Brief Definitive Report

Highly Biased CDR3 Usage in Restricted Sets of β Chain Variable Regions During Viral Superantigen 9 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.

Mouse mammary tumor virus (MMTV) is a retrovirus that uses the immune system for its transmission by encoding a viral superantigen (vSAG) (1). MMTV vSAGs can stimulate a large pool of T cells by interacting with the hypervariable region 4 (HV4) of the TCR β chain, which is germline encoded by individual β chains (2, 3) and is distinct from the CDR3 hypervariable region involved in recognition of antigens presented by MHC molecules (4). Since SAG-responsive cells can express different TCR α and β combinations, it is believed that an intimate TCR–MHC interaction is not required for recognition, as most T cells bearing appropriate β chain variable regions (BVs) can respond to vSAGs (1). Potential contributions of non-HV4 elements have been proposed since vSAG response can be influenced by α chain usage (5, 6), and nonrandom joining region usage has been described in SAG-responsive BVs (7). T cell repertoire analysis in mice differing by the presence of vSAG7 has shown that particular β chain junctional regions (BJs) and CDR3 lengths were underrepresented in BVs having survived deletion (8), but this indirectly infers a CDR3 selection by analyzing the structure of TCRs in nonresponding cells. These data suggest that TCR–MHC contacts might be required for productive interaction. To assess which TCRs would be amplified by vSAG stimulation, we subjected T cells from individuals, differing at the BV6S7 allele, to vSAG9 stimulation. The structure of TCRs amplified by vSAG9 provides evidence that CDR3s are selected in BVs having lower responsiveness towards the superantigen. This suggests that additional contacts provided by CDR3–peptide interaction allow stabilization of the complex and implies that vSAGs cross-link MHC and TCR in a way that preserves interactions seen in conventional antigen recognition (4, 9), in marked contrast with what occurs during bacterial SAG (bSAG) recognition (10).

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)

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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/BIOTECX Laboratories, Houston, TX) and 10 μg were reverse transcribed with 5 μg of oligodT 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 BamHI, and fractionated on 2% gels.

T Cell Stimulation with vSAG9. Human T cells were purified from four donors homozygous for either BV6S7 allele using rosetting with sheep red blood cells (Quélab, Montréal, Canada) and PBMCs separated by Ficoll centrifugation. Purity was assessed by staining with anti-CD3 (OKT3-FITC) and anti–HLA-DR (L-243) mAbs (Becton Dickinson, Mountain View, CA) and T cell purity was >99%, whereas the non–T cell fraction contained <15% of T cells. T cells were stimulated in the presence of irradiated autologous feeder cells with DAP-DR1 cells, which are murine fibroblasts transfected with DR1 α and β chain cDNAs, or DAP-DR1 expressing vSAG9 (13). DAP-DR1 and DR1-vSAG9 cells were treated for 1 h at 37°C with 100 μg/ml mitomycin C (Sigma Chemical Co., St. Louis, MO) and cocultured in 96-well plates in DMEM, 5% FCS, 2 mM l-glutamine, and 20 μg/ml gentamycin. 6×10^5 human T cells were cultured with 2×10^5 DAP-DR1 or DR1-SAG9 in the presence of 5×10^5 autologous irradiated feeder cells. After 2 d, 10 U/ml of recombinant human IL-2 was added and the culture proceeded for an additional 7 d. For PHA stimulation, 10^6 T cells were cocultured in 24-well plates with 2×10^5 irradiated feeder cells and 1 μg/ml PHA in 1.5 ml. Proliferation was measured by [^{3}H]thymidine incorporation (Dupont Co.–New England Nuclear, Boston, MA) after 3 d and determined with a β plate counter (Pharmacia Biotech AB).

FACS® and Quantitative PCR Analysis of the BV Repertoire. FACS® analysis was performed after T cell stimulation with DR1-SAG9 or PHA, using CD4, CD8, and BV-specific mAbs. The BV-specific mAbs used were anti-BV2, 3, 8, BV13S3 (JU74), 17, 19, 21 (Immunotech Coulter, St. Laurent, Canada), BV5 (MH3-2), BV5 (3D11), BV6S7*1 (OT145), BV9 (MKB1), BV12 (S511), BV13 (BAMB), BV13S1 (H131), BV13S2 (H132), and BV23 (HUT-78). Cells were stained for CD4 (OKT4-PE) and CD8 (OKT8-PerCP) (Becton Dickinson) and 3×10^5 live cells were gated according to forward and side light scatter and analyzed on a FACScan®. For quantitative PCR (qPCR), cDNAs were synthesized as described above and PCR was performed using conditions and primers described elsewhere (14). PCR products were fractionated on 12% polyacrylamide gels and exposed overnight on PhosphorImager screens (Molecular Dynamics, Sunnyvale, CA). Quantification of signals was performed on a PhosphorImager running the IMAGEQUANT software (Molecular Dynamics) and normalized against a TCR α chain constant region internal control (14).

Cloning and Analysis of BV6 Subfamily Members. BV6 members were amplified by PCR using DeepVent (New England Biolabs, Mississauga, Canada) using the constant region primer GGTGTGGGAGATGTCGACTTTTGATGGCTCAAAC and the pan-BV6 primer: CCTTTACTGGTACCGACAGAGCCTGG. PCR products were digested with KpnI and SalI, cloned into pBSKS+ (Stratagene, La Jolla, CA), and recombinants were screened by PCR using reverse and universal primers (15). BV6 members were subdivided by RFLP based on the presence of BamHI and ApaLI sites (BamHI+ApaLI+: BV6S7*1; BamHI−ApaLI+: BV6S7*2; BamHI−ApaLI−: BV6S5; BamHI+ApaLI−: BV6S1, BV6S3, BV6S4, BV6S11, and BV6S14). 10 μl of PCR reactions were digested with BamHI and ApaLI and fractionated on 2% gels. Sequence determination was performed using the T7 Sequencing Kit (Pharmacia Biotech AB).

Results and Discussion

Since vSAG9 can stimulate BV6S7+ human T cells and polymorphism between BV6S7 alleles is located within the HV4 region (16), we assumed that responsiveness to vSAG9 might differ between individuals homozygous for either allele and that analysis of BVs 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 BV6S7*1 (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). FACS® 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 vSAG9 stimulated BV6S7*1+ 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 BV6S7*1 homozygous individual (data not shown). T cells derived from two individuals homozygous for the BV6S7*2 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 BV6S7*1 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 HV4 regions (18). HLA typing for MHC haplotypes was performed and showed that donor J was DR1/DR1, donor S was DR7/DR7, and donor M was DR2/DR7. Although donors S and M express MHC molecules different from DR1, T cell proliferation after 3 d of coculture with the control DR1 transfectant was similar between the DR1 donor (3.2×10^4 cpm) and the non-DR1 donors (3.1×10^4 and 3.0×10^4 cpm), indicating that no significant allogeneic response occurred, which is not surprising since DAP cells are known to be poor at eliciting alloresponses (19). From qPCR analysis, it is apparent that the overwhelming majority of T cells that responded to vSAG9 belonged to the BV6 family (Figs. 1 *B*, 2, *A* and *B*). Since the human BV6 locus contains seven expressed genes (18) and our qPCR did not allow discrimination between subfamily members, BV6s were amplified using a pan-BV6 primer and cloned for analysis. Approximately 200 clones from PHA- and vSAG9-stimulated cells were analyzed by

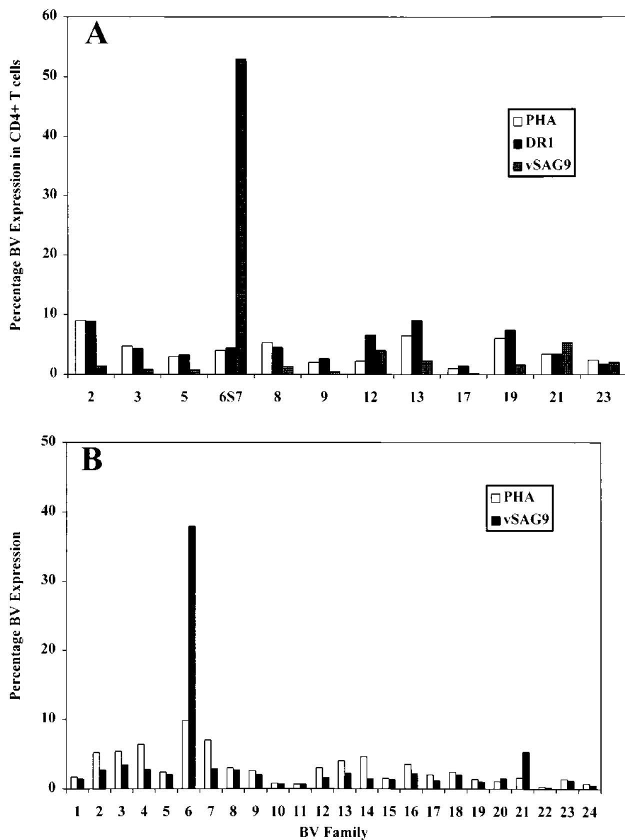

Figure 1. (*A*) Cytofluorometric analysis of TCR BV usage in T cells stimulated by vSAG9. T cells from donor J (homozygous for BV6S7*1) were stained with a panel of human BV-specific antibodies. Shown is the percentage of BV expression in $CD4^+$ T cells after PHA, DR1, or vSAG9 stimulation. (*B*) Percentage of TCR BV usage determined by qPCR analysis. The signal intensity for each BV obtained by volume integration using the IMAGEQUANT software was normalized against the α chain internal control, and the relative percentage of BV expression was calculated by dividing the individual normalized values by the sum of all BV normalized values.

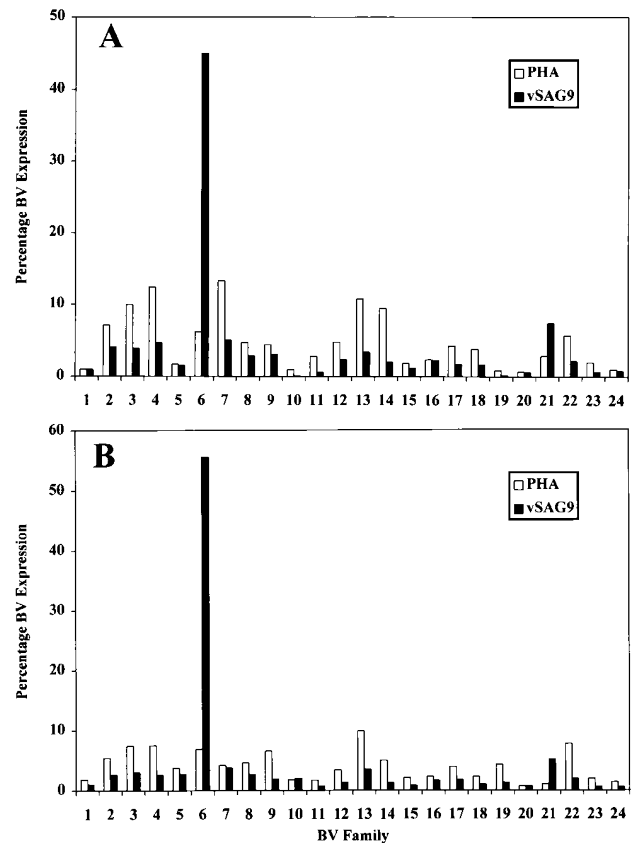

Figure 2. qPCR analysis of TCR BV usage in vSAG9-stimulated T cells from BV6S7*2 donors. (*A*) Donor S, homozygous for the BV6S7*2 allele; (*B*) donor M, homozygous for the BV6S7*2 allele. The values were calculated as described in the legend of Fig. 1 *B*.

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 $BV6S5^+$, whereas in donor M, 5% of BV6 gene segments were $BV6S5^+$. 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.

CDR3 Structure of BV6 Subfamily Members Amplified by vSAG9. To evaluate the possible contribution of the β chain CDR3 in recognition of the vSAG9–MHC complex, TCR junctional regions from T cells stimulated by vSAG9 or PHA were sequenced. Sequence analysis of the BV6S7 clones revealed no particular BJ selection from either vSAG9- or PHA-stimulated cells, with a random BJ usage and CDR3 length distribution (data not shown, Table 2). In contrast, the BV6S5 clones obtained after vSAG9 stimulation in donor S revealed a striking bias; 89% of

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

Donor	PHA			vSAG9		
	BV-6S7	BV-6S5	BV6S1, S3, S4, S11, S14	BV-6S7	BV-6S5	BV6S1, S3, S4, S11, S14
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.

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*
6S5	CASS	LPTGGVED	TIYFG	1S3	1/18	6S5	CASS	FPSGGL	YEQYFG	2S7	1/9
6S5	CASS	**SLNSG**	**NQPQHFG**	**1S5**	1/18	**6S5**	CASS	**LEHSTRP**	**YEQYFG**	**2S7**	**8/9**
6S5	CASS	**PQNSG**	**NQPQHFG**	**1S5**	**9/18**						
6S5	CASS	**PENSG**	**NQPQHFG**	**1S5**	**4/18**	6S7*2	CASS	YRVG	EKLFFG	1S4	1/25
6S5	CASS	**PNSG**	**NQPQHFG**	**1S5**	1/18	6S7*2	CASS	LVGTTDQG	KLFFG	1S4	1/25
6S5	CASS	WGLAW	NEQFFG	2S1	1/18	6S7*2	CASS	LDGRN	NSPLHFG	1S6	1/25
6S5	CASS	LDRGS	EQFGG	2S1	1/18	6S7*2	CASS	QDTS	SYNEQFFG	2S1	1/25
						6S7*2	CASS	PGAGGPP	YNEQFFG	2S1	1/25
6S7*2	CASS	LNQLKH	TEAFFG	1S1	1/22	6S7*2	CASS	KGGAGP	YNEQFFG	2S1	1/25
6S7*2	CASS	LSAGTI	EAFFG	1S1	1/22	6S7*2	CASS	WRGSSTN	NEQFFG	2S1	1/25
6S7*2	CASS	LTRD	YGYTFG	1S2	1/22	6S7*2	CASS	LVAGGQ	NEQFFG	2S1	1/25
6S7*2	CASS	LTRGA	GNTIYFG	1S3	1/22	6S7*2	CASS	AGTSGGG	EQFFG	2S1	1/25
6S7*2	CASS	RPEGQYR	NTIYFG	1S3	1/22	6S7*2	CASS	LLPFQ	QFFG	2S1	1/25
6S7*2	CASS	TQTG	QPQHFG	1S5	1/22	6S7*2	CASS	YKGGGPT	TDTQYFG	2S3	1/25
6S7*2	CASS	LQWGG	NSPLHFG	1S6	1/22	6S7*2	CASS	SRFVAGG	TDTQYFG	2S3	1/25
6S7*2	CASS	LANGGIG	SPLHFG	1S6	1/22	6S7*2	CASS	FRSVG	TDTQYFG	2S3	1/25
6S7*2	CASS	RALLREQD	SYNEQFFG	2S1	1/22	6S7*2	CASS	PFSSR	TDTQYFG	2S3	1/25
6S7*2	CASS	YQS	YNEQFFG	2S1	1/22	6S7*2	CASS	SRGFR	TDTQYFG	2S3	1/25
6S7*2	CASS	LTGRVN	NEQFFG	2S1	1/22	6S7*2	CASS	PRGSGR	DTQYFG	2S3	1/25
6S7*2	CASS	SEYVTI	EQFFG	2S1	1/22	6S7*2	CASS	STVV	DTQYFG	2S3	1/25
6S7*2	CASS	SGTSS	EQFFG	2S1	1/22	6S7*2	CASS	SMGR	QETQYFG	2S5	1/25
6S7*2	CASS	QGGGQA	GELFFG	2S2	1/22	6S7*2	CASS	LTLGGY	ETQYFG	2S5	1/25
6S7*2	CASS	PRFI	TDTQYFG	2S3	1/22	6S7*2	CASS	ARRV	ETQYFG	2S5	1/25
6S7*2	CASS	SRLVTLGS	FG	2S3	1/22	6S7*2	CASS	SDH	YEQYFG	2S7	1/25
6S7*2	CASS	SGLAGV	AKNIQYFG	2S4	1/22	6S7*2	CASS	GT	YEQYFG	2S7	1/25
6S7*2	CASS	LAPRD	YEQYFG	2S7	1/22	6S7*2	CASS	LSLGSS	EQYFG	2S7	1/25
6S7*2	CASS	FMDT	YEQYFG	2S7	1/22	6S7*2	CASS	QTV	EQYFG	2S7	1/25
6S7*2	CASS	AGLALR	EQYFG	2S7	1/22	6S7*2	CASS	QP	YFG	2S7	1/25
6S7*2	CASS	FGGSG	QYFG	2S7	1/22						
						6S1	CASS	GG	TDTQYFG	2S3	2/9
6S1	CASS	LFKG	SYNEQFFG	2S1	1/7	6S3	CASS	EGQGASD	EQFFG	2S1	1/9
6S1	CASS	YRDTF	SGANVLTFG	2S6	1/7	6S4	CASS	PERL	SGNTIYFG	1S3	1/9
6S3	CASS	LIGG	SYEQYFG	2S7	1/7	6S4	CAST	LRTGN	NEQFFG	2S1	1/9
6S3	CASS	TSRST	EQYFG	2S7	1/7	6S4	CASS	LTSGRAR	DTQYFG	2S3	1/9
6S4	CASS	LESTGAR	NTIYFG	1S3	1/7	6S4	CASS	GARGSGE	QETQYFG	2S5	1/9
6S4	CASS	HKGG	TGELFFG	2S2	1/7	6S4	CASS	LGR	ETQYFG	2S5	1/9
6S4	CASS	LAAGA	DTQYFG	2S3	1/7	6S4	CASS	PQEGG	YEQYFG	2S7	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.

BV6S5 clones amplified used the BJ1S5 element (Table 2), whereas BJ1S5 usage in BV6S5 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 BV6S5 clonotype, having the totally N-encoded CDR3 region, LEHSTRP, represented 89% of the BV6S5^{+} 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

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 BV6S5 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 can not 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 BV6S7s is located next to that residue in which the glycine present in BV6S7*1 is replaced by a glutamic acid in BV6S7*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 shown to abrogate both vSAG7 and conventional antigen recognition (20), this argues in favor of a similar MHC–TCR topology. In addition, mutagenesis in CDR1 (but not CDR2) affects vSAG responsiveness, and mutagenesis in CDR2 (but not CDR1) affects bSAG reactivity, indicating that vSAGs and bSAGs probably bind differently to the TCR (21). Mutagenesis studies have shown the importance of CDR2 in bSAG recognition (21, 22) and the structure of SEC3 bound to TCR shows that it contacts the CDR2 peptide backbone and should act like a wedge between TCR and MHC, allowing only part of the MHC to contact the TCR (10). In addition, the structure of SEB bound to DR1 predicts that the SAG-MHC-TCR orientation should differ by 45° compared to MHC-peptide-TCR orientation (23).

Our results and those from others (7, 8) indicate that BJ usage impacts vSAG recognition. Although this region might be a contact site for vSAGs, we feel this is unlikely given it is located opposite to HV4 (4, 9) and that the two BV6S7*2 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. Waanders, 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-1[a]. *Cell.* 61:1365–1374.
3. Cazenave, P.-A., P.N. Marche, E. Jouvin-Marche, D. Voegtlé, F. Bonhomme, D. Bandeira, and A. Couthino. 1990.

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

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. 1992. Influence of T cell receptor Vα expression on Mls[a] superantigen-specific T cell responses. *J. Exp. Med.* 175:1405–1408.
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.* 174:989–1000.
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[a] mice. A role for the third complementarity-determining region (CDR3) of the T cell receptor β chain in superantigen recognition. *J. Immunol.* 155:4171–4178.
9. Garcia, K.C., M. Degano, R.L. Stanfield, A. Brunmark, M.R. Jackson, P.A. Peterson, L. Teyton, 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. Malchiodi, H. Li, X. Ysern, C.V. Stauffacher, P.M. Schlievert, K. Karjalainen, and R.A. Mariuzza. 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 V beta 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. McGrath, 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. Marinescu, A. Molano, C. Ciurli, R.-P. Sékaly, J.D. Fraser, A. Popowicz, and D.N. Posnett. 1996. TCR binding differs for a bacterial superantigen (SEE) and a viral superantigen (Mtv-9). *J. Exp. Med.* 184:1471–1482.
17. Wong, F.S., M.L. Hibberd, L. Wen, B.A. Millward, and A.G. Demaine. 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. 1988. 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 1 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. Jorgensen, and M.M. Davis. 1993. Transfer of putative complementarity determining region loops of T cell receptor V domains confers toxin reactivity but not peptide/MHC specificity. *J. Immunol.* 150:2281–2294.
23. Jardetzky, T.S., J.H. Brown, J.C. Gorga, L.J. Stern, R.G. Urban, Y.I. Chi, C. Stauffacher, 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. Mariuzza, 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.