Brief Definitive Report

Influence of T Cell Receptor Vα Expression on Mls^a Superantigen-specific T Cell Responses

By Melanie S. Vacchio,* Osami Kanagawa,‡ Kyuhei Tomonari,§ and Richard J. Hodes*

*From the *Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892; the ‡Department of Pathology, Washington University School of Medicine, St. Louis, Missouri 63110; and the §Transplantation Biology Section, Medical Research Council Clinical Research Center, Harrow, Middlesex, HA1 3UJ England*

Summary

Recognition of conventional foreign antigen by T cells is determined by the expression of multiple variable regions of both α and β chains of the T cell receptor (TCR) α/β heterodimer. In contrast, there exists a class of antigens that appears to interact with the TCR α/β heterodimer through the variable region on the β chain (Vβ), independent of other TCR components, a property that has led to their designation as superantigens. The goal of the present study was to analyze Vα use in Vβ6$^+$ T cells responsive to the superantigen, Mla^a. Results indicate that while deletion of T cells expressing Vβ6 in Mls^a-expressing mice is essentially complete and therefore appears to occur regardless of Vα usage, in vitro Mls^a stimulation of T cells from Mls^a-negative mice results in significant skewing of Vα use among responding Vβ6$^+$ T cells. This indicates that Vα expression influences recognition of the superantigen, Mls^a by mature peripheral T cells.

T cell recognition of conventional antigens is influenced by multiple variable regions of the α/β TCR (1). There exists, however, a class of antigens that appears to interact with the TCR α/β heterodimer through the V region on the β chain (Vβ), regardless of other variable regions (V, D, J, or N regions) expressed on either the α or β chain. The ability of these antigens to elicit vigorous primary proliferative responses from T cells based solely on Vβ use has led to their designation as superantigens (2). These superantigens occur endogenously in the mouse (3), and as bacterial, mycoplasma, and viral products that act as exogenous superantigens to murine and human T cells (4–6). TCR residues implicated in superantigen recognition have been mapped to regions of the TCR β chain distant from residues that appear to be critical for conventional antigen recognition (7–9).

Endogenously expressed superantigens in the mouse, now believed to be products of mouse mammary tumor viruses (10), have extensive effects on formation of the T cell repertoire. In the majority of instances, virtually all T cells expressing a superantigen-specific Vβ are deleted in mice which express that superantigen. In other instances, e.g., Vβ11 expression, only partial deletion occurs in some strains of mice (11). A potential explanation for this nonsusceptibility to negative selection is that variable regions of the TCR other than Vβ are involved in superantigen recognition.

The present study analyzed Vα use in Vβ6$^+$ T cells responsive to the superantigen Mls^a. Results indicate that whereas deletion of T cells expressing Vβ6 in Mls^a-expressing mice is essentially complete, regardless of Vα use, in vitro stimulation of T cells with Mls^a results in significant skewing of Vα use among responding Vβ6$^+$ T cells, indicating that Vα expression influences recognition of Mls^a.

Materials and Methods

Animals. BALB/c Mls^a mice were generated by Berumen et al. (12). Other mice were obtained from the Frederick Cancer Research Facility (Frederick, MD).

Reagents. Anti-Vα2 (B20.1) (13), anti-Vα3.2 (RR3-16) (14), anti-Vβ8 (KT50) (15), and anti-Vα11 (RR8-1) (16) antibodies were as previously reported. Purified and directly conjugated anti-Vβ6 were purchased from Pharmingen (La Jolla, CA). Biotin- and FITC-conjugated anti-Thy1.2 were obtained from Becton Dickinson & Co. (San Jose, CA). FITC-conjugated goat anti-rat Ig was purchased from Caltag, (San Francisco, CA).

Flow Cytometry Analysis. T cells were analyzed as previously described (17).

T Cell Stimulation. Splenocytes were enriched for T cells by panning on rabbit anti-mouse Ig coated plates (17). 3×10^6 T cells were cultured for 48 h with 6×10^6 mitomycin C-treated spleen cells of the indicated strain, or in wells that had been coated with 10 μg/ml anti-Vβ6 mAb. After 48 h, RIL2 (Cetus Corp., Emeryville, CA) (final concentration 50 U/ml) was added directly to the cultures. Cultures were harvested 6–7 d after initiation.

Results

T cells expressing Vβ6 are clonally deleted during development in an environment in which Mls^a is expressed as a

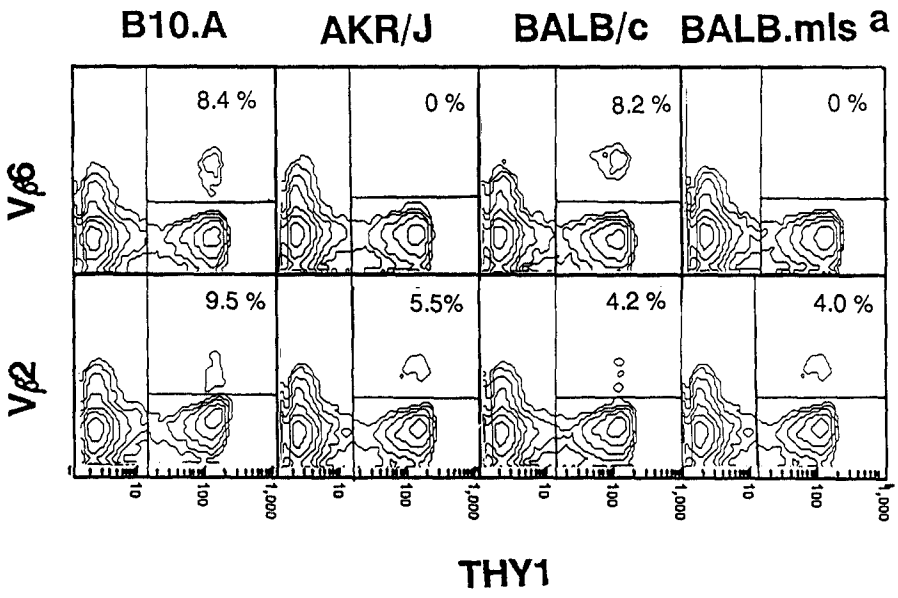

Figure 1. Expression of Mls^a results in essentially complete deletion of Vβ6$^+$ T cells. Lymph node cells were stained with anti-Vβ2 or anti-Vβ6 culture supernatant and goat anti-rat-FITC followed by biotin-labeled anti-Thy 1, and Texas Red-conjugated avidin. Background staining with an irrelevant antibody has been subtracted from the percentages shown.

self-antigen (18). We attempted to analyze the influence of TCR components other than Vβ in such instances of Vβ-specific negative selection by analyzing Vα use in the few undeleted Vβ6$^+$ T cells remaining in AKR/J or BALB/c Mls^a (Mls^a strains) mice. However, the deletion of Vβ6$^+$ T cells in Mls^a mice was so complete that it was impossible to reliably analyze Vα expression in the remaining Vβ6$^+$ T cells (Fig. 1).

To determine whether the response of mature T cells to endogenous superantigens such as Mls^a is determined solely by Vβ expression, or whether Vα use also plays a role, primary anti-Mls^a T cell cultures were analyzed by flow cytometry for simultaneous Vα and Vβ expression. Activation of heterogeneous T cells with Mls^{a+} stimulators consistently resulted in preferential expansion of Vβ6$^+$ T cells (Fig. 2 *A*). Analysis of Vα expression in C3H Vβ6$^+$ T cells demonstrated that there was a significant and consistent decrease in expression of Vα2 and Vα8 on the Vβ6$^+$ T cells that were expanded in anti-Mls^a primary cultures and stimulated with either AKR/J or CBA/J cells, as compared with expression in normal Vβ6$^+$ spleen cells (Fig. 3). Vβ6$^+$ T cells generated in BALB/c anti-BALB/c Mls^a cultures, in which responder and stimulator cells differed only by Mls^a-linked genes, showed similar decreases in Vα2 and Vα8 (Fig. 3).

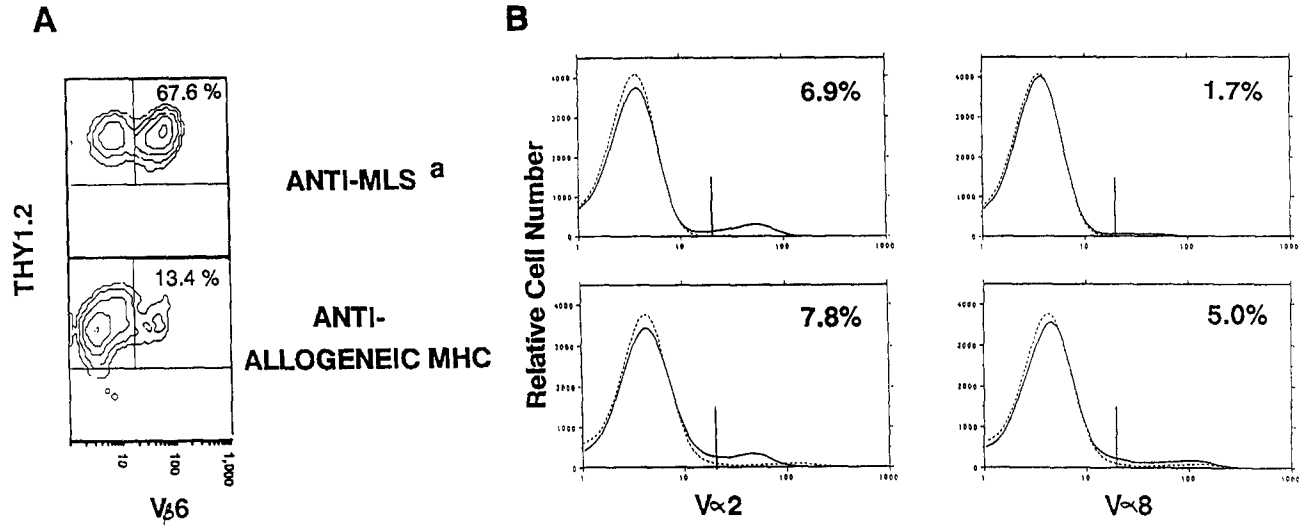

Figure 2. Selective expansion of Vβ6$^+$ T cells and analysis of Vα expression by Vβ6$^+$ T cells in anti-Mls cultures. (*A*) BALB/c T cells from 7-d cultures stimulated with either Mls^{a+} (BALB/c Mls^a) or allogeneic-MHC (B10.A) stimulator cells were analyzed for expression of Vβ6. Background staining with an irrelevant antibody has been subtracted from the percentages shown. (*B*) These cultures were analyzed for Vα expression by incubation with anti-Vα culture supernatant and goat anti-rat biotin followed by FITC-labeled anti-Thy-1 or anti-Vβ, and Texas Red-conjugated avidin. The two-parameter profiles were then software-gated to determine Vα expression (red fluorescence) on Vβ6$^+$ cells. (*Dotted lines*) control staining. (*Solid lines*) indicate staining with the appropriate anti-Vα mAb.

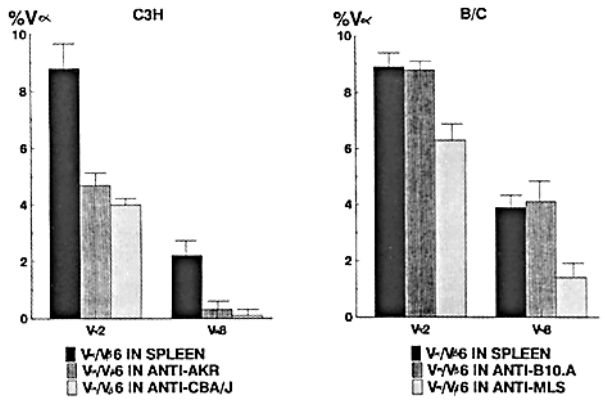

Figure 3. Altered Vα expression in Mls^a-specific C3H and BALB/c Vβ6$^+$ T cells. T-enriched spleen cells were cultured with mitomycin-treated spleen cells for 7 d before analysis of Vα expression in Vβ6$^+$ T cells and were compared with freshly isolated spleen cells. T cells were stained as in Fig. 2. % Vα = 100% × (#Vα$^+$/#Vβ$^+$) or (#V$^+$/Thy1$^+$). Results shown are means ± SEM for results obtained with four mice per strain.

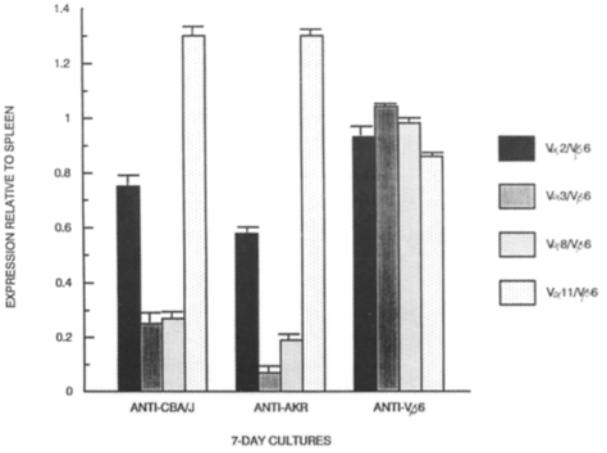

Figure 4. Altered Vα expression in Mls^a-specific B10.A Vβ6$^+$ T cells. T-enriched spleen cells were cultured with mitomycin-treated spleen cells for 7 d before analysis for Vα expression in Vβ6$^+$ T cells and were compared with freshly isolated spleen cells. T cells were stained as in Fig. 2. Expression of specific Vα/Vβ6 pairs in the 7-d cultures was expressed relative to that seen in uncultured Vβ6$^+$ spleen cells. Relative expression in spleen = Vα/Vβ6 in 7-d cultures/Vα/Vβ6 in spleen cells. Expression of specific Vα/Vβ6 pairs as a percentage of uncultured Vβ6$^+$ spleen cells was: Vα2/Vβ6, 7.2%; Vα3/Vβ6, 2.8%; Vα8/Vβ6, 5.0%; and Vα11/Vβ6, 4.4%. Results shown are derived from means obtained with three to nine mice per group.

To determine whether the observed changes in Vα expression were unique to Mls^a-specific responses, BALB/c responder cells were cultured with either Mls^a-congenic stimulators or with MHC-disparate, Mls^a-negative B10.A stimulators (Fig. 2 *B*, and Fig. 3). Vα expression in Vβ6$^+$ T cells isolated from anti-B10.A cultures was not significantly different from Vα expression in Vβ6$^+$ T cells from naive spleen cells, in contrast to the reduced Vα2 and Vα8 expression in anti-Mls^a cultures (Fig. 3).

Of the four available Vα-specific antibodies, only two are reactive with BALB/c and C3H T cells because of the allelic specificity of these reagents. Vα expression was therefore examined in Mls^a-reactive B10.A T cells, a strain in which T cells are reactive with all four Vα-specific mAbs (Fig. 4). Expression of Vα2, Vα3, and Vα8 in Vβ6$^+$ cells, expressed in Fig. 4 relative to that observed in uncultured Vβ6$^+$ spleen cells, was reduced in Mls^a-stimulated Vβ6$^+$ B10.A T cells, whereas there was an increase in Vα11 expression. In contrast, when Vβ6$^+$ T cells were selectively expanded by activation with an anti-Vβ6 antibody, Vα expression of the resulting Vβ6$^+$ T cells was equivalent to that seen in uncultured spleen, arguing that the difference in Vα expression noted between Mls^a-activated Vβ6$^+$ T cells and unstimulated Vβ6$^+$ T cells was not due to a generalized decrease in the responsiveness of Vα2-, Vα8- and Vα3-expressing T cells. Analysis of Vα expression in CD4$^+$ and CD8$^+$ T cells indicated that the differences observed could not be accounted for by selective expansion of T cell subsets in response to Mls stimulation (data not shown). These results indicate that Vβ6$^+$ T cells expressing certain Vα's are selectively more or less responsive to the Mls^a superantigen.

Discussion

It has previously been thought, based on Vβ-specific deletion of T cells by superantigens (11), that superantigen recognition by T cells is dependent solely on the TCR Vβ gene product expressed on the T cell. However, analysis in this study of Vα expression in Mls^a-responsive T cells demonstrates that skewing of Vα use occurs. Although expression of a particular Vβ may be sufficient for clonal deletion, it appears that Vα expression plays a significant role in the ability of T cells to respond functionally to superantigens. These results are consistent with the previous studies of Pullen et al. (8) and Yui et al. (19), which suggested that TCR variable regions other than Vβ might play a role in determining responsiveness to Mls^a.

Expression of TCR elements other than Vα and Vβ was not analyzed in this study, and it remains possible that dominant influences of J, D, or N products on the response to Mls^a may exist. TCR variable regions other than Vβ could influence T cell recognition of the Mls^a superantigen either directly or indirectly. Superantigen may interact with a recognition site consisting of both α and β chain variable regions such that inappropriate expression of one or the other would result in lack of binding and activation by the superantigen. Studies evaluating the binding site of superantigens on the TCR (7–9) have suggested that Vβ residues critical for superantigen binding are distant from the α chain. However, the actual binding site has not yet been directly demonstrated. Alternatively, it is possible that superantigen does not directly contact variable regions of the TCR other than Vβ, but that expression of other regions conformationally affects the TCR and consequently affects the ability of superantigen to bind to the TCR Vβ.

Although alloreactive Mls^a-specific Vβ6$^+$ T cells showed skewed Vα use in the present study, deletion of Vβ6$^+$ T

cells in Mls^{a+} mice appears to affect essentially all Vβ6$^+$ T cells, regardless of the Vα expressed. The in vitro differential expansion of T cells expressing specific Vα products reflects the outcome of a competitive process in which those cells that survive and/or proliferate most efficiently predominate at the end of culture. In contrast, clonal deletion of Vβ6$^+$ cells during in vivo development may not reflect any such competitive process. Thus, it is possible that essentially all Vβ6$^+$ T cells have sufficient affinity to mediate negative selection in vivo, despite the fact that Vα expression affects T cell recognition of Mls^a in a manner that is reflected in differential in vitro responsiveness. The TCR requirements for superantigen-mediated clonal deletion may differ either quantitatively or qualitatively from the requirements for activation of mature Mls^a-specific T cells.

The authors would like to acknowledge the excellent animal care provided by Mr. Francis Jones and the staff of Bioqual. The authors would also like to thank Michael Sheard and Larry Granger, National Institutes of Health, for flow cytometry analysis, and Drs. J. Ashwell, B. Malissen, and A. Singer for critical review of the manuscript.

Address correspondence to Melanie S. Vacchio, Bldg. 10, Rm. 1B40, Biological Response Modifiers Program, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Received for publication 11 December 1991 and in revised form 18 February 1992.

References

1. Matis, L.A. 1990. The molecular basis of T cell specificity. *Annu. Rev. Immunol.* 8:65.
2. White, J., A. Herman, A.M. Pullen, R. Kubo, J.W. Kappler, and P. Marrack. 1989. The Vβ-specific superantigen staphylococcal enterotoxin B: stimulation of mature T cells and clonal deletion in neonatal mice. *Cell.* 56:27.
3. Abe, R., and R.J. Hodes. 1989. T cell recognition of minor lymphocyte stimulating (Mls) gene products. *Annu. Rev. Immunol.* 7:683.
4. Janeway, C.A., J. Yagi, P.J. Conrad, M.E. Katz, B. Jones, S. Vroegop, and S. Buxser. 1989. T-cell responses to Mls and to bacterial proteins that mimic its behavior. *Immunol. Rev.* 107:61.
5. Lynch, D., B.C. Colé, J.A. Bluestone, and R.J. Hodes. 1986. Cross-reactive recognition by antigen-specific, major histocompatibility complex-restricted T cells of a mitogen derived from *Mycoplasma arthriditis* mitogen (MAS). *Eur. J. Immunol.* 18:1733.
6. Hugin, A.W., M.S. Vacchio, and H.C. Morse III. 1991. A retrovirus-encoded "superantigen" in a immune deficiency syndrome in the mouse. *Nature (Lond.).* 252:424.
7. Pullen, A.M., T. Wade, P. Marrack, and J.W. Kappler. 1990. Identification of the region of the T cell receptor β chain that interacts with the self-superantigen Mls-1^a. *Cell.* 61:1365.
8. Choi, Y., A. Herman, D. DiGiusto, T. Wade, P. Marrack, and J. Kappler. 1990. Residues of the variable region of the T cell receptor β chain that interact with *S. aureus* toxin superantigens. *Nature (Lond.).* 346:471.
9. Cazenave, P.A., P.N. Marche, E. Jouvin-Marche, D. Voegtle, F. Bonhomme, A. Bandeira, and A. Coutinho. 1990. Vβ17 gene polymorphism in wild-derived mouse strains: two amino acid substitutions in the Vβ17 region greatly alter T cell receptor specificity. *Cell.* 63:717.
10. Janeway, C.A. 1991. Mls: makes a little sense. *Nature (Lond.).* 349:459.
11. Tomonari, K., and S. Fairchild. 1991. The genetic basis of negative selection of Tcrb-V11$^+$ T cells. *Immunogenetics.* 33:157.
12. Berumen, L., O. Halle-Pannenko, and H. Festenstein. 1983. Strong histocompatibility and cell-mediated cytotoxic effects of a single Mls difference demonstrated using a new congenic mouse strain. *Eur. J. Immunol.* 13:292.
13. Pircher, H., N. Rebai, M. Groettrup, C. Gregoire, D.E. Speiser, M.P. Happ, E. Palmer, R.M. Zinkernagel, H. Hengartner, and B. Malissen. 1992. Preferential positive selection of Vα2$^+$CD8$^+$ T cells in mouse strains expressing both H-2^k and T cell receptor Vα^a haplotypes: determination with a Vα2 specific monoclonal antibody. *Eur. J. Immunol.* 22:399.
14. Utsunomiya, Y., J. Bill, E. Palmer, K. Gollub, Y. Takagaki, and O. Kanagawa. 1989. Analysis of a monoclonal antibody directed to the α-chain variable region (Vα3) of the mouse T cell antigen receptor. *J. Immunol.* 143:2602.
15. Tomonari, K., E. Lovering, S. Fairchild, and S. Spencer. 1989. Two monoclonal antibodies specific for the T cell receptor Vα8. *Eur. J. Immunol.* 19:1131.
16. Jameson, S.C., P.B. Nakajima, J.L. Brooks, W. Heath, O. Kanagawa, and N.R.J. Gascoigne. The T cell receptor Vα11 gene family. Analysis of allelic sequence polymorphism and demonstration of Jα region-dependent recognition by allele-specific antibodies. *J. Immunol.* 147:3185.
17. Vacchio, M.S., J.J. Ryan, and R.J. Hodes. 1990. Characterization of the ligand(s) responsible for negative selection of Vβ11- and Vβ12-expressing T cells: effects of a new Mls determinant. *J. Exp. Med.* 172:807.
18. 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.
19. Yui, K., S. Komori, M. Katsumata, R.M. Siegel, and M.I. Greene. 1990. Self-reactive T cells can escape clonal deletion in T-cell receptor Vβ8.1 transgenic mice. *Proc. Natl. Acad. Sci. USA.* 87:7135.