 334:395.