with buffer A containing 0.4 M NaCl, and proteins were eluted with buffer A containing 65% ethylene glycol. Fractions were diluted 1:10 in buffer B (buffer A with 0.1% Triton X-100) and incubated with Affi-Gel blue gel (equilibrated in buffer A) for 90 min. The resin was washed with buffer B, and proteins were eluted with 0.75 M NaCl in buffer B. Fractions containing protein were pooled and concentrated with Centriprep 30 (Amicon), and the concentrate was diluted with 10 volumes of buffer B and loaded on a Mono Q column. Proteins were eluted with a linear NaCl gradient (0 to 0.375 M) in buffer B, and column fractions were assayed for CREB kinase activity (*8*). Fractions with high CREB kinase activity were pooled, desalted by size exclusion chromatography, and applied to a Mono S column equilibrated in buffer B. Proteins were eluted with a linear gradient of 0 to 0.375 M NaCl in buffer B, and fractions were assayed for CREB kinase activity. Peak fractions were then pooled and concentrated in a Centricon-30, and proteins from the concentrated sample were separated on SDS-PAGE. The CREB kinase band (identified with an in-gel kinase assay) was excised and eluted from the gel by electroelution. The eluates from several preparations were pooled, concentrated, and subjected to SDS-PAGE. The CREB kinase polypeptide was then transferred to a polyvinylidene difluoride membrane for protein digestion and peptide sequencing.

14. Protease digestion of purified CREB kinase with the endoproteinase Lys-C, peptide isolation by high-performance liquid chromatography, and peptide sequencing were done by the Harvard Microchemistry Facility (Cambridge, Massachusetts). Sequences of four different peptides were obtained: EIAITHHVK, ISG_DARQ_YAMK, L_YAFQTEGK, ATNMEF_V (*25*). These sequences matched 100% with those of amino acids 49 to 57, 88 to 100, 133 to 142, and 443 to 450 of human RSK2, respectively, and shared 75% overall identity with corresponding regions of human RSK1 or RSK3. Underlined blanks in peptide sequences represent residues that could not be determined unambiguously.
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20. To generate the RSK2 expression vector pMT2-HA-RSK2, we cloned a sequence encoding the HA-tag (YPYDVPDYA) (*25*) into the NH_2-terminus of murine RSK2 using the polymerase chain reaction, and the HA-tagged RSK2 cDNA was cloned into the expression vector pMT2. We constructed the mutant RSK2 expression vector pMT2-HA-RSK2(KR100) by changing the coding determinants of Lys^{100} of RSK2 to Arg by site-directed mutagenesis [M. P. Weiner *et al.*, *Gene* **151**, 119 (1994)]. The following plasmids have been described: CMV-GAL4-CREB encoding a hybrid protein with the NH_2-terminal 147 amino acids of the yeast transcription factor GAL4 fused to the full-length CREB protein [L. A. Berkowitz and M. Z. Gilman, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5258 (1990)], pSG5-MKP1 (*24*), pCDNA3-MEK1(KA97) [R. Seger *et al.*, *J. Biol. Chem.* **269**, 25699 (1994)], and pCMV-Elk-1 [C. K. Miranti *et al.*, *Mol. Cell. Biol.* **15**, 3672 (1995)]. The constitutively active MEK expression vector pCDNA3-MEK1(SE218/222) was made by replacement of Ser^{218} and Ser^{222} with Glu residues.
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25. Abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; and Y, Tyr.
26. For immune complex kinase assays, cells were washed once in cold phosphate-buffered saline (PBS) and then quickly collected in Triton X-100 lysis buffer [20 mM tris (pH 6.8), 137 mM NaCl, 50 mM β-glycerophosphate, 1 mM Na_3VO_4, 2 mM EDTA, 2 mM EGTA, 1 mM DTT, 1% Triton X-100, 10% glycerol, 1 mM benzamidine, leupeptin (10 μg/ml), pepstatin (10 μg/ml), aprotinin (20 μg/ml), and 1 mM PMSF]. The lysates were mixed and centrifuged at 13,000*g* for 10 min at 4°C. Antibodies to RSKs and protein A–Sepharose beads (washed in lysis buffer) were then added to the supernatants. After a 1-hour incubation at 4°C, the immune complex was collected by centrifugation and washed twice with lysis buffer and twice with kinase buffer [50 mM Pipes (pH 7.3), 10 mM $MgCl_2$, and 1 mM DTT]. Washed immune complexes were used to phosphorylate CREBtide or recombinant CREB in kinase reactions as described (*8*).
27. Transfection of COS cells was done by the DEAE-dextran method [F. M. Ausubel *et al.*, Eds., *Current Protocols in Molecular Biology* (Wiley, New York, 1994)]. Two days after transfection, cells were treated as described (Figs. 3 and 4). Cells were washed in ice-cold PBS and lysed in boiling SDS sample buffer for in-gel kinase assay or immunoblotting analysis. Alternatively, cells extracts were prepared and subjected to an immune complex kinase assay (*26*).
28. Assays for CAT and β-galactosidase were done as described [J. Sambrook *et al.*, Eds., *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989)].
29. We thank D. E. Moller and Y. Zhao for providing antibodies to RSK3, R. L. Erikson for murine RSK2 cDNA, E. G. Krebs for plasmids pCDNA3-MEK1(SE218/222) and pCDNA3-MEK1(KA97), N. K. Tonks and H. Sun for pSG5-MKP1, M. Z. Gilman for CMV-GAL4-CREB, S. H. Orkin for cloning vector pMT2, members of the Greenberg laboratory for critical reading of the manuscript, A. Bonni and Z. Xia for helpful discussions, and S. E. Kim and Y. Zhang for technical assistance. D.D.G. is a recipient of a Klingenstein Award in Neuroscience, an American Cancer Society Junior Faculty Research Award, and an Alfred Sloan Research Fellowship. Supported by National Institutes of Health grants NS34814-01 (D.D.G.) and CA43855 (M.E.G.), Mental Retardation Research Center grant NIH P30-HD18655, and an American Cancer Society Faculty Research Award (FRA-379) (M.E.G.).

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Control of MHC Restriction by TCR V_α CDR1 and CDR2

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Individual T cell receptor (TCR) V_α elements are expressed preferentially in CD4 or CD8 peripheral T cell subsets. The closely related V_α3.1 and V_α3.2 elements show reciprocal selection into CD4 and CD8 subsets, respectively. Transgenic mice expressing site-directed mutants of a V_α3.1 gene were used to show that individual residues in either the complementarity-determining region 1 (CDR1) or CDR2 were sufficient to change selection from the CD4 subset to the CD8 subset. Thus, the germline-encoded V_α elements are a major influence on major histocompatibility class complex (MHC) restriction, most likely by a preferential interaction with one or the other class of MHC molecule.

Thymocytes that are positively selected on MHC class I proteins become peripheral $CD8^+$ cells and those positively selected on MHC class II proteins become $CD4^+$ peripheral T cells (*1*). An αβ TCR transgene from a $CD8^+$ T cell causes most T cells bearing that receptor to be positively selected into the $CD8^+$ compartment (*2*), whereas a transgenic TCR from a $CD4^+$ cell shows similarly skewed expression in the $CD4^+$ population (*3*). Less extreme skewing into the CD4 or CD8 peripheral T cell subset is also seen with individual V regions—most noticeably with V_α regions, which are preferentially expressed in one or the other subset (*4–9*). This phenomenon is largely independent of MHC haplotype and suggests either that individual V_α regions react preferentially with class I or class II molecules or that particular V_α regions associate with the CD4 or CD8 coreceptors.

The structure of the TCR V_α domain has recently been determined (*10*). The CDR3 segments of TCR V_α and V_β [produced by VJ or V(D)J recombination, respectively] are predicted to lie centrally in the combining site of the TCR (*10*, *11*). If CDR3 interacts with the peptide bound in the MHC groove, the less variable germline-encoded CDR1- and CDR2-equivalent regions would be available to interact with the MHC α helices. Thus, the skewed expression of V_α elements in CD4 and CD8 subsets suggests that the CDR1 and CDR2 of V_α could play a role in distinguishing between class I and II. Closely related members of the V_α3 family undergo selection by different MHC classes (*7*, *8*), allowing determination of the residues involved in MHC class discrimination. In B6 mice,

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V_α3.1 is expressed predominantly in CD4 peripheral T cells (6.3 ± 0.7% versus 2.4 ± 0.8% in CD8), whereas V_α3.2 is expressed mainly in CD8 cells (3.6 ± 0.2% versus 0.9 ± 0.1% in CD4) (*7, 8, 12*). A comparison of the sequences of V_α3.1 and V_α3.2 showed that four amino acid residues (at positions 27, 51, 85, and 92) differ between them (*8, 13*). Two out of the four differences lie within the CDR1 and CDR2. Thus, residue 27 (CDR1) and residue 51 (CDR2) are candidates for interaction with the α helices of the MHC molecules. The equivalent positions in V_α4 have side chains pointing out of the structure (*10*). Residue 85 lies at the base of β strand F and is slightly solvent-exposed. It could potentially be available to interact with the coreceptor molecules. Residue 92 is buried and is not part of CDR3.

To determine if the residues differing between V_α3.1 and V_α3.2 control selection on MHC class I or II, we altered a wild-type V_α3.1 gene to resemble V_α3.2 by making the mutations Ser^{27}→Phe (S27F), S51P, and S85W (*14*) and producing transgenic lines (*15, 16*). The wild-type V_α3.1 transgene showed skewed expression in the CD4 subset (Fig. 1) (*7*). In contrast, three independent lines carrying the mutant α chain (V_α3.1m) showed skewed expression in the CD8 subset (*17*). The V_α3.2-specific monoclonal antibody RR3-16 reacts with the V_α3.1m transgene (*18*). The CD8/CD4 ratio of expression of the V_α3.1m transgene ranged from 5.7 to 2.1. In both V_α3.1 and V_α3.1m transgenic mice, TCR V_α2 remained skewed into the $CD4^+$ cells (6), indicating that this phenomenon was limited to the transgenic α chain (*12*). Therefore, the three mutations created in the V_α3.1 gene were sufficient to change selection from CD4 into the CD8 subset.

We tested the CDR1 and CDR2 polymorphisms separately with transgenic mice in which the V_α3.1 gene was mutated to the V_α3.2 sequence only in CDR1 (CDR1m; S27F) or CDR2 (CDR2m; S51P). All three of the CDR1m transgenic lines overexpressed CDR1m in the CD8 subset with a CD8/CD4 ratio of 2 to 3 (Fig. 2, A and B) (*18, 19*). Similarly, CDR2m was expressed predominantly in the CD8 subset (Fig. 2, C

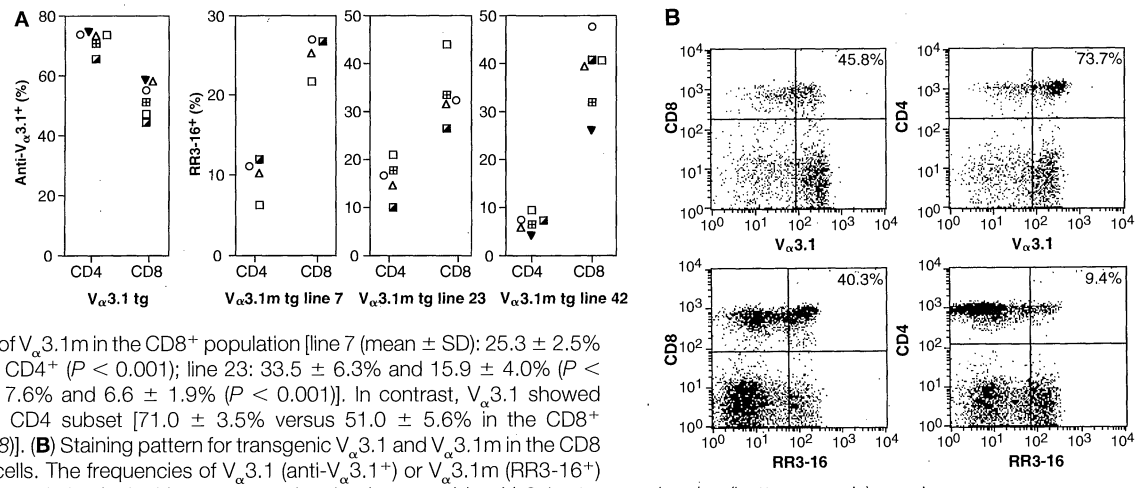

Fig. 1. (**A**) Three mutations in the V_α3.1 gene are sufficient to change class I and class II selection. Comparison of V_α3 expression in mice transgenic for V_α3.1 and in three independent V_α3.1m lines (*15, 16*). Symbols represent individual transgenic mice. All three V_α3.1m transgenic lines (lines 7, 23, and 42) showed a higher percentage of V_α3.1m in the $CD8^+$ population [line 7 (mean ± SD): 25.3 ± 2.5% of $CD8^+$ and 9.9 ± 2.5% of $CD4^+$ ($P < 0.001$); line 23: 33.5 ± 6.3% and 15.9 ± 4.0% ($P < 0.001$); and line 42: 37.6 ± 7.6% and 6.6 ± 1.9% ($P < 0.001$)]. In contrast, V_α3.1 showed reciprocal selection into the CD4 subset [71.0 ± 3.5% versus 51.0 ± 5.6% in the $CD8^+$ population ($P < 0.001$) (*17, 18*)]. (**B**) Staining pattern for transgenic V_α3.1 and V_α3.1m in the CD8 and CD4 subsets of $Thy1^+$ cells. The frequencies of V_α3.1 (anti-V_α3.1$^+$) or V_α3.1m (RR3-16$^+$) (*18*) cells in the CD8 or CD4 population in the V_α3.1 transgenic mice (top panels) or V_α3.1m transgenic mice (bottom panels) are shown.

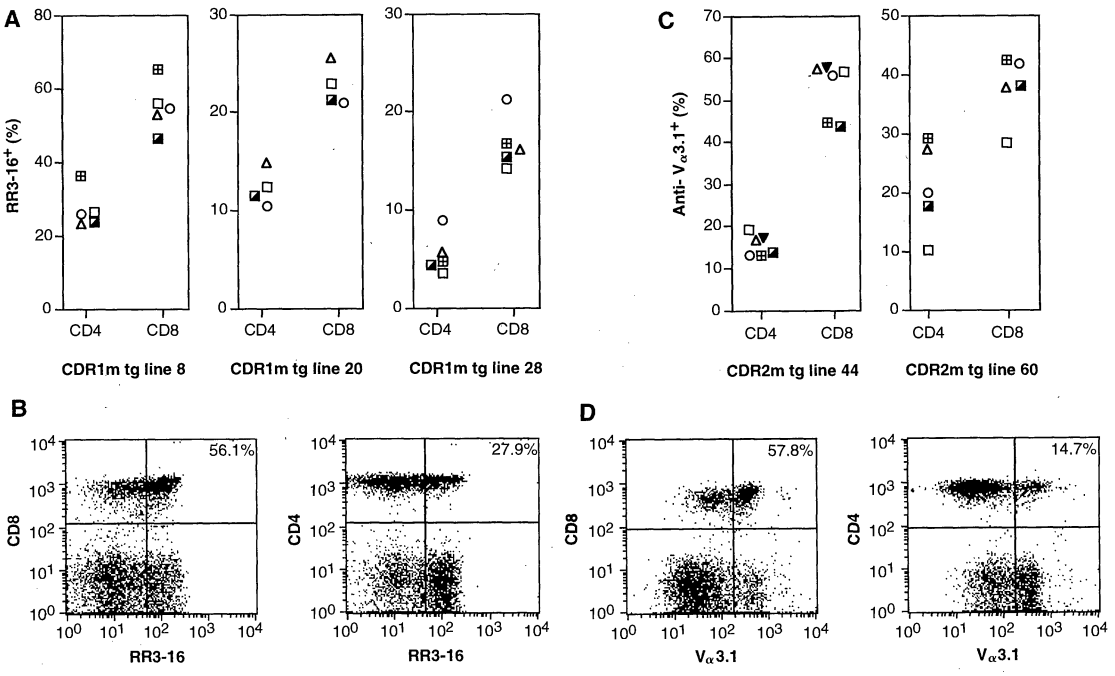

Fig. 2. Point mutations in CDR1 or CDR2 are sufficient to change class I and class II selection. The panels represent independent lineages of mice transgenic for CDR1m (**A**) and CDR2m (**C**). Symbols represent individual mice. The frequency of peripheral $CD4^+$ or $CD8^+$ T cells expressing CDR1m or CDR2m V_α chains was determined (*16*). CDR1m transgenic lines 8, 20, and 28 expressed a higher percentage of RR3-16^+ T cells (*18*) in the $CD8^+$ subset [line 8: 54.9 ± 6.7% in $CD8^+$ versus 27.0 ± 5.3% in $CD4^+$ ($P < 0.001$); line 20: 22.6 ± 2.0% versus 12.3 ± 1.8% ($P < 0.001$); and line 28: 16.7 ± 2.7% versus 5.5 ± 2.1% ($P < 0.001$)]. Both CDR2m transgenic lines showed similarly skewed expression in $CD8^+$ [line 44, 52.7 ± 6.4% versus 15.6 ± 2.6% in CD4 ($P < 0.001$); and line 60: 37.8 ± 5.6% versus 20.8 ± 7.7% ($P = 0.002$)]. An alternative method for calculating the skewed expression gave similar results (*19*). An example is shown of CDR1m (**B**) and CDR2m (**D**) staining frequencies in the $Thy1.2^+CD8^+$ and $Thy1.2^+CD4^+$ subsets.

and D) with a CD8/CD4 ratio of 3.4 for line 44 and 1.8 for line 60 (*19*). Therefore, either of the single–amino acid substitutions in the CDR1 or CDR2 region of the V_α3.1 gene was sufficient to alter its selection from a predominantly class II–restricted to a predominantly class I–restricted phenotype. As before, selection of V_α2 was unaffected (*12*). No clear difference in the strength of selection was noted between CDR1 or CDR2 regions.

Because skewing of other V_α elements appears to be caused by positive selection (*4*–*6*), we stained thymocytes for expression of the V_α3 proteins in the CD4, CD8-defined subsets (*20*). These showed a pattern consistent with increased positive selection into the relevant mature subset, although increased negative selection in the other cannot be ruled out. Bone marrow chimeras made with the panel of V_α transgenic mice (*21*) showed that preferential expression of the V_α3 transgenes in peripheral CD4 or CD8 cells was determined by the genetic origin of the bone marrow cells (Fig. 3). In the same thymic environment, the T cells bearing mutant forms of V_α3.1 (V_α3.1m, CDR1m, and CDR2m) were all selected preferentially into the CD8 subset, whereas the wild-type V_α3.1 transgene was skewed into the CD4 subset.

It is possible that the altered selection of V_α is caused by changes in TCR αβ pairing (*22*). Therefore, we investigated the participation of the β chain in the observed skewed expression of the various forms of V_α3.1 by breeding the panel of V_α transgenic mice with V_β5 and V_β3 transgenic mice (*23*). If there is no β chain effect on the V_α skewing, the resultant first generation mice will not be expected to show selection for or against the various forms of the $V_\alpha3V_\beta5$ or $V_\alpha3V_\beta3$ pairs other than the consistent bias of V_α3.1 usage in the CD4 subset and of mutant V_α3.1 in the CD8 subset. Alternatively, the fact that the V_β5 gene was derived from a class I–restricted cell and the V_β3 chain from a class II–restricted cell might cause changes in the selection. (Because the α and β chains came from different cells, they have no predictable peptide specificity.) When forced to pair with either V_β3 or V_β5, the various mutant forms of V_α3.1 were all selected preferentially into the CD8 subset, whereas the wild-type V_α3.1 was skewed into the CD4 subset (Fig. 4) (*24*). Therefore, the V_β5- or V_β3-chain element did not change the preference of the V_α3 proteins for selection on class I or class II.

Our data demonstrate that a single amino acid residue present in either the CDR1 or CDR2 regions of the V_α element is sufficient to change selection from the CD4 (class II–restricted) to the CD8 (class I–restricted) subset. Skewed selection of V_α can be explained either by a better interaction between a particular V_α and class I or class II molecules (*5*) or by preferential association with CD4 or CD8 coreceptors (*25*). The idea that a V_α element could interact better with all molecules of one MHC class than with the other is feasible because potential TCR contact residues along the α helices are remarkably conserved within MHC class, and there are significant structural differences between class I and class II (*26*). There is some evidence that coreceptors interact with the TCR as well as with the MHC (*27*). Although V_α3 has a motif associated with CD8 interaction (*25*), those residues are identical between V_α3.1 and V_α3.2 (*13*), which are selected into different subsets. The position of the residues that control selection of V_α3 (in CDRs 1 and 2) makes them unlikely to be involved in interactions with coreceptors, but likely to be involved in interactions with MHC (*10*, *11*). Therefore, the changes in V_α selection in the mutant transgenics are explained better by the interaction between the TCR and MHC (*5*) than by the coreceptor interaction model (*25*). These data, and the conservation of skewed selection across different MHC haplotypes (*4*–*6*), suggest that the orientation of the TCR-MHC interaction is fixed and conserved both within and between MHC class. Polymorphism in V_α CDR1 and CDR2 is likely to result in biased usage of the various members of a V_α family in class I– or class II–restricted T cells, which could explain the maintenance of large families of closely related V_α genes and the concentration of within-family diversity in the CDRs (*28*).

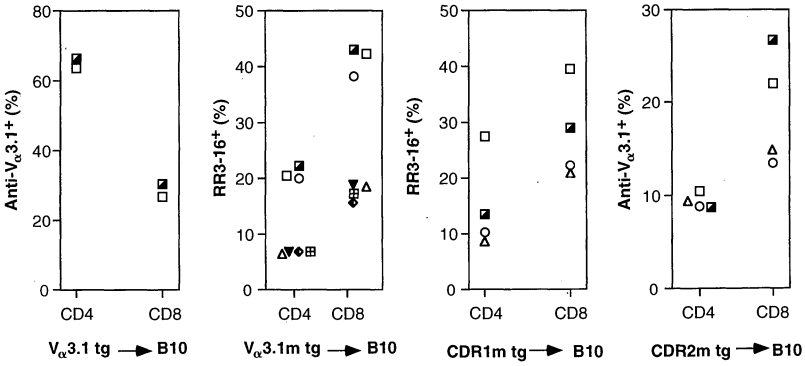

Fig. 3. CDR1 and CDR2 sequences determine MHC class restriction. Expression of the V_α3 transgenes was compared in radiation bone marrow chimeras. The frequencies of $V_\alpha3.1^+$ or RR3-16$^+$ cells in CD4 or CD8 subsets of the donor-derived cells are shown. Data from two separate experiments were pooled. Data (mean ± SD) from each experiment are as follows [CD4 cells, CD8 cells, *P* (Student's *t* test)]: V_α3.1: 64.9 ± 2.0, 28.5 ± 2.5, $P < 0.01$; V_α3.1m (experiment 1): 20.8 ± 1.3, 41.0 ± 2.5, $P < 0.01$; V_α3.1m (experiment 2): 6.5 ± 0.3, 17.3 ± 1.4, $P < 0.01$; CDR1m (experiment 1, one mouse): 27.5, 39.5; CDR1m (experiment 2): 10.7 ± 2.5, 23.9 ± 4.3, $P = 0.01$; CDR2m (experiment 1): 9.6 ± 1.3, 24.3 ± 3.3, $P < 0.03$; and CDR2m (experiment 2): 9.1 ± 0.4, 14.2 ± 0.9, $P < 0.02$. Donor-derived cells from nontransgenic littermate controls reciprocally selected V_α3.1 into the CD4 subset and RR3-16 in the CD8 population as expected (*12*).

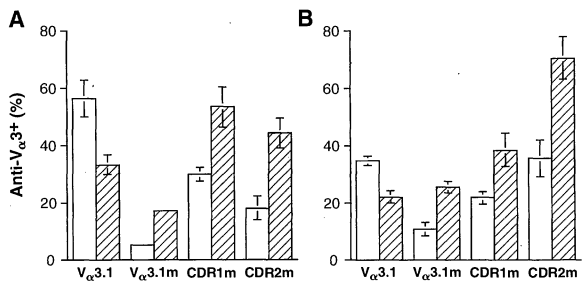

Fig. 4. Preferential selection of mutant V_α3 is not influenced by transgenic V_β3 and V_β5 chains. Mice analyzed were F_1 offspring derived by mating the panel of V_α3 transgenic mice to V_β3 or V_β5 transgenic mice. The MHC haplotypes were H-2^b for the V_β5 cross and H-$2^{b/k}$ for the V_β3 cross. Only F_1 mice bearing both $V_\alpha3V_\beta3$ (A) or $V_\alpha3V_\beta5$ (B) transgenes are shown. Peripheral blood T cells were stained for three-color FACS analysis (*24*). The cells were gated on $V_\beta3^+$ (A) or $V_\beta5^+$ (B) cells, and the percentage of cells positive for anti-V_α3.1 (V_α3.1 and CDR2m) or RR3-16 (V_α3.1m and CDR1m) in CD4 (open bars) or CD8 (cross-hatched bars) populations was determined.

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14. Mutations are designated by the wild-type residue, followed by its position, and the mutant residue. Abbreviations for the amino acid residues are as follows: F, Phe; P, Pro; S, Ser; and W, Trp.
15. The T cell clone Ar-5, specific for arsonate/I-A^d, expresses V_α3.1J_α31 (AV3S5J31) [K.-N. Tan *et al.*, *Cell* **54**, 247 (1988)]. Nomenclature follows (*13*) and B. F. Koop *et al.* [*Genomics* **19**, 478 (1994)]. This α chain was used to raise the antiserum to V_α3.1 (anti-V_α3.1) and to generate V_α3.1 transgenic mice (*7*). The monoclonal antibody RR3-16 recognizes V_α3.2 (AV3S2) (*8*). The AV3S5J31 genomic clone was mutated by polymerase chain reaction overlap extension [S. N. Ho, H. D. Hunt, R. M. Horton, J. K. Pullen, L. R. Pease, *Gene* **77**, 51 (1989)] and cloned into a genomic α–chain vector [V. Kouskoff, K. Signorelli, C. Benoist, D. Mathis, *J. Immunol. Methods* **180**, 273 (1995)]. Transgenic mice were generated at The Scripps Research Institute (TSRI) with FVB oocytes and backcrossed to C57BL/6J (B6) mice. All animals were treated in accordance with TSRI institutional guidelines.
16. Transgenic mice were screened by fluorescent-activated cell sorting (FACS) on peripheral blood lymphocytes, gating on viable Thy1.2$^+$ cells. The percentage of V_α-expressing cells in CD4 or CD8 populations was determined. Mice expressing greater than threefold above the endogenous level of V_αs were considered transgenic. Antibodies were purchased from PharMingen (San Diego, CA) unless otherwise stated. Biotinylated anti-V_α3.2 (RR3-16) (*8*) and anti-V_α2 (B20.1) (*6*) were used with streptavidin–fluorescein isothiocyanate (FITC). Anti-V_α3.1 (*7*) was used with goat anti-rabbit (H+L)-FITC. Red613-conjugated anti-CD4 (H129.19), anti-CD8α (53-6.7), and anti-Thy1.2-PE (30-H12) were obtained from Gibco BRL (Grand Island, NY).
17. The skewing pattern remained unchanged when analyzed as percent $V_\alpha3^+$ cells expressing CD4 or CD8. In the V_α3.1 transgenics, 20.1 ± 1.2% of $V_\alpha3.1^+$ cells were CD8$^+$ and 47.8 ± 2.4% were CD4$^+$. In the V_α3.1m (line 42) mice, 63.0 ± 6.9% of RR3-16$^+$ cells were CD8$^+$ and 14.9 ± 3.0% CD4$^+$. Peripheral CD4$^-$CD8$^-$ T cells were also present, as in other TCR transgenic lines (*29*). Of the Thy1$^+$ cells in the V_α3.1 transgenic mice, 40.8 ± 2.7% were CD4$^+$ and 24.2 ± 1.8% CD8$^+$, compared with 63.5 ± 6.7% and 30.3 ± 3.0% in the littermate controls. In V_α3.1m transgenic mice, 52.2 ± 5.5% of Thy1$^+$ cells were CD4$^+$ and 33.8 ± 3.4% were CD8$^+$, compared with the littermate values of 54.1 ± 5.8% and 34.3 ± 3.1%. The frequency of $V_\alpha3.1^+$ or RR3-16$^+$ cells within each T cell subset in nontransgenic littermates of each line was comparable to values obtained in wild-type B6 mice (*12*). Each line was second to fourth generation backcross to B6. All mice were homozygous for H-2^b.
18. The V_α3.1m and CDR1m proteins, but not the wild-type V_α3.1 or CDR2m, were detectable with the V_α3.2-specific monoclonal antibody, RR3-16 (*8*). Therefore, the specificity of RR3-16 resides in Phe^{27} in the CDR1 region.
19. Similar results were obtained by calculating the data as percent $V_\alpha3^+$ cells expressing CD4$^+$ or CD8$^+$. The RR3-16$^+$ T cells in CDR1m transgenic line 8 were 46.3 ± 6.1% CD8$^+$ and 29.3 ± 5.8% CD4$^+$. The CDR2m-expressing T cells in line 44 were 56.0 ± 5.0% CD8$^+$ and 22.8 ± 3.4% CD4$^+$. In the CDR1m transgenic mice, 48.7 ± 5.8% of Thy1$^+$ cells were CD4$^+$ and 36.3 ± 3.2% CD8$^+$. In CDR2m transgenic mice, 50.7 ± 4.5% of Thy1$^+$ cells were CD4$^+$ and 32.5 ± 5.7% CD8$^+$.
20. Expression of transgenic V_α3 on CD4$^+$CD8$^+$, CD4$^+$CD8$^-$, and CD4$^-$CD8$^+$ thymocytes, respectively. V_α3.1: 40.7, 43.4, and 39.0%; V_α3.1m (line 7): 10.4, 11.2, and 23.4%; CDR1m (line 8): 26.4, 17.4, and 29.5%; and CDR2m (line 44): 24.9, 16.4, and 31.1%.
21. Radiation bone marrow (BM) chimeras were prepared by standard techniques. Erythrocyte-free BM cells were isolated from the femur and tibia of donor mice and depleted of T cells by killing with anti-CD4, anti-CD8, and anti-Thy1 (clones RL172, 3-168, and T24.2.5, respectively) plus guinea pig complement (Gibco). Donor cells (3.0×10^6 to 1.0×10^7) were injected intravenously into irradiated host B10 mice [11 gray (Gy) of γ-irradiation]. Donor BM cells for V_α3.1m, CDR1m, and CDR2m were extracted from transgenics on the FVB background (Thy1.1, H-2^q). V_α3.1 BM was obtained from BALB/c background (Thy1.2, H-2^d) transgenic mice. Reconstituted splenocytes were examined by FACS after 8 to 10 weeks as described (*16*) with phycoerythrin–anti-mouse Thy1.1 (OX-7) or PE-H-$2K^d$ (SF1-1.1) to distinguish donor cells.
22. We stained these mice for V_β3-8, 10, 11, and 13, but did not see any significant differences.
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24. Three-color FACS analysis was done as described (*16*). Data were analyzed as a percentage of the appropriate V_α3 in the $V_\beta3^+$ (KJ-25$^+$) or $V_\beta5^+$ (MR9-4$^+$) transgenic population of CD4 and CD8 T cells. The frequency of transgenic β-chain expression in the αβ transgenic mice was similar to that in the β-chain transgenics, as was the percentage of T cells in the periphery (*12*), indicating that this αβ combination was selectable by some endogenous peptide-MHC complexes. The V_β transgenic mice were bred at TSRI.
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Protection Against Atherogenesis in Mice Mediated by Human Apolipoprotein A-IV

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Apolipoproteins are protein constituents of plasma lipid transport particles. Human apolipoprotein A-IV (apoA-IV) was expressed in the liver of C57BL/6 mice and mice deficient in apoE, both of which are prone to atherosclerosis, to investigate whether apoA-IV protects against this disease. In transgenic C57BL/6 mice on an atherogenic diet, the serum concentration of high density lipoprotein (HDL) cholesterol increased by 35 percent, whereas the concentration of endogenous apoA-I decreased by 29 percent, relative to those in transgenic mice on a normal diet. Expression of human apoA-IV in apoE-deficient mice on a normal diet resulted in an even more severe atherogenic lipoprotein profile, without affecting the concentration of HDL cholesterol, than that in nontransgenic apoE-deficient mice. However, transgenic mice of both backgrounds showed a substantial reduction in the size of atherosclerotic lesions. Thus, apoA-IV appears to protect against atherosclerosis by a mechanism that does not involve an increase in HDL cholesterol concentration.

The role of apoA-IV in lipid transport and lipoprotein metabolism is not clear. In humans, apoA-IV is associated with triglyceride-rich lipoproteins and HDL, and also occurs in a lipoprotein-free form (*1–4*). ApoA-IV has been proposed to play a role in reverse cholesterol transport (cholesterol transport from tissues back to the liver for elimination) on the basis of in vitro properties: It activates lecithin cholesterol acyltransferase (*5*, *6*), promotes cholesterol efflux from cholesterol-preloaded cells (*7–9*), and binds to hepatocytes (*10*).

To investigate the function of apoA-IV, we generated transgenic mice that express human apoA-IV in the liver. The transgene comprised an 8.4-kb human genomic DNA fragment encoding apoA-IV linked to the 1.7-kb Pst I–Pst I fragment of the hepatic control region of the apoE/C-I gene (*11*). The DNA fragment was injected into one-cell embryos of C57BL/6 mice (IFFA-Credo, Lyon,