Direct Evidence for the Role of COOH Terminus of Mouse Mammary Tumor Virus Superantigen in Determining T Cell Receptor Vβ Specificity

By Karina Yazdanbakhsh, Chae Gyu Park,* Gary M. Winslow,† and Yongwon Choi

From the Howard Hughes Medical Institute, *The Rockefeller University, New York 10021; and the †Howard Hughes Medical Institute, Division of Basic Immunology, Department of Medicine, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado 80206

Summary

It has recently been shown that open reading frames in the 3' long terminal repeats of mouse mammary tumor viruses encode superantigens. These viral superantigens (vSAGs) stimulate most T cells expressing appropriate Vβs almost regardless of the rest of the variable components of the T cell receptors (TCR) expressed by those cells. vSAGs produce a type II integral membrane protein with a nonessential short cytoplasmic domain and a large glycosylated extracellular COOH-terminal domain, which is predicted to interact with major histocompatibility complex class II molecules and the TCR. The transmembrane region of vSAG also has an internal positively charged lysine residue of unknown significance. A set of chimeric and mutant vSAG genes has been used in transfection experiments to show that only the extreme COOH-terminal portion of vSAGs determine their TCR Vβ specificities, and to show that the lysine residue in the transmembrane domain is not essential for the function of vSAG.
other unusual feature of this protein is the positively charged lysine residue in the transmembrane region (see Fig. 1). By analogy to the transmembrane region of TCR α and β chains, it has been suggested that the lysine residue in the transmembrane region of the vSAG may contribute to the function of this protein (26, 27).

To test the importance of polymorphic regions I and II of vSAG, and the importance of the lysine residue in the transmembrane region for the superantigenic property of vSAG, we have constructed various chimeric and mutant vSAG genes and studied the properties of their protein products. We report here that only polymorphic region II is important in determining the TCR Vβ specificity of the vSAG, and that the lysine residue in the transmembrane region of the vSAG is not essential for the function of this protein as a superantigen.

Materials and Methods

Construction of Chimeric and Mutant vSAG Genes. The vSAG-1 and vSAG-7 genes have been described previously (22, 23). Chimeric vSAG-ln7c and -7nc series of genes were constructed by restriction enzyme digestion and ligation of EcoRI-vSAG-1 and EcoRI-vSAG-7 (22, 23). The vSAG-7/KL was constructed by PCR-mediated, site-directed mutagenesis as described previously (13, 14).

The primer set of 5'-ATACCATGTCCTGTCCTCTG-3' (5' primer) and 5'-TATGCCAAGAGGCAGCAGAG-3' (3' primer) was used to replace the lysine residue to leucine residue. The mutant vSAGs were amplified with a set of primers (5'-GGGATCCCTGACCTGGAAGGAGGACGAGGACGAGGAATGAGTAT-3') cloned into pBluescript KS+ (Stratagene, La Jolla, CA), sequenced, and ligated into the mammalian expression vector pHBAPr-1 as previously described (28).

Transfection of vSAG Genes. Wild-type vSAGs and their derivative genes were linearized with PvuI and electroporated into the B cell lymphoma, CH12.1, using a Gene Pulser Transfection Apparatus (Bio-Rad Laboratories, Cambridge, MA) at 240 V, 500 μF. The transfectants were selected by growth in G418 (700 μg/ml) and were screened for expression of transfected genes by Northern blot analysis or flow cytometric analysis using biotinylated VS7 mAb as previously described (23).

Assay of vSAG Function. CH12.1 transfectants were screened for their ability to stimulate the T cell hybridomas KMLs-8 (Vβ6*) and 5K-73.8 (Vβ3*) because the vSAG-1 and vSAG-7 are known to stimulate mouse T cells bearing these Vβs, respectively. The T cell hybridoma KOX15-8.3 (Vβ15*) was used as negative control (18). The stimulation of T hybridomas was assayed by lymphokine production as previously described (29).

Results and Discussion

Regions of vSAG Involved in Determining TCR Vβ Specificity. The sequence analysis of vSAGs revealed two regions that are highly polymorphic among vSAGs (Fig. 1) (16–18, 22). For example, vSAG-7 and vSAG-1 have 46 amino acid residue differences, of which eight residues are located in the polymorphic region I and 31 residues in the polymorphic region II (Fig. 1). Based on the sequence comparison of different vSAGs that have same the TCR Vβ specificity, it was suggested that polymorphic region I and/or II would determine the TCR Vβ specificity of a given vSAG (16–18, 22). To test this hypothesis directly, we have constructed several chimeric vSAGs from vSAG-1 and vSAG-7, Vβ3 and Vβ6 associated, respectively. The predicted amino acid sequences of vSAG-1 and vSAG-7 genes are shown in Fig. 1.

There are three convenient restriction enzyme sites (BsmI, StuI, and PpuMI) at the same location in vSAG-1 and vSAG-7 (Fig. 1). Therefore, these restriction enzymes were used to generate chimeric vSAG genes, vSAG-ln7c, in which the gene fragment 5' to a restriction enzyme site was from vSAG-1 and the gene fragment 3' to a restriction enzyme site was from vSAG-7 (Fig. 2). These chimeric vSAG genes (named vSAG-ln7c-Bsm, -Stu, and -Ppu, Fig. 2) were cloned into the mammalian expression vector, pHBAPr-1, and transfected into MHC class II-expressing CH12.1 lymphoma cells by electroporation. Transfectants were assayed for stimulation of T cell hybridomas expressing Vβ3 and Vβ6, the targets of vSAG-1 and vSAG-7, respectively.

The results of representative experiments are illustrated in Fig. 3. As previously shown, the untransfected cell line, CH12.1, failed to stimulate KMLs-8 (Vβ6) and 5K-73.4 (Vβ3) (30, 31). The transfectants expressing vSAG-1,
Is the Positively Charged Lysine Residue in the Transmembrane Region of the vSAG Essential for Its Function? All the MMTV encoded vSAGs known to date have an internal positively charged lysine residue in the transmembrane region (Fig. 1) (25, 26). By analogy to the transmembrane region of TCR α and β chains, it has been proposed previously that the ly-

Figure 2. Construction of chimeric vSAG genes. The positions of restriction enzyme sites are indicated. (*) Termination codon. (Shaded box) Portion from vSAG-1. (Solid vertical line) Differences in amino acid residues between vSAG-1 and vSAG-7.

Figure 3. The importance of polymorphic region II in determining TCR Vβ specificity. CH12.1 cells transfected with different vSAG genes were tested for their ability to stimulate various T cell hybridomas, KMls-8 (Vβ8.1), 5KC-73.8 (Vβ3), and KOX15-8.3 (Vβ15) as described previously (18, 30, 31). Stimulation was assayed 24 h later by levels of secreted lymphokines in the supernatants.
To test this hypothesis, we have generated a mutant vSAG-7 by changing the lysine residue to a leucine by site-directed mutagenesis. This mutant vSAG, vSAG-7/KL, was ligated into a mammalian expression vector and transfected into CH12.1 cells as described above. The transfectedants expressing similar surface vSAG-7 expression were selected and tested for their ability to stimulate T cells. Representative experiments are shown in Fig. 4. The transfected, CH12.1/vSAG7-KL.1, expressing the mutant vSAG-7, stimulated KMs-8 as efficiently as CH12.1/vSAG7-A5C, which expresses wild-type vSAG-7.

These data suggest that the internal positively charged lysine residue in the transmembrane region of vSAG-7 is not essential for its function. Since the transfectedants expressing similar surface levels of wild-type and mutant vSAG stimulated KMs-8 with approximately the same efficiency, it is also unlikely that the lysine residue is involved in the interaction of vSAG antigen with MHC class II molecules.

The experiments in this study have conclusively shown that only polymorphic region II at the extreme COOH-terminus of vSAGs determines the TCR Vβ specificity of a given vSAG. Polymorphic region I of vSAG is not involved in determining the TCR Vβ specificity. However, possible involvement of polymorphic region I for the function of vSAG is not excluded as yet. Since chimeric vSAGs of different polymorphic regions I and II produce functional proteins, it seems unlikely that polymorphic region I interacts directly with amino acid residues in polymorphic region II which interact with the Vβ element of TCR. It has been noticed for some time that different vSAGs show variable degrees of stimulation of target T cells in mixed lymphocyte reactions, even though all vSAGs are very efficient in clonal deletion of target T cells during T cell development in the thymus (1-7). Therefore, it is possible that polymorphic region I may be involved in presentation of vSAG to T cells, either by affecting the interaction of vSAGs with MHC class II molecules or the transport of vSAG to the cell surface.

By making a mutant vSAG-7 lacking an internal positively charged residue in the transmembrane region, we have shown that the lysine residue at position 51 is not important for the surface expression of vSAG. Since the mutant vSAG stimulated target T cells as efficiently as the wild-type vSAG, it is unlikely that the lysine residue is involved in the association of vSAG with MHC class II molecules, which is a prerequisite for the stimulation of T cells by vSAG.

The authors would like to thank Drs. J. Kappler, P. Marrack, and the members of the MK laboratory for their continuous help and critical discussion. The authors also would like to thank Dr. M. Sanchez (The Rockefeller University) for her help with flow cytometric analysis.

This work is supported in part by National Cancer Institute grant 1 R29 CA59751-01. Y. Choi is a recipient of a Cancer Research Institute Investigator Award.

Address correspondence to Dr. Youngwon Choi, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

Received for publication 13 April 1993.
References

1. Herman, A., J.W. Kappler, P. Marrack, and A.M. Pullen. 1991. Superantigens: mechanism of T-cell stimulation and role in immune responses. Annu. Rev. Immunol. 9:745.

2. Herrmann, T., and H.R. MacDonald. 1991. T cell recognition of superantigens. Curr. Top. Microbiol. Immunol. 174:21.

3. Abe, R., and R.J. Hodes. 1989. Properties of the Mls system: a revised formulation of Mls genetics and an analysis of T-cell recognition of Mls determinants. Immunol. Rev. 107:5.

4. Janeway, C.J., J. Yagi, P.J. Conrad, M.E. Katz, B. Jones, S. Vreugdenhil, and S. Buxser. 1989. T-cell responses to Mls and to bacterial proteins that mimic its behavior. Immunol. Rev. 107:61.

5. Kappler, J.W., N. Roehm, and P. Marrack. 1987. T cell tolerance by clonal elimination in the thymus. Cell. 49:273.

6. MacDonald, H.R., R.K. Schneider, R.C. Lees, H. Howe, H. Acha-Orbea, H. Feinstein, R.M. Zinkernagel, and H. Hengartner. 1988. T-cell receptor Vβ use predicts reactivity and tolerance to Mls-encoded antigens. Nature (Lond.). 332:40.

7. Pullen, A.M., P. Marrack, and J.W. Kappler. 1988. The T-cell repertoire is heavily influenced by tolerance to polymorphic self-antigens. Nature (Lond.). 335:796.

8. Babbitt, B.P., P.M. Allen, G. Matsueda, E. Haber, and E.R. Unanue. 1985. Binding of immunogenic peptides to Ia histocompatibility molecules. Nature (Lond.). 317:359.

9. Davis, M.M., and P.J. Bjorkman. 1988. The T-cell receptor genes and T cell recognition. Nature (Lond.). 334:39.

10. Hedrick, S., L. Matis, T. Hecht, L. Samelson, D. Longo, E. Herber-Katz, and R. Schwartz. 1982. The fine specificity of antigen and Ia determinant recognition by T cell hybridomas clones specific for cytochrome c. Cell. 30:141.

11. Jorgensen, J.L., P.A. Reay, E.W. Ehrich, and M.M. Davis. 1992. Molecular components of T-cell recognition. Annu. Rev. Immunol. 10:835.

12. Cazenave, P.A., P.N. Marche, M.E. Jouvin, D. Voegtl~, F. Bonhomme, A. Bandeira, and A. Coutinho. 1990. V beta 17 gene polymorphism in wild-derived mouse strains: two amino acid substitutions in the V beta 17 region greatly alter T cell receptor specificity. Cell. 63:717.

13. Choi, Y., A. Herman, D. DiGiusto, T. Wade, P. Marrack, and J. Kappler. 1990. Residues of the variable region of the T-cell receptor beta-chain that interact with S. aureus toxin superantigens. Nature (Lond.). 346:471.

14. Pullen, A.M., T. Wade, P. Marrack, and J.W. Kappler. 1990. Identification of the region of T cell receptor beta chain that interacts with the self-superantigen Mls-1. Cell. 61:1365.

15. Woodland, D.L., F.E. Lund, M.P. Happ, M.A. Blackman, E. Palmer, and R.B. Corley. 1991. Endogenous superantigen expression is controlled by mouse mammary tumor proviral loci. J. Exp. Med. 174:1255.

16. Acha-Orbea, H., A.N. Shakho, L. Scarpellino, E. Kolb, V. Müller, S.A. Vessaz, R. Fuchs, K. Böschinger, R. Pollini, J. Billotte, et al. 1991. Clonal deletion of V beta 14-bearing T cells in mice transgenic for mammary tumour virus. Nature (Lond.). 350:207.

17. Beutner, U., W.N. Frankel, M.S. Cote, J.M. Coffin, and B.T. Huber. 1991. Mls-1 is encoded by the long terminal repeat open reading frame of the mouse mammary tumor provirus Mtv-7. Proc. Natl. Acad. Sci. USA. 89:5432.

18. Choi, Y., J.W. Kappler, and P. Marrack. 1991. A superantigen encoded in the open reading frame of the 3' long terminal repeat of mouse mammary tumour virus. Nature (Lond.). 350:203.

19. Dyson, P.J., A.M. Knight, S. Fairchild, E. Simpson, and K. Tomonari. 1991. Genes encoding ligands for deletion of V beta 11 T cells cosegregate with mammary tumour virus genomes. Nature (Lond.). 349:531.

20. Frankel, W.N., C. Rudy, J.M. Coffin, and B.T. Huber. 1991. Linkage of Mls genes to endogenous mammary tumour viruses of inbred mice. Nature (Lond.). 349:526.

21. Marrack, P., E. Kushnir, and J. Kappler. 1991. A maternally inherited superantigen encoded by a mammary tumour virus. Nature (Lond.). 349:524.

22. Pullen, A.M., Y. Choi, E. Kushnir, J. Kappler, and P. Marrack. 1992. The open reading frames in the 3' long terminal repeats of several mouse mammary tumor virus integrants encode Vβ 3-specific superantigens. J. Exp. Med. 175:41.

23. Winslow, G.M., M.T. Scherer, J.W. Kappler, and P. Marrack. 1992. Detection and biochemical characterization of the mouse mammary tumor virus 7 superantigen (Mls-1a). Cell. 71:719.

24. Coffin, J.M. 1992. Superantigens and endogenous retroviruses: a confluence of puzzles. Science (Wash. DC). 255:411.

25. Korman, A.J., P. Bourgarel, T. Meo, and G.E. Rieckhof. 1992. The mouse mammary tumor virus long terminal repeat encodes a type II transmembrane glycoprotein. EMBO (Eur. Mol. Biol. Organ.). 11:1901.

26. Choi, Y., P. Marrack, and J.W. Kappler. 1992. Structural analysis of a mouse mammary tumor virus superantigen. J. Exp. Med. 175:847.

27. Davis, M.M. 1985. Molecular genetics of the T cell-receptor beta chain. Annu. Rev. Immunol. 3:537.

28. Gunning, P., J. Leavitt, G. Muscat, S.-Y. Ng, and L. Kedes. 1987. A human β-actin expression vector system directs high-level accumulation of antisense transcripts. Proc. Natl. Acad. Sci. USA. 84:4831.

29. Kappler, J., B. Skidmore, J. White, and P. Marrack. 1981. Antigen-inducible, H-2-restricted, interleukin 2-producing T cell hybridomas. Lack of independent antigen and H-2 recognition. J. Exp. Med. 153:1198.

30. Kappler, J.W., U. Staerz, J. White, and P.C. Marrack. 1988. The mouse mammary tumor virus long terminal repeat encodes of the major histocompatibility complex. Nature (Lond.). 332:35.

31. White, J., A. Pullen, K. Choi, P. Marrack, and J.W. Kappler. 1993. Antigen recognition properties of mutant Vβ 3+ T cell receptors are consistent with an immunoglobulin-like structure for the receptor. J. Exp. Med. 177:119.