Highly Biased CDR3 Usage in Restricted Sets of β Chain Variable Regions During Viral Superantigen Response

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Summary

Superantigens encoded by the mouse mammary tumor virus can stimulate a large proportion of T cells through interaction with germline-encoded regions of the T cell receptor β chain like the hypervariable region 4 (HV4) loop. However, several lines of evidence suggest that somatically generated determinants in the CDR3 region might influence superantigen responses. We stimulated T cells from donors differing at the BV6S7 allele with vSAG9 to assess the nature and structure of the T cell receptor in amplified T cells and to evaluate the contribution of non-HV4 elements in vSAG recognition. This report demonstrates that vSAG9 stimulation caused the expansion of TCR BV6-expressing T cells, although to varying degrees depending on the BV6 subfamily. The BV6S7 subfamily was preferentially expanded in all donors, but in donors homozygous for the BV6S7*2 allele, a significant number of BV6S5 T cells were amplified and showed a highly biased β chain junctional region (BJ) and CDR3 usage. As CDR3 regions are involved in major histocompatibility complex (MHC)–peptide interaction, such a selection is highly suggestive of an intimate MHC–TCR interaction and would imply that the topology of the MHC-vSAG-TCR complex is similar to the one occurring during conventional antigen recognition.

Materials and Methods

Genotyping for BV6S7 Alleles. The nomenclature used for BV genes is according to Wei et al. (11). PBMCs were separated by Ficoll centrifugation (Pharmacia Biotech AB, Uppsala, Sweden) on May 2, 2019.
and stimulated for 24 h with 1 μg/ml of PHA (Murex Diagnostics, Guelph, Canada). Total RNA was extracted from 4 × 10^7 cells with RNazol B (CINNA/BIOTEX Laboratories, Houston, TX) and 10 μg were reverse transcribed with 5 μg of oligo(dT) and 60 U of AMV-RT (Life Sciences, St. Petersburg, FL). The BV6S7 cDNAs were amplified by PCR using primers and conditions described elsewhere (12), digested with BamH1 and cloned into vector pBluescript SK+ (Stratagene, La Jolla, CA), and recombinants were amplified as described above and PCR was performed using DeepVent (New England Biolabs, Mississauga, Canada) using the constant region primer GGT-AGAGCCTGG.

Results and Discussion

Since vSAG9 can stimulate BV6^+ human T cells and polymorphism between BV 657 alleles is located within the HV4 region (16), we assumed that responsiveness to vSAG9 might differ between individuals homozygous for either allele and that stimulation of BV6s expanded in donors bearing a less responsive allele might reveal the contribution of non-HV4 elements in vSAG9 response. vSAG9 Preferentially Expands the TCR BV6 Family.

Since the murine cell line DAP, expressing both HLA-DR1 (DAP-DR1) and vSAG7, has been shown to stimulate human T cells in a BV-restricted manner (14), we used DAP-DR1 transfected with vSAG9 (DR1-SAG9; reference 13) to stimulate T cells from an individual homozygous for BV657^+ (donor J). Proliferation measured by thymidine incorporation peaked after 4 d of coculture and was reproducibly fourfold higher compared to control DR1 cells (data not shown). FACScan® analysis of BV usage in human CD4^+ T cells in response to vSAG9 or PHA, a mitogen that stimulates T cells independently of BV usage (17 and data not shown), shows that BV6S7 stimulated BV657^+ and BV21^+ T cells (Fig. 1 A). Since the BV-specific mAbs currently available cover ~65% of the repertoire, qPCR analysis was performed and this confirmed that only BV6 and BV21 responded to vSAG9 and were amplified four- and threefold, respectively (Fig. 1 B). This selective expansion was reproduced on a second BV657^+ homozygous individual (data not shown). T cells derived from two individuals homozygous for the BV657^+ allele (donors S and M) were stimulated with vSAG9 and proliferation was six- and fourfold higher compared to control DAP-DR1 cells (data not shown). Quantitative PCR analysis was performed and, as with the BV657^+ donors, only BV6^+ and BV21^+ T cells were amplified (Fig. 2, A and B). The responsiveness of BV6 and BV21 is not surprising given that they share significant homology, notably in the CDR1 and CDR2 region of BV6 (18). From qPCR analysis, it is apparent that the over-
RFLP. For donor J (BV6S7*1 homozygous), 98% of the clones obtained after vSAG9 stimulation used BV6S7. With donors S and M (BV6S7*2 homozygous), RFLP analysis showed that 64 and 87% of the clones used BV6S7, although a significant number of other BV6 gene segments were also obtained after stimulation (Table 1). For donor S, 27% of the BV6s present after vSAG9 stimulation were BV6S5*1, whereas in donor M, 5% of BV6 gene segments were BV6S5*2. For all donors, BV6S7 was clearly the best responder to vSAG9, as evidenced by the low numbers of BV6 non-BV6S7 cells present after stimulation. For donor M, the proportion of BV6S7*2-positive clones present in the PHA-stimulated population was twice that of the other donors (Table 1) and appear to have dominated the vSAG9 response, perhaps explaining the lower number of BV6S5 found after stimulation.

Table 1. Percentage of BV6 Subfamily Members Assessed by RFLP Analysis

| Donor  | PHA | vSAG9 |
|--------|-----|-------|
|        |BV6S1, BV6S5|BV6S1, BV6S5|
|        | S3, S4 | S3, S4 |
| J (BV6S7*1) | 21 | 69 | 10 | 98 | 0 | 2 |
| S (BV6S7*2) | 17 | 68 | 15 | 64 | 27 | 9 |
| M (BV6S7*2) | 42 | 43 | 15 | 87 | 5 | 8 |

Donor J PHA n = 200 clones, vSAG9 n = 200 clones; donor S PHA n = 194 clones, vSAG9 n = 198 clones; donor M PHA n = 143 clones, vSAG9 n = 143 clones.
BV 655 clones amplified used the BJ155 element (Table 2), whereas BJ155 usage in BV 655 clones from PHA-stimulated T cells was not elevated (10%; data not shown). Two dominant clonotypes were found, with CDR3 regions bearing a P(Q/E)NSG motif (Table 2), created entirely by N-additions at the V–J junction. In donor M, a dominant BV 655 clonotype, having the totally N-encoded CDR3 region, LEHSTRP, represented 89% of the BV 655 clones present after vSAG9 stimulation (Table 2), whereas this clonotype could not be detected in PHA-stimulated cells (data not shown). The number of BV6S1, S3, and S4 present after vSAG9 stimulation was low in all donors, and

| Table 2. Junctional Regions of BV6s from vSAG9-stimulated Cells of Donors S and M |
|---|
| **Donor S** | **Donor M** |
| BV | N-D-N | BJ | n* | BV | N-D-N | BJ | n* |
| 655 | CASS LPTGVED | T1YFG | 153 | 1/18 | 655 | CASS FPSCGL | YEQQFG | 257 | 1/9 |
| 655 | CASS SLNSG | NQPQHFG | **155** | 1/18 | 655 | CASS LEHSTRP | YEQQFG | **257** | 8/9 |
| 655 | CASS PQNSG | NQPQHFG | **155** | 9/18 | 655 | CASS PVQSD | YEQQFG | 257 | 1/9 |
| 655 | CASS PENSG | NQPQHFG | **155** | 4/18 | 655 | CASS LVGTGDIQ | KLFFG | 154 | 1/25 |
| 655 | CASS WGLAW | NEQQFG | 251 | 1/18 | 655 | CASS LDRGN | NSPLHFG | 156 | 1/25 |
| 655 | CASS LDRGS | EQFFG | 251 | 1/18 | 655 | CASS QDTS | SYNEQQFG | 251 | 1/25 |
| 655 | CASS LNLKDH | T1AEFFG | 151 | 1/22 | 655 | CASS YKGGP | YNENQQFG | 251 | 1/25 |
| 655 | CASS LSAGTL | E1AFFG | 151 | 1/22 | 655 | CASS YRGSST | NEQQFG | 251 | 1/25 |
| 655 | CASS LTRDK | YG1TFG | 152 | 1/22 | 655 | CASS LAVGGP | NEQQFG | 251 | 1/25 |
| 655 | CASS LTRGA | G1TYFG | 153 | 1/22 | 655 | CASS AGTSSG | EQFFG | 251 | 1/25 |
| 655 | CASS RPEQYR | NT1YFG | 153 | 1/22 | 655 | CASS LLFFQ | QFQQ | 251 | 1/25 |
| 655 | CASS TQ1TG | QPQHFG | 155 | 1/22 | 655 | CASS YKGGP | TDTQYFG | 253 | 1/25 |
| 655 | CASS LQWGG | NSPLHFG | 156 | 1/22 | 655 | CASS SRFVAGG | TDTQYFG | 253 | 1/25 |
| 655 | CASS LANGG | S1LHFG | 156 | 1/22 | 655 | CASS FRSGV | TDTQYFG | 253 | 1/25 |
| 655 | CASS RA1LEQD | SYNEQQFG | 251 | 1/22 | 655 | CASS PPSSR | TDTQYFG | 253 | 1/25 |
| 655 | CASS YQ5 | YNENQQFG | 251 | 1/22 | 655 | CASS SRGFR | TDTQYFG | 253 | 1/25 |
| 655 | CASS LTRGNV | NEQQFG | 251 | 1/22 | 655 | CASS PRGSGR | DTQYFG | 253 | 1/25 |
| 655 | CASS SEYVTI | EQFFG | 251 | 1/22 | 655 | CASS STVW | DTQYFG | 253 | 1/25 |
| 655 | CASS SGTSS | EQFFG | 251 | 1/22 | 655 | CASS SMGR | QTQYFG | 255 | 1/25 |
| 655 | CASS QCGQQA | GLEFFG | 252 | 1/22 | 655 | CASS LTLGGY | ETQYFG | 255 | 1/25 |
| 655 | CASS PRFI | TDTQYFG | 253 | 1/22 | 655 | CASS ARRV | ETQYFG | 255 | 1/25 |
| 655 | CASS SRLVQG | FG | 253 | 1/22 | 655 | CASS SDH | YEQYFG | 257 | 1/25 |
| 655 | CASS SGLAVG | AK1NQYFG | 254 | 1/22 | 655 | CASS GT | YEQYFG | 257 | 1/25 |
| 655 | CASS LAPRD | YEQQFG | 257 | 1/22 | 655 | CASS LSLGG | YEQQFG | 257 | 1/25 |
| 655 | CASS P1MDT | YEQQFG | 257 | 1/22 | 655 | CASS QTV | EQYFG | 257 | 1/25 |
| 655 | CASS AGLALR | EQQFG | 257 | 1/22 | 655 | CASS QP | YFG | 257 | 1/25 |
| 655 | CASS FGGSG | QYFG | 257 | 1/22 | 651 | CASS GG | TDTQYFG | 253 | 2/9 |
| 651 | CASS LFKG | SYENQQFG | 251 | 1/7 | 653 | CASS EQQGADD | EQQFG | 251 | 1/9 |
| 651 | CASS YRTDF | SG1NLTPG | 256 | 1/7 | 654 | CASS PERL | SGN1YFG | 153 | 1/9 |
| 653 | CASS LIGG | SYEQYFG | 257 | 1/7 | 654 | CASS CAST | LRTG | NEQQFG | 251 | 1/9 |
| 653 | CASS TSRST | EQYFG | 257 | 1/7 | 654 | CASS LTSGRAR | DTQYFG | 253 | 1/9 |
| 654 | CASS LESTQR | NT1YFG | 153 | 1/7 | 654 | CASS GARGSGE | QTQYFG | 255 | 1/9 |
| 654 | CASS HKGG | TG1LPPG | 252 | 1/7 | 654 | CASS LGR | ETQYFG | 255 | 1/9 |
| 654 | CASS LAAGA | DTQYFG | 253 | 1/7 | 654 | CASS PQ1ECC | YEQQFG | 257 | 1/9 |

*n, the number of times a given sequence was found. The sequences data are available from EMBL/GenBank/DDBJ under accession numbers AF011574–AF011643.

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the total lack of CDR3 bias seen in these clones suggests that they have not been amplified. Thus, the bias observed in the CDR3 region of BV66S clones suggests that intimate MHC-TCR contacts might exist during vSAG response. Other studies have yielded similar results, as sequence analysis of TCR-β junctional regions in BV6+CD4+ T cells that survived deletion mediated by vSAG7 showed a BJ usage and CDR3 length distribution that differed significantly from H2-Matched mice lacking vSAG7 (8). Introduction of an Eα transgene in SJL mice, which do not express I-E and therefore cannot present the endogenous vSAG9, restores deletion of BV17+ T cells when compared to nontransgenic mice, T cells having survived negative selection showed increased BJ2S5 and decreased BJ1S1 usage, indicating that the nature of the BJ segment can have an impact on T cell deletion (7). Our results are in agreement with these studies and extend them by providing evidence that the structure of the CDR3 plays a positive role in vSAG responsiveness.

Model of MHC-vSAG-TCR Interaction. Comparison of HV4 sequences between BV6s and BV21s reveals that they are highly homologous except for the presence of a glutamic acid at the tip of the HV4 loop in all nonresponding BV6s. The allelic polymorphism between the BV65S7s is located next to that residue in which the glycine present in BV65S7*1 is replaced by a glutamic acid in BV65S7*2. These differences might partially explain the differential reactivity observed between BV6 subfamily members. Although HV4 is clearly the overriding determinant in vSAG recognition, it appears that a BV with a suboptimal HV4 would become more dependent on stabilization provided by TCR-MHC contacts. As mutagenesis of the CDR1 in murine BV6 was more dependent on stabilization provided by TCR-MHC contacts, it appears that a BV with a suboptimal HV4 would become a contact site for vSAGs, we feel this is unlikely given it is located opposite to HV4 (4, 9) and that the two BV65S7 homozygous individuals used different BJs. The BJ bias observed during vSAG9 response might be due to a TCR interaction with a peptide present in the MHC groove, since the BJ gene segment contributes for a significant portion of the CDR3 (4, 9). The different CDR3s found in the two donors could be due to recognition of different peptides or a dominant peptide being recognized by both TCRs, since TCRs having identical BV segments, but different CDR3 sequences, can recognize the same peptide-MHC complex (24). Since the TCR α and β chains CDR1 and CDR2 are involved in MHC contacts (4), this would explain α chain biases (5, 6) and the contribution of the β chain CDR1 (20, 21), whereas the skewed BJ usage observed (7, 8) could be explained by CDR3 contacts with peptide-MHC complexes. Thus, the data in the literature about the role of non-HV4 regions in vSAG responses could be readily reconciled using a model in which vSAGs cross-link MHC and TCR in a way that allows the interactions occurring during conventional antigen recognition to exist.

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References

1. Acha-Orbea, H., W. Held, G.A. Wanders, A.N. Shakhov, L. Scarpellino, R.K. Lees, and H.R. MacDonald. 1993. Exogenous and endogenous mouse mammary tumor virus superantigens. Immunol. Rev. 131:5–25.

2. 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-I+. Cell. 61:1365–1374.

3. Cazenave, P.-A., P.N. Marche, E. Jouvin-Marche, D. Voegtlé, F. Bonhomme, D. Bandeira, and A. Couthino. 1990.

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Vβ17 gene polymorphism in wild-derived mouse strains: two amino acid substitutions in the Vβ17 region greatly alter T cell receptor specificity. C. el. 63:717–728.

4. Garboczi, D.N., P. Ghosh, U. Utz, Q.R. Fan, W.E. Biddison, and D.C. Wiley. 1996. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. Nature. 384:134–141.

5. Smith, H.P., P. Le, D.L. Woodland, and M.A. Blackman. 1992. T cell receptor α-chain influences reactivity to Mls-1 in Vβ8.1 transgenic mice. J. Immunol. 149:887–896.

6. Vacchio, M.S., O. Kanagawa, K. Tomonari, and R.J. Hodes. 1996. Crystal structure of a T-cell receptor β-chain in superantigen recognition. J. Immunol. 155:4171–4178.

7. Candéias, S., C. Waltzinger, C. Benoist, and D. Mathis. 1991. The Vβ17+ T cell repertoire: skewed Jβ usage after thymic selection; dissimilar CDR3s in CD4+ versus CD8+ cells. J. Exp. Med. 174:899–1000.

8. Chies, J.A.B., G. Marodon, A.-M. Joret, A. Regnault, M.-P. Lembezat, B. Rocha, and A.A. Freitas. 1995. Persistence of Vβ 6+ T cells in Mls-1+ mice. A role for the third complementarity-determining region (CDR 3) of the T cell receptor β-chain in superantigen recognition. J. Immunol. 155:4171–4178.

9. García, K.C., M. Degano, R.L. Stanfield, A. Brunmark, M.R. Jackson, P.A. Peterson, L. Tytton, and I.A. Wilson. 1996. An αβ T cell receptor structure at 2.5 Å and its orientation in the TCR-MHC complex. Science. 274:209–219.

10. Fields, B.A., E.L. Macchiardi, H. Li, X. Yersin, C.V. Stauffer, P.M. Schlievert, K. Kanai, and A.R.A. Mariuzzo. 1996. Crystal structure of a T-cell receptor β-chain complexed with a superantigen. Nature. 384:188–192.

11. Wei, S., P. Charmley, M.A. Robinson, and P. Concannon. 1994. The extent of the human germline T-cell receptor Vbeta gene segment repertoire. Immunogenetics. 40:27–36.

12. Vissinga, C.S., P. Charmley, and P. Concannon. 1994. Influence of coding region polymorphism on the peripheral expression of a human TCR Vβ gene. J. Immunol. 152:1222–1227.

13. Thibodeau, J., N. Labrecque, F. Denis, B.T. Huber, and R.-P. Sékaly. 1994. Binding sites for bacterial and endogenous retroviral superantigens can be dissociated on major histocompatibility complex class II molecules. J. Exp. Med. 179:1029–1034.

14. Labrecque, N., H. McGregor, M. Subramanyam, B.T. Huber, and R.-P. Sékaly. 1993. Human T cells respond to mouse mammary tumor virus-encoded superantigen: Vβ restriction and conserved evolutionary features. J. Exp. Med. 177:1735–1743.

15. White, B.A., and S. Rosenzweig. 1989. The polymerase chain reaction colony miniprep. Biotechniques. 7:696–698.

16. Liao, L., A. Mairancescu, A. Molano, C. Ciurl, R.-P. Sékaly, J.D. Frazer, A. Popowicz, and D.N. Posnett. 1996. TCR binding-dimers for a bacterial superantigen (SEE) and a viral superantigen (Mtv-9). J. Exp. Med. 184:1471–1482.

17. Wong, F.S., M.L. Hieber, L. Wen, B.A. Millward, and A.G. Demain. 1993. The human T cell receptor Vβ repertoire of normal peripheral blood lymphocytes before and after mitogen stimulation. Clin. Exp. Immunol. 92:361–366.

18. Rowen, L., B.F. Koop, and L. Hood. 1996. The complete 685-kilobase DNA sequence of the human β T cell receptor locus. Science. 272:1755–1762.

19. Lechler, R.I., V. Bal, J.B. Rothbard, R.N. Germain, R. Sékaly, E.O. Long, and J. Lamb. 1989. Structural and functional studies of HLA-DR restricted antigen recognition by human helper T lymphocyte clones by using transfected cell lines. J. Immunol. 9:3003–3009.

20. Kang, J., C.A. Chambers, J. Pawling, C. Scott, and N. Hozumi. 1994. Conserved amino acid residues in the complementarity-determining region 3 of the TCR β-chain are involved in the recognition of conventional Ag and Mls-1 superantigen. J. Immunol. 152:5305–5317.

21. Hong, S.-C., G. Waterbury, and C.A. Janeway, Jr. 1996. Different superantigens interact with distinct sites in the Vβ domain of a single T cell receptor. J. Exp. Med. 183:1437–1446.

22. Patten, P.A., E.P. Rock, T. Sonoda, B. Fazekas de St. Groth, J.J. Jongesen, and M.M. Davis. 1993. Transfer of putative complementarity-determining region loops of T cell receptor V domains confers toxin reactivity but not peptide/HMC specificity. J. Immunol. 150:2281–2294.

23. Jaraczewsky, T.S., J.H. Brown, J.C. Gorga, L.J. Stern, R.G. Urry, Y.I. Chi, C. Stauffer, J.L. Strominger, and D.C. Wiley. 1994. Three-dimensional structure of a human class II histocompatibility molecule complexed with a superantigen. Nature. 368:711–718.

24. Boitel, B., M. Ermonval, P. Panina-Bordignon, R.A. Mariuzzza, A. Lanzavecchia, and O. Acuto. 1992. Preferential Vβ usage and lack of junctional sequence conservation among T cell receptors specific for a tetanus toxin-derived peptide: evidence for a dominant role of a germline-encoded V region in antigen/major histocompatibility complex recognition. J. Exp. Med. 175:765–777.