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

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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 pfZ18R-vSAG-1 and -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'-ATACTCATTCTCTGCTGCCTCCTT-

AATGAGTAT-Y (3' primer) was used to replace the lysine residue in the polymorphic region I and/or II (Fig. 1). To test the importance of polymorphic regions I and II 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.

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 the same 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, Stul, 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, -Stul, and -Ppu, Fig. 2) were cloned into the mammalian expression vector pHIΛPr-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 5KC-73.4 (Vβ3) (30, 31). The transfectants expressing vSAG-1,
Figure 2. Construction of chimeric vSAG genes. The positions of restriction enzyme sites are indicated. (*) Termination codon. (Shaded boxes) Portion from vSAG-1. (Solid vertical lines) Differences in amino acid residues between vSAG-1 and vSAG-7.

CH12.1/vSAG1-1, stimulated 5KC-73.4. Likewise, the transfectants expressing vSAG-7, CH12.1/vSAG7-A5C, stimulated KMLS-8 (23). All the transfectants expressing chimeric vSAG-ln7c-Bsm, -Stu, and -Ppu (CH12.1/Bsm-1N7C.5, CH12.1/Stu-1N7C.9, and CH12.1/Ppu-1N7C.10, respectively) stimulated KMLS-8, but failed to stimulate 5KC-73.8. A Vβ15+ T cell hybridoma, KOX15-8.3, was not stimulated by any of the B cell lines, as expected. Since vSAG-ln7c-Ppu is essentially identical to vSAG-1 except in polymorphic region II, the data provide direct evidence that the polymorphic region II of the vSAG-7 is sufficient to determine the Vβ specificity of vSAG-7. These data also suggest that polymorphic region I is not involved in determining TCR Vβ specificity of vSAG.

Reciprocal experiments have also been done in this study, using the chimeric vSAG, vSAG-7nlc-Ppu, in which the gene fragment 3' to the PpuI site in vSAG-1 is replaced with that in vSAG-7. The transfectants expressing the chimeric vSAG-7nlc-Ppu gene, CH12.1/Ppu-7N1C.1, stimulated 5KC-73.8 but not KMLS-8, which is consistent with the data described above (Fig. 3).

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 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.

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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 transfectants with similar surface vSAG-7 expression were selected and tested for their ability to stimulate T cells. Representative experiments are shown in Fig. 4. The transfectant, CH12.1/vSAG7-KL.1, expressing the mutant vSAG-7, stimulated KMls-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 transfectants expressing similar surface levels of wild-type and mutant vSAG stimulated KMls-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.
References


