Extraction and characterization of the rhesus macaque T-cell receptor β-chain genes

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Rhesus macaque models have been instrumental in the development and testing of vaccines before human studies and have provided fundamental insights into the determinants of immune efficacy in a variety of infectious diseases. However, the characterization of antigen-specific T-cell receptor (TCR) repertoires during adaptive immune responses in these models has earlier relied on human TCR gene assignments. Here, we extracted and characterized TCR β-chain (TRB) genes from the recently sequenced rhesus macaque genome that are homologous to the human TRB genes. Comparison of the rhesus macaque TRB genes with the human TRB genes showed an average best match similarity of 92.9%. Furthermore, we confirmed the usage of most rhesus macaque TRB genes by expressed TCRβ sequences within epitope-specific TCR repertoires. This primary description of the rhesus macaque TRB genes will provide a standardized nomenclature and enable better characterization of TCR usage in studies that use this species.

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**Keywords:** T-cell; T-cell receptor; T-cell receptor repertoire

The rhesus macaque is widely used as a nonhuman primate model to study infection and immunity due to the close genetic relationship with humans (~93% average human–macaque sequence identity) and owing to the homology between human and rhesus pathogen genomes. Indeed, rhesus macaques have been used to study fundamental aspects of immunology, including the development and maintenance of T-cell memory, immunodominance and the aging immune system. There have also been many studies of immune responses in rhesus macaque models of human infections such as human immunodeficiency virus (HIV), influenza virus, tuberculosis, Epstein–Barr virus, cytomegalovirus, smallpox, measles and severe acute respiratory syndrome. Furthermore, rhesus macaques have been instrumental in the design and testing of vaccines against infections such as HIV and smallpox.

The various roles of T lymphocytes in adaptive immune responses to infection, which include the provision of helper functions to other immune cells and cytolytic control of infected cells, require that T-cell populations recognize a large variety of foreign peptides bound to major histocompatibility complex molecules. This recognition is facilitated by a diverse repertoire of T-cell receptors (TCRs). The TCR repertoires that respond to different peptide–major histocompatibility complex epitopes can vary greatly. Indeed, diversity estimates range from ∼10 to >1000 different TCRs responding to a specific epitope. Moreover, some epitope-specific TCR repertoires can feature biased usage of TCR Vβ (TRBV) or TCR Jβ (TRBJ) genes, or distinct patterns of amino-acid usage within the third complementarity-determining region (CDR3).

Studies of the TCR repertoire can provide valuable information about the molecular evolution of an immune response and the factors that shape clonotype selection *in vivo*. Furthermore, it is becoming increasingly apparent that the clonotypic structure of an epitope-specific T-cell response can have important implications for the immune control of some viral infections. For example, one issue of current debate that has important implications for the rational design of immunotherapeutic and vaccination strategies is whether a restricted TCR repertoire responding to a highly variable pathogen could be associated with the emergence of viral mutants that escape T-cell recognition at this epitope.

Many studies of T-cell immunity in rhesus macaque models of infection have used TCR repertoire data to gain additional insights. In particular, a large number of studies have characterized the TCR repertoires of target CD4+ T-cell populations or CD8+ T-cell populations involved in the control of simian immunodeficiency virus (SIV) in rhesus macaques. Most of these studies have relied on human TCR gene homology to identify V and J gene usage. Although the rhesus macaque TCR Dβ (TRBD) and TRBJ genes have been sequenced, the TRBV genes were not available earlier. Here, we present the TRBV, TRBD and TRBJ genes extracted...
Table 1 Comparison of the rhesus macaque TRBV genes with their best human homologs

| Rhesus macaque gene | Best human homolog | Gene nucleotide sequence | Exon nucleotide sequence | Exon amino-acid sequence |
|---------------------|--------------------|--------------------------|--------------------------|--------------------------|
|                     | Percentage identity | Alignment length | Percentage identity | Alignment length | Percentage identity | Alignment length |
| TRBV1-1*            | TRBV1              | 93.3 489                | 93.1 333                | 86.5 111           |
| TRBV1-2*            | TRBV1              | 91.0 490                | 90.1 334                | 64.0 111           |
| TRBV1-3*            | TRBV1              | 85.5 496                | 82.8 337                | 89.6 115           |
| TRBV2-1             | TRBV2              | 93.5 475                | 94.8 347                | 89.6 115           |
| TRBV2-2             | TRBV2              | 93.7 475                | 93.7 347                | 87.8 115           |
| TRBV2-3             | TRBV2              | 94.3 475                | 93.9 347                | 86.8 114           |
| TRBV3-1             | TRBV3-1            | 92.6 499                | 93.6 344                | 84.2 114           |
| TRBV3-2             | TRBV3-1            | 92.8 499                | 92.4 344                | 86.0 114           |
| TRBV3-3             | TRBV3-1            | 90.8 499                | 93.9 344                | 89.5 114           |
| TRBV3-4             | TRBV3-1            | 92.6 499                | 93.6 344                | 86.0 114           |
| TRBV4-1             | TRBV4-1            | 92.7 493                | 94.8 344                | 90.4 114           |
| TRBV4-2             | TRBV4-1            | 94.7 493                | 93.0 344                | 87.7 114           |
| TRBV4-3             | TRBV4-1            | 91.2 502                | 93.9 343                | 91.2 114           |
| TRBV5-1*            | TRBV5-1            | 93.5 509                | 93.9 343                | 86.1 115           |
| TRBV5-2*            | TRBV5-1            | 84.8 512                | 83.7 343                | 88.6 114           |
| TRBV5-3             | TRBV5-3            | 93.1 506                | 93.4 346                | 91.8 114           |
| TRBV5-4             | TRBV5-8            | 93.1 506                | 93.6 343                | 91.2 114           |
| TRBV5-5             | TRBV5-6            | 90.9 507                | 91.6 344                | 86.0 114           |
| TRBV5-6             | TRBV5-6            | 94.7 505                | 95.0 343                | 91.2 114           |
| TRBV5-7             | TRBV5-6            | 93.7 506                | 95.0 343                | 90.4 114           |
| TRBV5-8             | TRBV5-6            | 94.7 506                | 95.3 343                | 88.6 114           |
| TRBV5-9             | TRBV5-6            | 94.5 505                | 92.1 343                | 87.7 114           |
| TRBV5-10            | TRBV5-6            | 92.1 506                | 92.1 343                | 87.7 114           |
| TRBV6-1             | TRBV6-1            | 91.9 472                | 93.6 344                | 87.7 114           |
| TRBV6-2*            | TRBV6-1            | 94.3 351                | 93.9 295                | 87.6 97            |
| TRBV6-3             | TRBV6-5            | 93.9 475                | 94.2 344                | 88.6 114           |
| TRBV6-4             | TRBV6-5            | 92.0 475                | 92.4 344                | 84.2 114           |
| TRBV6-5*            | TRBV6-9            | 91.5 424                | 90.8 355                | 84.7 111           |
| TRBV6-6             | TRBV6-5            | 92.2 475                | 94.5 344                | 87.7 114           |
| TRBV6-7             | TRBV6-5            | 92.4 475                | 93.9 344                | 86.8 114           |
| TRBV6-8*            | TRBV6-5            | 91.0 390                | 91.3 293                | 83.8 99            |
| TRBV7-1*            | TRBV7-1            | 83.6 329                | 93.1 290                | 83.3 96            |
| TRBV7-2*            | TRBV7-3            | 87.7 261                | 88.3 222                | 83.6 73            |
| TRBV7-3             | TRBV7-3            | 90.6 500                | 91.9 347                | 86.1 115           |
| TRBV7-4             | TRBV7-7            | 89.8 549                | 90.8 347                | 84.3 115           |
| TRBV7-5             | TRBV7-8            | 91.8 525                | 92.8 347                | 87.0 115           |
| TRBV7-6             | TRBV7-6            | 89.7 532                | 92.8 347                | 87.8 115           |
| TRBV7-7             | TRBV7-4            | 89.6 509                | 90.8 347                | 82.6 115           |
| TRBV7-8*            | TRBV7-6            | 87.4 546                | 89.2 361                | 37.2 121           |
| TRBV7-9             | TRBV7-6            | 92.8 538                | 94.5 347                | 89.6 115           |
| TRBV7-10            | TRBV7-9            | 90.7 516                | 92.2 347                | 82.6 115           |
| TRBV8-1*            | TRBV8-2            | 86.7 511                | 85.1 336                | 30.4 115           |
| TRBV9-1             | TRBV9              | 94.7 514                | 96.2 343                | 93.9 114           |
| TRBV10-1            | TRBV10-1           | 96.5 489                | 96.2 344                | 93.9 114           |
| TRBV10-2            | TRBV10-1           | 95.1 489                | 95.3 344                | 93.9 114           |
| TRBV10-3            | TRBV10-3           | 95.5 489                | 96.5 344                | 93.0 114           |
| TRBV11-1            | TRBV11-1           | 93.3 490                | 93.9 347                | 87.0 115           |
| TRBV11-2            | TRBV11-2           | 94.4 480                | 94.5 347                | 91.3 115           |
| TRBV11-3            | TRBV11-3           | 93.9 477                | 94.2 347                | 88.7 115           |
| TRBV12-1            | TRBV12-2           | 92.7 482                | 93.0 344                | 85.2 115           |
| TRBV12-2            | TRBV12-3           | 91.8 486                | 93.1 347                | 87.8 115           |
| TRBV12-3            | TRBV12-3           | 91.8 486                | 92.5 347                | 87.8 115           |
| TRBV12-4            | TRBV12-5           | 94.9 486                | 94.5 347                | 92.2 115           |
| TRBV13-1            | TRBV13             | 94.5 524                | 95.7 374                | 89.5 124           |
| TRBV14-1            | TRBV14             | 93.6 482                | 95.1 347                | 89.6 115           |
| TRBV15-1            | TRBV15             | 94.3 508                | 94.8 344                | 89.5 114           |
| TRBV16-1            | TRBV16             | 94.7 493                | 94.8 347                | 88.7 115           |
from the rhesus macaque genome\(^1\) on the basis of their homology with the human TRB genes. In addition, we show the extracted TRB gene usage in expressed TCR\(^\beta\)-chain genes (see Supplementary Table S1).

### RESULTS

#### Rhesus macaque TRBV genes

A total of 72 TRBV genes were extracted from chromosome 3 of the rhesus macaque genome (Table 1 and Rhesus_macaque_TRBV.fsa in Supplementary Information). The human TRBV gene corresponding most precisely to each rhesus macaque TRBV gene was identified on the basis of the highest percentage match between the nucleotide sequences for the TRBV genes (that is, V-GENE in the IMGT standardized labels). The percentage similarity between the nucleotide sequences for the rhesus macaque and the best match human TRBV genes ranged between 78.3 and 96.5%, with an average similarity of 92.2%. We could not identify a one-to-one correspondence between all rhesus macaque and human TRBV genes (Figure 1). In many cases, one human TRBV gene was found to be the best match to more than one of the TRBV genes extracted from the rhesus macaque genome. For example, the human TRBV6-5 gene had the highest percentage similarity of all human TRBV genes to five of the rhesus macaque TRBV genes; in contrast, the human TRBV6-6 gene was not the best match to any of the rhesus macaque TRBV genes. For 5 of the 72 TRBV genes, only partial sequences were available from the rhesus macaque genome (Supplementary Table S1), and only 2 of these partial TRBV genes were incomplete at the 3'-end, which would influence their use in the analysis of the CDR3. The human TRBV17 subgroup, consisting of just one gene, was the only one for which no corresponding TRB gene was found in the rhesus macaque genome (with a cutoff of 75% similarity).

We also compared the TRBV exons (that is, L-PART1+V-EXON in the IMGT standardized labels) between the rhesus macaque and best match human TRBV\(^\gamma\) genes (Table 1). The percentage identities between the nucleotide sequences for the rhesus macaque and human TRBV exons ranged between 72.7 and 96.5%, with an average of 92.9%. The similarities between the rhesus macaque and human TRBV exons at the amino-acid sequence level ranged between 19.5 and 94.7%, with an average of 85.3%.

#### Rhesus macaque TRBD genes

The two TRBD genes extracted from the rhesus macaque genome were found to have an agreement of 95.0 and 92.8%, respectively, at the nucleotide level with the corresponding human TRBD genes (Table 2 and Rhesus_macaque_TRBD.fsa in Supplementary Information). The percentage similarities between the rhesus macaque and human TRBD exons (that is, D-REGION in the IMGT standardized labels) nucleotide sequences were 84.6 and 75.0%.

The rhesus macaque TRBD genes have been sequenced in an earlier study.\(^4\) The TRBD1 gene extracted from the rhesus macaque genome does not differ from that reported in this earlier study. A 1.2%
difference was found between the TRBD2 gene reported here and that reported earlier, with a single nucleotide difference occurring in the 5’-spacer. Thus, there are no differences in the TRBD2 D-REGION extracted from the rhesus macaque genome compared with that reported earlier.44

Rhesus macaque TRBJ genes
For each of the 14 human TRBJ genes, there was one corresponding TRBJ gene found on chromosome 3 of the rhesus macaque genome (Table 3 and Rhesus_macaque_TRBJ.fsa in Supplementary Information). The percentage similarities between the rhesus macaque TRBJ genes and the corresponding human TRBJ genes are shown in Table 3 (range: 92.1 and 98.7%; average: 96.1%).

A comparison of the rhesus macaque with human TRBJ exons (that is, J-REGION in the IMGT standardized labels) indicated percentage similarities of nucleotide sequences ranging between 90.2 and 100%, with an average similarity of 95.4% (Table 3). The similarities between the translated TRBJ exons of the rhesus macaque and human genes
ranged between 81.3 and 100%, with an average similarity of 92.3% (Table 3).

We compared the TRBJ genes extracted from the rhesus macaque genome with those reported in an earlier study.44 The only differences found were in the TRBJ1-6 and TRBJ2-1 genes, which differed by 1.9 and 2.0%, respectively. A single nucleotide difference in the 20th nucleotide position of the TRBJ1-6 exon resulted in a difference of a single amino acid (that is, the TRBJ1-6 exon from the rhesus macaque genome contained H in the 7th amino-acid position instead of Y). In the TRBJ2-1 gene, a single nucleotide difference in the 31st nucleotide position of the exon did not result in any amino-acid differences between the TRBJ2-1 exon extracted from the rhesus macaque genome and that reported by Cheynier et al.44

Use of the rhesus macaque TRB genes by expressed TCRβ sequences

To show the use of the TRB genes extracted from the rhesus macaque genome by expressed TCRβ sequences, we used an existing database of 7218 TCRβ sequences involved in CD8+ T-cell responses specific for the immunodominant Mamu-A*01-restricted SIV-SL8/TL8 and SIV-CM9 epitopes in 20 rhesus macaques.30,45 Each of these TCRβ sequences was aligned with the TRB gene exons to determine the most likely TRBV, TRBJ and TRBD gene usage. In Tables 4 and 5, we show the rhesus macaque TRB genes that were found to be most likely used by at least one of the TCRβ sequences. The genes used by the TCRβ sequences included 54 of the 72 TRBV genes, both TRBD genes and 13 of the 14 TRBJ genes. The highest percentage homology and the longest match between each TRB gene and a TCRβ sequence are also shown. Of the 18 rhesus macaque TRBV genes not used by the TCRβ sequences, 12 either did not begin with a start codon or contained stop codons when translated (Table 1). The rhesus macaque TRBJ2-2P gene, which is homologous to the human TRBJ2-2P gene (qualified by IMGT as having an ‘open reading frame’ functionality), was the only TRBJ gene not used by the TCRβ sequences. Deviations between the rhesus macaque TRB genes and TCRβ sequences were mostly attributed to the full-length genes not being used by the TCRβ sequences, owing to nucleotides being cleaved during TCR gene recombination. However, allelic differences could also exist between the single rhesus macaque sequenced in the genome project and the 20 SIV-infected macaques from which the TCRβ sequences were obtained.

Possible allelic variants of the TRB genes used by the TCRβ sequences were not identified because of the level of uncertainty associated with distinguishing allelic variants from sequencing errors.

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### Table 2 Comparison of the rhesus macaque TRBD genes with their human homologs

| Rhesus macaque gene | Human gene homolog | Gene nucleotide sequence | Exon nucleotide sequence |
|---------------------|--------------------|--------------------------|--------------------------|
| TRBD1               | TRBD1              | 98.7%                    | 97.9%                    |
| TRBD2               | TRBD2              | 94.9%                    | 96.2%                    |
| Average percentage identity | | 96.1% | 95.4% |

Abbreviation: TRBD, T-cell receptor Dβ gene.

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### Table 3 Comparison of the rhesus macaque TRBJ genes with their human homologs

| Rhesus macaque gene | Human gene homolog | Gene nucleotide sequence | Exon amino-acid sequence |
|---------------------|--------------------|--------------------------|--------------------------|
| TRBJ1-1             | TRBJ1-1            | 98.7%                    | 98.7%                    |
| TRBJ1-2             | TRBJ1-2            | 97.4%                    | 97.4%                    |
| TRBJ1-3             | TRBJ1-3            | 94.9%                    | 94.9%                    |
| TRBJ1-4             | TRBJ1-4            | 97.5%                    | 97.5%                    |
| TRBJ1-5             | TRBJ1-5            | 97.5%                    | 97.5%                    |
| TRBJ1-6             | TRBJ1-6            | 97.5%                    | 97.5%                    |
| TRBJ2-1             | TRBJ2-1            | 98.7%                    | 98.7%                    |
| TRBJ2-2             | TRBJ2-2            | 98.7%                    | 98.7%                    |
| TRBJ2-3             | TRBJ2-3            | 97.4%                    | 97.4%                    |
| TRBJ2-4             | TRBJ2-4            | 97.5%                    | 97.5%                    |
| TRBJ2-5             | TRBJ2-5            | 97.1%                    | 97.1%                    |
| TRBJ2-6             | TRBJ2-6            | 97.5%                    | 97.5%                    |
| TRBJ2-7             | TRBJ2-7            | 97.5%                    | 97.5%                    |
| Average percentage identity | | 97.5% | 97.5% |

Abbreviation: TRBJ, T-cell receptor Jβ gene.

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*The alignment length is the total length across both the aligned rhesus macaque and human gene/exon sequences.
in either the rhesus macaque genome or TCRβ sequences, when there were
often small numbers of TCRβ sequences per rhesus macaque
using a particular TRB gene. However, we investigated whether the
nucleotide sequence variants of the TRB1-6 and TRB2-1 genes
reported by Cheynier et al.44 were used in our collection of epitope-
specific TCRβ sequences. The earlier reported variant of the TRB1-6
gene was found to be used by some TCRβ sequences, suggesting that
this is an allelic variant of the TRB1-6 gene extracted from the rhesus
macaque genome. The TRB2-1 gene variant was not used by any of
the TCRβ sequences. It may be an allelic variant that was not present
in any of the 20 rhesus macaques in which Mamu-A*01-restricted
SIV-SL8/TL8- and SIV-CM9-specific TCRβ repertoires were studied,
but it is also possible that the single nucleotide difference in the
TRB2-1 gene reported by Cheynier et al.44 is due to a sequencing
error.

**DISCUSSION**

The assembly of reference TCR gene data sets for many species has
often relied on the *ad hoc* sourcing of different TRC genes from
various studies over time. Here, we report a reference set of TRβ genes
extracted from the rhesus macaque genome, most of which were
expressed by TCRβ sequences in our extensive database of TCRβ
repertoires involved in CD8⁺ T-cell responses to the immunodomi-
nant Mamu-A*01-restricted SL8/TL8 and CM9 epitopes derived from
SIV. Although there is a high degree of similarity (93.0%) between the
exons of the rhesus macaque and human TRβ genes, important
interspecies differences exist. These interspecies differences are empha-
sized by the lack of a one-to-one correspondence between the rhesus
macaque and human TRβ genes, and could potentially limit the
accuracy of studies that rely on human TCR genes to characterize
rhesus macaque TCR repertoires.

The rhesus macaque TRβ genes described herein will not only aid in
the identification of the TRBV and TRBJ genes used by TCRβ
sequences but will also improve the accuracy of studies that aim to
characterize the V(D)J recombination mechanisms that produce
TCRβ repertoires. Indeed, several of the extracted rhesus macaque
TRB genes have already been used in a study of TCRβ sequence
sharing between macaques in the SIV-SL8/TL8-specific and SIV-CM9-

**Table 4** Usage of the rhesus macaque TRBV genes by expressed
TCRβ sequences

| Rhesus macaque gene | Highest % usage by a TCRβ sequence | Alignment length a |
|---------------------|------------------------------------|-------------------|
| TRBV2-1             | 100                                | 182               |
| TRBV2-2             | 99.1                               | 116               |
| TRBV2-3             | 100                                | 185               |
| TRBV3-1             | 100                                | 274               |
| TRBV3-2             | 100                                | 278               |
| TRBV3-3             | 100                                | 179               |
| TRBV3-4             | 100                                | 278               |
| TRBV4-1             | 100                                | 167               |
| TRBV4-2             | 100                                | 282               |
| TRBV4-3             | 100                                | 273               |
| TRBV5-1             | 98.7                               | 232               |
| TRBV5-2             | 98.1                               | 281               |
| TRBV5-3             | 98.9                               | 272               |
| TRBV5-4             | 98.9                               | 159               |
| TRBV5-5             | 97.9                               | 280               |
| TRBV5-6             | 98.7                               | 307               |
| TRBV6-1             | 100                                | 189               |
| TRBV6-2             | 100                                | 277               |
| TRBV6-3             | 100                                | 274               |
| TRBV7-2             | 98.9                               | 281               |
| TRBV7-3             | 98.7                               | 297               |
| TRBV7-4             | 99.3                               | 289               |
| TRBV7-5             | 98.6                               | 289               |
| TRBV7-6             | 99.7                               | 287               |
| TRBV7-7             | 97.1                               | 68                |
| TRBV7-9             | 97.9                               | 290               |
| TRBV7-10            | 98.6                               | 284               |
| TRBV9-1             | 99.4                               | 180               |
| TRBV10-1            | 96.7                               | 276               |
| TRBV10-2            | 100                                | 279               |
| TRBV10-3            | 99.2                               | 238               |
| TRBV11-1            | 99.6                               | 276               |
| TRBV11-2            | 98.6                               | 282               |
| TRBV11-3            | 98.9                               | 282               |
| TRBV12-2            | 99.3                               | 277               |
| TRBV12-3            | 98.8                               | 168               |
| TRBV12-4            | 95.9                               | 74                |
| TRBV13-1            | 98.9                               | 182               |
| TRBV14-1            | 99.7                               | 294               |
| TRBV15-1            | 99.2                               | 262               |
| TRBV16-1            | 94.6                               | 74                |
| TRBV18-1            | 97.6                               | 167               |
| TRBV19-1            | 100                                | 279               |
| TRBV20-1            | 99.5                               | 197               |
| TRBV21-1            | 98.2                               | 274               |
| TRBV23-1            | 100                                | 298               |
| TRBV24-1            | 97.7                               | 214               |
| TRBV25-1            | 98.5                               | 268               |
| TRBV27-1            | 100                                | 281               |
| TRBV28-1            | 99.2                               | 119               |
| TRBV29-1            | 100                                | 282               |
| TRBV30-1            | 98.2                               | 170               |

**Table 5** Usage of the rhesus macaque TRBD and TRBJ genes by
expressed TCRβ sequences

| Rhesus macaque gene | Highest % usage by a TCRβ sequence | Alignment length a |
|---------------------|------------------------------------|-------------------|
| TRBD1               | 100                                | 13                |
| TRBD2               | 100                                | 14                |
| TRBJ1-1             | 100                                | 48                |
| TRBJ1-2             | 100                                | 48                |
| TRBJ1-3             | 100                                | 50                |
| TRBJ1-4             | 100                                | 51                |
| TRBJ1-5             | 100                                | 50                |
| TRBJ1-6             | 96.2                               | 53                |
| TRBJ2-1             | 100                                | 50                |
| TRBJ2-2             | 100                                | 51                |
| TRBJ2-3             | 100                                | 49                |
| TRBJ2-4             | 100                                | 49                |
| TRBJ2-5             | 100                                | 48                |
| TRBJ2-6             | 100                                | 53                |
| TRBJ2-7             | 100                                | 47                |

Abbreviation: TRBV, T-cell receptor VB gene; TRBD, T-cell receptor DB gene; TRBJ, T-cell receptor JB gene.
*The alignments were performed over the total length of the TRBD or TRBJ exon.
specific CD8+ T-cell responses.39 This study required predictions of the potential V(D)J recombination mechanisms involved in producing the observed epitope-specific TCRβ repertoires, which were more reliable using the rhesus macaque TRB genes instead of the human TRB genes.

Rhesus macaques are frequently used to study fundamental aspects of immunology and to investigate vaccine efficacy in various infectious diseases. Increasing evidence, much of which has come from studies conducted with this nonhuman primate model, indicates that the clonotypic architecture of antigen-specific T-cell populations is a fundamental determinant of immune control and disease outcome.26,45 Thus, the rhesus macaque TRB genes presented here provide a valuable tool for dissecting the molecular features of TCRβ repertoires that underlie such associations in this model.

METHODS

Extraction of TRB gene sequences from the rhesus macaque genome

The published rhesus macaque (Macaca mulatta) genome1 is available from the National Center for Biotechnology Information (NCBI) Rhesus Macaque Genome Resources website (http://www.ncbi.nlm.nih.gov/projects/genomeguide/rhesus_macaque/). The TRB gene locus is located on chromosome 3 (accession no.: NC_007860.1). The rhesus macaque chromosome 3 sequence was queried against all human TRB reference genes (obtained from the NCBI Human Resources website http://www.ncbi.nlm.nih.gov/projects/genomeguide/human/) using BLAST (Basic Local Alignment Search Tool)46 to identify regions in the rhesus macaque sequence that resembled human TRB genes. Results were filtered to those with e-value \( \leq 0.001 \), total alignment length \( \geq 35\% \) of the human reference gene and total percentage identity \( \geq 75\% \) with the human reference gene. These parameters were chosen to minimize false-positive search results. Overlapping regions were merged and all regions were extended in both the 5'- and 3'-directions to account for regions missed in BLAST's local alignment search. Sequence alignments using ClustalW47 were then performed to compare each region of the rhesus macaque genome with each human TRB gene from the NCBI human reference set. The best human match to each macaque region was identified and then used as a guide to determine the exact length and terminal ends of the rhesus macaque TRB gene sequences, as well as intron and exon positions.

Comparison of rhesus macaque with human TRB gene sequences

We assessed the similarity between the rhesus macaque and the NCBI human TRB reference gene sequences (or the IMGT human TRB reference gene if the NCBI reference gene sequence was partial) by identifying the human TRB gene that had the highest overall percentage identity with each rhesus macaque TRB gene using a ClustalW alignment. We encountered the following scenarios:
(i) a clearly identifiable one-to-one correspondence between a rhesus macaque and a human TRB gene; (ii) a rhesus macaque TRB gene with reasonable similarity to a group of human TRB genes; and (iii) a human TRB gene with no reasonable correspondence to a rhesus macaque TRB gene. We therefore adopted the following approach to labeling the rhesus macaque TRB genes. For each rhesus macaque TRB gene, we first identified the group of human TRB genes to which it was most similar (for example, TRBV1). We then numbered all rhesus macaque TRB genes that were most similar to this same group of human TRB genes according to the order in which the TRB sequences were found in the rhesus macaque genome (for example, TRBV1-1 and TRBV1-2). The IMGT48 nomenclature for TCR genes was used throughout.

Analysis of expressed epitope-specific TCRβ sequences using the rhesus macaque TRB genes

For all Mamu-A*01-restricted SIV-SL8/TL8-specific and SIV-CM9-specific TCRβ sequences, we performed a complete alignment analysis using the identified rhesus macaque TRB genes. This analysis determined for each epitope-specific TCRβ sequence the best percentage match TRBV, TRBD and TRBJ genes over the longest alignment length by initially aligning the TRBV gene at the 5'-end of the TCRβ sequence and then aligning the TRBJ gene at the 3'-end of the TCRβ sequence. A minimum percentage match of 77% over an alignment length of at least 50 nucleotides was required for alignment of the TRBV genes. For alignment of the TRBJ genes, a minimum percentage match of 70% was required over the length of the TRBJ exon. The TRBD genes were then aligned to the sequence interval between the identified TRBV and TRBJ regions. A match to a string of two or more nucleotides was considered to originate from the TRBD gene.

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