Contrasting contributions of complementarity-determining region 2 and hypervariable region 4 of rat BV8S2+ (V\textsubscript{B}8.2) TCR to the recognition of myelin basic protein and different types of bacterial superantigens

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Abstract

In experimental autoimmune encephalomyelitis (EAE) of LEW rats, BV8S2\textsuperscript{+} (V\textsubscript{B}8.2) T cells dominate the RT1Bl-restricted response to guinea pig myelin basic protein (gpMBP), and respond to the superantigens (SAg) Staphylococcus enterotoxin C1 (SEC1), Mycoplasma arthritidis SAg (MAS) and Yersinia pseudotuberculosis mitogen (YPM). T cells expressing the closely related BV8S4 differ from BV8S2 T cells in their response to gpMBP, and the SAg SEC1 and MAS, but not in their response to YPM. The functional differences between BV8S2 and BV8S4, which vary in complementarity-determining/hypervariable region 4 (CDR4/HV4) and CDR2, were analyzed by cloning and mutating a TCR with features typical for gpMBP-specific BV8S2 TCR. The wild-type BV8S2 receptor and the BV8S4-like CDR2\textsuperscript{b} + 4\textsuperscript{b} double mutant of BV8S2 showed the same differences in ligand specificity as polyclonal BV8S2\textsuperscript{+} and BV8S4\textsuperscript{+} lymphocyte populations. The CDR2\textsuperscript{b} mutant lost its reactivity for SEC1 and gpMBP\textsubscript{68-88}, but the CDR4/HV4\textsuperscript{b} mutation abolished only activation by SEC1. Thus, CDR2 and HV4 contribute not only differently to recognition of peptide antigens, but also to recognition of different types of bacterial SAg.

Introduction

The V gene usage of peripheral T cells changes not only after encounter with superantigens (SAg), which by definition interact with TCR comprising certain BV, but also in the response to many peptide antigens (1,2). One striking example for the latter is the RT1Bl-restricted CD4 T cell response of LEW rats to guinea pig myelin basic protein (gpMBP) (peptide 68-88), which leads to MBP-specific autoimmunity and the development of experimental autoimmune encephalomyelitis (EAE), a model for some aspects of multiple sclerosis.

TCR of the encephalitogenic BV8S2\textsuperscript{+} CD4 cells share some structural features. Most CDR3\textsuperscript{b} bear only few or no N nucleotides and have an AsnSer or XSer adjacent to the CysAlaSerSer as a sequence motif (3-5). Frequently, BV8S2\textsuperscript{b}
chains are also associated with certain TCR chains. These α chains preferentially comprise AV1, AV2, AV23 and a complementarity-determining region (CDR) 3 motif of two to four Asn starting 5 amino acids after the Cys90 of the V segment (6,7).

Two alleles of BV8S2 have been described so far: BV8S2A1 for the Tcrlb haplotype (e.g. in LEW, PVG), and BV8S2A2 for Tcrlb and related haplotypes (e.g. DA, F344, LER, AS). Both alleles differ by 3 amino acids (Arg105Ser, Leu14Val and Asn64Asp) (8-10) and in binding to the mAB R78 (BV8S2A1 binds, BV8S2A2 does not bind) (9,11). RNase protection assays, analysis of cloned RT-PCR products as well as spectratyping provide compelling evidence for the lack of major differences between both alleles with respect to CDR3 motifs in a gpMBP response (12,13), encephalitogenic potential or response to the SAgl SEB (11).

The closest homologue of BV8S2 is BV8S4 (10,11). The Tcrlb haplotype carries a non-functional allele (BV8S4A1), but BV8S4A2, which is found in all other haplotypes, is functional. Surprisingly, like the BV8S2A1, BV8S4A2 also binds mAB R78. Consequently, in different Tcrlb haplotypes, mAB R78 identifies different BV members rather than alleles of the same BV (9,11). BV8S4A2 varies from BV8S2A1 by 7 amino acids and from BV8S2A2 by 6 amino acids. As shown in this paper, six of them are of particular importance for ligand recognition. These amino acids are clustered in the CDR2 (2 amino acids) and in the CDR/hypervariable (HV) 4 (4 amino acids), and differences between the CDR have been suggested to cause differential reactivity of BV8S2 and BV8S4 cells for the SAgl of mtv7 (mtv7-SAgl or Mls1a), Staphylococcus enterotoxin C1 (SEC1) and SEB (9,11). In addition, as a consequence of differential thymic selection, CD4 cells express BV8S4A2 more frequently than BV8S2A1 (10). Finally, as shown with the LEW variant LER, BV8S4 cells also differ from BV8S2 cells in their gpMBP response. The gpMBP-specific BV8S4A2 cells vary considerably in CDR3 length and BV8S4A2 cell lines carry neither the characteristic CDR3 motifs of BV8S2 cells nor do they respond to the encephalitogenic peptide (gpMBP68-88) (12-14). In short, BV8S2 and BV8S4 comprising TCR differ in their response to mtv7-SAgl and SEB/SEC1, their MHC class preference during thymic selection as well as in their contribution to the encephalitogenic MBP response.

SAg are characterized by their capability to induce a massive BV-specific primary T cell response and are presented by MHC class II molecules (15). Interaction with MHC class II and TCR is quite well characterized for many SAg of Gram-positive bacteria (15,16). The staphylococcal enterotoxins, toxic shock syndrome toxin 1 of Staphylococcus aureus as well as the streptococcal pyrogenic toxins A and C share as overall structural features two globular β sheet domains providing the binding sites for TCR and MHC, which are connected by a central α helix. (16). Nevertheless, crystallographic data and molecular modeling [reviewed in (16)] showed that these domains bind in quite different ways to MHC and TCR.

Probably the best-defined SAg–ligand interaction is that of SEB and the highly homologous SEC3 to TCR and MHC class II. Both SAg bind to the α helix of the α1 domain of the MHC class II molecule and BV-encoded FR2, CDR2, FR3 and CDR4/HV4 areas. This interaction is supported by contacts of the CDR1 and 2 of the TCR α chain with the α helix of the β1 domain of MHC class II (16-19). The strength of binding of SEB (and of SEC1) varies between MHC iso- and allotypes (20,21), but there is a clear tendency of better binding to human MHC class II than to mouse or rat MHC class II. Homologues of BV are activated in all three species (9,22,23).

Very little is known about the structural requirements for recognition of SAg of Gram-negative bacteria, of which only two have been cloned and expressed so far: Mycoplasma arthritidis SAg (MAS, alternatively called mycoplasma arthritis mitogen (MAM)) (24) and Yersinia pseudotuberculosis mitogen (YPM) (25,26).

MAS is a soluble protein lacking sequence similarity (24) to other SAg. MAS stimulates homologous BV in mouse, human and rats (27), and, interestingly, an effect has also been reported of CDR3β composition on the response of human T cells to MAS (28).

YPM is a soluble product of the human pathogen Y. pseudotuberculosis, which bears no sequence homology to other SAg (25,26). In humans, YPM activates BV3, BV9, BV13S1 and BV13S2 cells (most of them are human homologues of mouse BV8), and, in mouse, BV8S1, BV8S2, BV8S3 and BV7+ T cells (25,29). Reactivity of rat T cells has not yet been analyzed.

This paper investigates the activation of rat T cells expressing closely related BV8 family members to highly divergent bacterial SAg and the peptide antigen gpMBP. The analysis of mutants revealed remarkable differences in the contribution of CDR2 and CDR4/HV4 to recognition of gpMBP, SEB/SEC1, MAS and YPM.

Methods

Animals

Lewis (LEW/Crl) rats were obtained from our own animal facilities (breeding pairs were from Charles River, Sulzfeld, Germany). Fischer (F344/Crl) rats were obtained from Charles River.

Antibodies, flow cytometry and cell sorting

mAB of the following specificity were used: R73 (30), rat TCR β chain; CA11, rat BV3S3 (31); R78, rat BV8S2A1 and BV8S4A2 (11,32); B73, rat BV8S3 (32); 18B11, rat BV13 (31); HIS42, rat BV16 (32,33); W3/25, rat CD4 (34), rat CD28 (35) and isotype-matched control antibodies. Polyclonal antibodies were: donkey anti-mouse Ig-phycoerythrin (PE) (Dako Diagnostika, Hamburg, Germany) and normal mouse Ig (Sigma, Deisenhofen, Germany). All mAB were purified and labeled by standard procedures from hybridoma culture supernatants or purchased from BD PharMingen (San Diego, CA). The RT1b- and H2-A-specific mAB OX6–PE (36) was used to detect surface expression of RT1b. PE-labeled rat y6 TCR-specific mAB V65 was used as an isotype control. Lymphocytes were characterized as described elsewhere by two-color immunofluorescence and four-parameter flow cytometry (9,11). Samples were analyzed on a FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA) with the use of CellQuest software. Light scatter gates were set to include all viable nucleated cells. Lymphoblasts were identified by their
forward scatter characteristics (9,11). The proportion of lymphoblasts determined by scatter characteristics correlated well with the number of cells with blast-like appearance in culture and using a hemocytometer. Cell sorting was performed either with a FACSVantage cell sorter (Becton Dickinson) or by magnetic sorting using the MACS system (Miltenyi, Bergisch Gladbach, Germany) according to the manufacturer’s instruction.

SAg and antigens

SEC1 was obtained from Toxin Technology (Saragota, FL). MAS was purified as described (37). YPM was purified as recombinant product from *Escherichia coli* XL1-blue carrying pQE30-6-hisypm by using Ni-NTA agarose, as reported previously (29,38). gpMBP was kindly provided by Dr Ralf Gold (Neurology Department, University of Würzburg) and gpMBP <sub>68-88</sub> peptide was obtained from Biotrend (Cologne, Germany).

Cells and cell culture

Suspensions of lymph node or spleen cells were prepared as previously described (30). P3/2 cells are mouse DAP-3 fibroblasts expressing DR1 (21) and were kindly provided by Dr R. Accolla (University of Verona, Italy). Cells were cultured at 37°C in 5% CO<sub>2</sub> and humidified atmosphere in RPMI 1640, supplemented as described (30). The T cell hybridoma 35/1 was generated by polyethylene glycol-mediated fusion of the encephalitogenic gpMBP-specific cell line MBP13 (40), provided by Dr Ralf Gold, and a TCR<sup>−/−</sup> variant of the BW5147 thymoma (41) using standard methods. Hybridomas were tested for expression of the R78 epitope and IL-2 production filling the inserts for cloning the TCR chains into the template for site-directed mutagenesis as well as for preparation of the inserts for cloning the TCR chains into the expression vectors

Stimulation of primary lymphocytes

The stimulation with SAg and concanavalin A (Con A) was essentially performed as previously described (9,11). Lymph node splenocytes (2 x 10<sup>6</sup>/ml) were stimulated with SEC1 (1 or 0.1 µg/ml), MAS (5 ng/ml) and YPM (1 µg/ml), and cultured for 4–5 days. Stimulation with SEC1 was performed in the presence of mitomycin C-treated P3/2 cells (9). For Con A stimulation, 2 x 10<sup>6</sup> cells/ml were incubated with 5 µg Con A/ml (Pharmacia, Freiburg, Germany) for 3 days, subsequently diluted 4-fold in fresh culture medium containing 200 U/ml IL-2 and cultured for 1–2 days more. BV and CD4² expression and blastoid transformation of cells were analyzed by immunofluorescence and flow cytometry (9). The proportion of blast cells varied from 50 to 70% for the SAg-stimulated and >90% for Con A-stimulated cells.

Stimulation of T cell lines and IL-2 assays

Ninety-six-well flat-bottomed plates were first coated with 10 or 40 µg/ml sheep anti-mouse Ig or rabbit anti-mouse Ig (9,11). Subsequently they were incubated with the indicated dilutions of RT73 culture supernatant and thoroughly washed thereafter. Stimulation was performed with 5 x 10<sup>4</sup> responder cells and 5 x 10<sup>4</sup> Raji RT1B<sup>+</sup> or 1 x 10<sup>4</sup> thymocytes as antigen-presenting cells (APC) in 96-well U-bottomed plates overnight. Culture supernatants were harvested 24 or 48 h later. The IL-2 content in the culture supernatants was tested using a commercially available sandwich ELISA for mouse IL-2 (BD Pharmingen).

Isolation of RT1B<sup>+</sup> cDNA clones and generation of retroviral expression vectors

RT1B<sup>+</sup> α and β chain sequences were obtained from cloned RT-PCR products using either lipopolysaccharide-activated B cells or total splenocytes of LEW rats as a source of RNA. Cell culture, isolation of RNA and RT-PCR were performed as described (11). The primers covered the C- and N-termini of the coding sequences. The α chain 5’ primer covered positions 35–55 and the 3’ primer was complementary to positions 805–786 [GenBank X14879 (42)]. The β chain 5’ primer covered positions 8–27 and the 3’ primer was complementary to positions 799–779 [GenBank X56596 (43)]. PCR products were directly TA cloned into PCR 2.1-TOPO vector (Invitrogen, Leiden, The Netherlands) for dye terminator sequencing by the ABI 377 sequencer.

The newly cloned genes were expressed in human B cell lymphoma Raji cells. Gene transduction was performed as described using a MMLV-based retroviral expression system (44). Cloning of the RT1B<sup>+</sup>α gene into the vector pSFGFPS65T was performed by PCR using 5’ primer CTC AGC AGA GCT CTG and 3’ primer CCC GGA AGA TCT of the coding sequences. The PCR fragment was purified with the Qiagen PCR purification kit (Qiagen, Hilden, Germany), digested with Bgl II, separated on an agarose gel, and ligated with a Klenow-filled Nco I site and a BamHI site of pSFGFPS65T. The RT1B<sup>+</sup>β chain gene was cloned accordingly. Primers used were 5’ primer CCC ATG CCA TGG CTC TGC and 3’ primer GCC GGG CAT CCT ACC TGT AGG AGC CCT GCT GG. The constructs were sequenced and directly used for transfection.

Isolation of 35/1 TCR cDNA clones and generation of retroviral expression vectors

The complete coding sequences of the TCR α and β chains were cloned by RT-PCR from the hybridoma 35.1. Primers used for the α chain were: 5’ primer CAT GTG ATC AGC AGT TCC CT and the 3’ primer GAC CAC AGC CCT AGC GTC A. Primers used for the β chain were located in the BV8S2A1 leader sequence and at the end of the TCB coding sequence: 5’ primer ATG GGC TCC AGG TTC CTC and 3’ primer GAA CCT TCT TTT TTG ACC ATA GC. PCR products were cloned into a PCScript vector, which was used as a template for site-directed mutagenesis as well as for preparation of the inserts for cloning the TCR chains into the expression vector pSFGFPS65T (45). A TCR α chain-containing PCR product was generated using the 5’ primer C GGC GGA TCC GCC ACC ATG GCC AGC TCC CTG GGG

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CDR2 and 4 in ligand recognition by rat BV8S2 TCR

GCC TT and the 3′ primer ATG CCG ATC CTC AAC TGG ACC ACA GCC TTA GCC TCA TGA G. For the TCR β chain, 5′ primer G CGC GGA TCC GCC ACC ATG GGC TCC AGG TTC CTC TTA GTG and 3′ primer ATG CCG ATC CTC AGG AAC TCT TTC TTT TGA CCA TAG C were used. Samples were denatured for 3 min before 35 cycles of amplification with Pfu polymerase (Promega) (94°C, 30 s; 55°C, 1 min; 72°C, 90 s) followed by a final extension at 72°C for 10 min. The PCR fragments were puriﬁed with the Qiagen PCR Puriﬁcation Kit, digested with Ncol and BamHI, separated on an agarose gel, and directly cloned into the Ncol and BamHI site of pSFGGFP565T. The constructs were sequenced and directly used for transfection.

Retroviral gene transduction

Recombinant retroviral particles containing either α or β chain of the TCR or of RT1B were generated separately by cotransfection of 293T cells with pHIT60 (44), the VSV envelope-encoding pcVGwt vectors (46) and with either pSFGGFP565T-based vector. Expression of transduced genes was tested by immuno-ﬂow cytometry. Expression of RT1B was determined with the mAb OX6; expression of TCR was determined with the mAb R73 and R78.

Site-directed mutagenesis

Mutations of the V segments of the TCR β chain were introduced by PCR using the Expand High ﬁdelity PCR System (Boehringer Mannheim, Mannheim, Germany; manufacturer’s protocol) and overlapping primers. Samples were denatured for 4 min before 30 cycles of ampliﬁcation (95°C, 30 s; 50°C, 30 s; 68°C, 6 min) followed by a ﬁnal extension at 72°C for 10 min. After digestion of the original template, the new PCR products were directly transformed into XL-1 blue bacteria and sequenced. Three mutants of the β chain of the 35.1-TCR containing CDR2 and/or CDR4/HV4 of BV8S4 (EMBL X77995.1) (12) were generated (for the amino acids sequences and localization of CDR, see Fig. 2). Primers used for the CDR2 mutant covered nucleotides 139–177 and 165–227 of the BV8S4 sequence (9,11). and those used for the CDR4 mutant covered nucleotides 199–239 and 227–189. The CDR2 + CDR4 mutant was generated by introducing the CDR4 of BV8S4 into the CDR2 mutant. All mutations were conﬁrmed by sequencing.

Molecular modeling

The 35/1 TCR was modeled using Swiss Model (47) (http://swissmodel.expasy.org/SM_TOPPAGE.html) and visualized using the Swiss PDB viewer in order to deﬁne the localization of mutated/substituted amino acids. First, single chains were modeled using the ‘ﬁrst approach mode’ and, subsequently, the ‘optimized mode’ for oligomeric molecules.

Results

Cloning and functional expression of the gpMBP-speciﬁc TCR 35/1 and its restriction element RT1B

In order to deﬁne the relative contribution of the CDR2 and CDR4/HV4 of BV8S2 and BV8S4 for recognition of the different types of SAg and the importance of either region for recog-
The sequence of the β chain (GenBank AY228549) was identical to that of two previously published chains at the protein level, but differed in its nucleotide sequence (3,51). The gpMBP specificity of the 35/1 hybridoma (total gpMBP as well as gpMBP 68-88) could be transplanted to a TCR−/− recipient cell line by retroviral transfer of the TCR-genes (Fig. 2). The reactivity of transduced 58C cells (58C-TCR) for gpMBP was very poor compared with original hybridoma 35/1, but could be considerably improved by expression of the TCR in 58C cells expressing a transgenic ratCD28 (58C-CD28-TCR). 58C-CD28-TCR cells responded nearly as well as the 35/1 hybridoma if RT1B-transduced Raji cells presented the gpMBP68-88 (Fig. 3). This improvement of sensitivity was much less striking when thymocytes were used as APC. Expression of the TCR and rat CD28 is shown in Fig. 1 for the 35/1 hybridoma and the 58C-CD28-TCR line.

Analysis of the contribution of CDR2 and CDR4/HV4 to antigen and SAg reactivity by site-directed mutagenesis of the TCR 35/1

The SAg SEB and SEC1 are known to differentially activate BV8S2 and BV8S4 (9). In addition, as described in Methods, we tested the response of LEW and F344 splenic and lymph node T cells to YPM and MAS. The following results were obtained in a series of four to six experiments. Activation with MAS yielded the following frequencies of CD4+ blast cells expressing the respective BV: 13.6 ± 2% (± SD) BV8S2A1 cells (R78∗CD4+ blasts from LEW) and 7.1 ± 0.2% BV8S4A2 cells (R78∗CD4+ blasts from F344); 6.3 ± 0.3% BV8S3A1 (B73∗CD4+ blasts from LEW) and 5.2 ± 1.5% BV8S3A2 (B73∗CD4+ blasts from F344) cells. For activation with YPM, the respective numbers were 65.4 ± 11% BV8S2A1 cells and 38.4 ± 10% BV8S4A2 cells. In striking contrast to the other BV8S2-activating SAg, no activation of BV8S3 cells was found (1.1 ± 1.5% BV8S3A1 and 1.5 ± 1.2% BV8S3A2 CD+ blasts). Neither SAg activated BV16 cells (<1% CD4+ blasts). The respective values for the BV-unspecific Con A stimulation were: BV8S2A1, 4.9 ± 0.5%; BV8S4A2, 7.4 ± 1.5%; BV8S3A1, 5.0 ± 0.5%; BV8S3A2, 3.5 ± 0.3%; BV16 (LEW), 9.2 ± 0.4%;
BV16 (F344), 9.9 ± 0.9%. A comparison of the sequences of the analyzed BV8 genes is shown in Fig 2. Assignment of the residues to CDR or framework regions has been confirmed by molecular modeling as described in Methods (not shown).

Site-directed mutagenesis of the CDR2 and CDR4/HV4 of the TCRβ chain gene of TCR 35/1 was performed as described in Methods in order to define the relative importance of CDR2 and CDR4/HV4 for the differences in antigen and SAg reactivity. TCR comprising wild-type or mutated β chains were expressed at similar levels (Fig. 1) in 58C-CD28 cells and tested for (S)Ag reactivity. Figure 4 shows one example of a set of experiments (three to five experiments for each ligand) in which all ligands were tested. Two types of presenting cells were used—thymocytes or Raji RT1Bl cells. Thymocytes, which are the classical source of APC for the maintenance of MBP-specific rat T cell lines, did not present SEB or SEC1 (not shown), probably due to the latter’s very poor binding to rat MHC class II (9). In contrast, Raji cells (not shown) as well as Raji RT1B1 cells presented SEC1. Consequently, the use of Raji RT1B1 cells and TCR-transduced 58C-CD28 cells allowed comparison of activation by all ligands, including SEC1, under the same experimental conditions.

The various TCR-transduced 58C-CD28 cell lines differ to some extent in their mAb R78 (or mAb R73, data not shown)-induced IL-2 production (Fig. 4), which prevented normalization of stimulation using mAb-induced stimulation as a reference. Instead, the IL-2 production by the individual cell lines in response to the various stimuli was compared for each T cell line. Figure 4(A) shows the responses using rat thymocytes as APC and Fig. 4(B) shows the results of an experiment with Raji RT1B1 as APC. For the stimulation with thymocytes as APC, concentrations of ligands were chosen such that wild-type TCR-expressing cells were stimulated to nearly the same extent by all ligands tested.

Cell lines expressing transduced wild-type and mutant TCR responded very well to YPM, which is in good agreement with the data obtained with BV8S2A1- and BV8S4A2-expressing polyclonal populations. Also, the YPM response was better with Raji RT1B1 cells (or Raji cells, data not shown) than with thymocytes as APC, suggesting a better binding of YPM to human than to rat MHC class II. The response to gpMBP was completely abrogated by the CDR2 and CDR2 + 4 mutations, while it appeared to be the same or sometimes better for the CDR4/HV4 mutant. The MAS response was abolished only by the CDR2 + 4 mutations. The CDR4/HV4 mutation as well as the CDR2 mutations had little, if any, negative effect for the activation by MAS.

The overall pattern of reactivity was as follows. The wild-type TCR responded to all ligands. The CDR2 mutant responded only to MAS and YPM, but not to gpMBP and SEC1. The CDR4/HV4 mutant responded to gpMBP, MAS and YPM, but not to SEC1. The CDR2 + 4 mutant responded only to YPM, but none of the other ligands.

**Discussion**

The TCR 35/1 which has been used in this study can be considered as typical for TCR found on encephalitogenic T cells in EAE of the LEW rat, with respect to antigen specificity (RT1B1-restricted, gpMBP69-88-specific), V gene usage, and
CDR3 motifs of α and β chains. (6,12,13,51,52). As demonstrated in this study, the cloned TCR provides a useful tool to study TCR–ligand interaction in this disease model. The same applies to the correct RT1Bl α chain sequence, the RT1Bl expression vectors and transduced cell lines, which will not only facilitate immunological analysis of LEW rats, but also of the widely used RT1B-expressing rat strains F344 and WKY.

Mutagenesis of CDR2 and CDR4/HV4 clearly demonstrated that replacement of the BV8S2 encoded part of the TCR by both CDR2 and CDR4/HV4 of BV8S4 completely abolished gpMBP specificity. Since changes in the CDR2, but not in the CDR4/HV4, destroyed gpMBP specificity, it seems quite likely that the CDR2 difference between BV8S2 and BV8S4 provides the molecular basis for the differential composition of BV8S2 and BV8S4 TCR in the RT1Bl-restricted gpMBP response. This interpretation is supported by crystallographic data of TCR–MHC complexes, which often show contacts between MHC (class II) and CDR2β, but never with the CDR4/HV4β [reviewed in (53)]. Finally, the strong effects of changes in the CDR2 on MHC-restricted peptide recognition support the idea that differences in the CDR2 of BV8S2 and BV8S4 TCR contribute to differences in MHC class I- versus II-specific selection of TCR (CD4 bias of BV8S4 cells) bearing either V segment. (10). Definite proof of this hypothesis will require in vivo studies with TCR comprising mutated BV of the kind presented in this paper.

The analysis of the SAg reactivity of BV8 family members of polyclonal T cell populations from two different Tcrβ haplotypes revealed overlaps in their SAg specificity, but also pronounced differences between closely related BV. Also, the TCR mutants varied considerably in their SAg reactivity, strongly suggesting major differences in their SAg binding sites.

For SEC1, the proposed role of CDR2 and CDR4/HV4 (9,11) in the differential reactivity of BV8S2 and BV8S4 TCR was confirmed by the loss of reactivity of the mutants of CDR2, CDR4/HV4 or CDR2 + 4. This is in line with a contribution of both CDR2 and CDR4/HV4 to SEB and SEC recognition, as has been found in homologous mouse and human TCR [reviewed in (16)].

For MAS, a BV8S2 response was found, although the majority of activated cells express BV not detected by the available rat BV-specific mAb, which is good agreement with RNase protection data suggesting BV6 cells as the major MAS-reactive population (27). BV8S3 T cells responded less than BV8S2 T cells and differences between BV8S3 alleles were not apparent. BV8S4 TCR, which differ from BV8S2 TCR mainly in CDR2 and 4, responded only marginal in the polyclonal stimulation, and the CDR2 + 4 mutant of the TCR 35/1 did not respond at all. Quite differently to SEC1, single CDR mutants had only a small or no effect on the MAS response and may play no important role in MAS recognition. Such small contributions of either CDR to SAg–MHC binding would be consistent with a unique mode of binding of MAS to TCR and MHC, which would be in line with the proposed role of the CDR3 in MAS recognition of human BV17 TCR (homologue to mouse and rat BV6). These residues form a sequence motif consisting of an Ile adjacent to the terminal CysAlaSerSer of the BV and a BV-encoded Tyr located opposite to the Ile in the CDR3 loop. The latter has been assigned to position 106 in the original description of the MAS reactive BV17 TCR (28), which corresponds to Tyr101 of the TCR 35/1 (28). This proposed SAg-binding site is distant from the CDR2 and 4.

For YPM, little is known so far about binding to MHC class II as well as to the TCR. Although not addressed in further detail, the strong discrepancy in the response of TCR-transduced lines to YPM presented either by human class II-bearing Raji RT1Bl or (Raj cells) or by rat thymocytes suggests a better binding of YPM to human than to rat MHC class II molecules. This differential binding could be related to the role of YPM as a virulence factor in human infection.

The difference between YPM and the other BV8-specific SAg in their reactivity for BV8 family members in different species and the response to BV8S2 and BV8S4 respectively is quite interesting. In contrast to the other SAg tested, BV8S2 and BV8S4 cells, respectively TCR wild-type and the CDR2 + 4 mutant T cell lines, respond very well to YPM, which may indicate no or little importance of either CDR2 or HV4/CDR4 in YPM recognition. In line with other sites of TCR being important for binding to YPM is the lack of a BV8S3 response of polyclonal rat T cells, which contrasts with the overlapping BV usage (BV7, 8S1, 8S2, 8S3) for YPM- and SEB-specific T cells in mice (29). Indeed, a sequence comparison of the non-responsive rat BV8S3 alleles with the responsive rat BV8S2, BV8S4 and mouse BV8S3 defines a number of amino acids scattered over the BV which are unique for the YPM-unresponsive BV8S3. Most of them are found in framework regions at the flank of the TCRBV, two at the tip of CDR1 and 2 respectively.

The analysis of the SAg response of rat BV8 family members highlights the variation in SAg recognition of different SAg by highly homologous or even identical TCR. Such differences have also been reported for staphylococcal enterotoxin- and mtv-SAg-specific TCR (54,55), and very different TCRβ-SAg contact sites have been identified by crystallographic analysis and molecular modeling of various complexes of TCR–SAg from Gram-positive bacteria [reviewed in (16,56)]. Nevertheless, these variant ways of binding to TCR and MHC still induce efficient T cell activation, suggesting that a certain strength of concomitant binding to MHC class II and BV suffices for the induction of agonistic TCR signals. The fact that such BV-specific TCR agonists evolved independently in highly divergent microorganisms may also be an indicator for a selective advantage provided by them for the microorganism.

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Abbreviations

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<tr>
<th>Acronym</th>
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<tr>
<td>APC</td>
<td>antigen-presenting cell</td>
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<tr>
<td>CDR</td>
<td>complementarity-determining region</td>
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<td>Con A</td>
<td>concanavalin A</td>
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<td>gp</td>
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<td>MAS</td>
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<td>MBP</td>
<td>myelin basic protein</td>
</tr>
</tbody>
</table>

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References


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