Conservation of Nonpeptide Antigen Recognition by Rhesus Monkey Vγ2Vδ2 T Cells[1]

Hong Wang,* Hoi K. Lee,* Jack F. Bukowski,† Hongmin Li,§ Roy A. Mariuzza,¶ Zheng W. Chen,‡ Ki-Hoan Nam,[2]* and Craig T. Morita[3]*

We have previously found that monkey Vγ2Vδ2$^+$ T cells mount adaptive immune responses in response to *Mycobacterium bovis* bacillus Calmette-Guérin infections. We have now analyzed rhesus monkey $\gamma\delta$ T cell responses to nonpeptide Ags and superantigens. Like human Vγ2Vδ2$^+$ T cells, rhesus monkey $\gamma\delta$ T cells are stimulated when exposed to prenyl pyrophosphate, bisphosphonate, and alkylamine Ags. Responsiveness was limited to $\gamma\delta$ T cells expressing Vγ2Vδ2 TCRs. Rhesus monkey Vγ2Vδ2$^+$ T cells also responded to the superantigen, staphyloccocal enterotoxin A. Sequencing of the rhesus monkey Vγ2Vδ2 TCR revealed a strong sequence homology to human Vγ2Vδ2 TCR that preserves important sequence motifs. Moreover, chimeric TCRs that pair human Vγ2 with monkey Vδ2 and monkey Vγ2 with human Vδ2 retain reactivity to nonpeptide Ags and B cell lymphomas. A molecular model of the rhesus monkey Vγ2Vδ2 TCR has a basic region in the complementarity-determining region 3 binding groove that is similar to that seen in the human Vγ2Vδ2 TCR and preserves the topology of the complementarity-determining region loops. Thus, recognition of nonpeptide prenyl pyrophosphate, bisphosphonate, and alkylamine Ags is conserved in primates suggesting that primates can provide an animal model for human $\gamma\delta$ T cell Ag responses. ***The Journal of Immunology*****, 2003, 170: 3696–3706.**

Gamma delta ($\gamma\delta$) T cells are a second subset of T cells with unique functional and recognition properties (1). Murine $\gamma\delta$ T cells play important roles in immunity to infections and tumors (2), tissue homeostasis (3), immunoregulation (4), and in controlling autoimmune $\alpha\beta$ T cell responses (5). In so doing, $\gamma\delta$ T cells likely function as a bridge between the innate and adaptive immune systems. Although few Ags have been defined for murine $\gamma\delta$ T cells, human $\gamma\delta$ T cells can recognize nonpeptide Ags. $\gamma\delta$ T cells expressing Vδ1 TCRs can recognize self and foreign lipids presented by CD1 (6) and the nonclassical MHC class Ib protein, MHC class I-related chain A/MHC class I-related chain B (7). Most human $\gamma\delta$ T cells express Vγ2Vδ2 TCRs that recognize nonpeptide prenyl pyrophosphates (8–10), bisphosphonates (11–13), and alkylamines (14). The recognition of these nonpeptide Ags requires the pairing of Vγ2 (also termed Vγ9) with Vδ2 (15). Although cell-cell contact is required for recognition (16), recognition does not require classical MHC class I or class II molecules (16), prior antigenic exposure (8, 17, 18), or Ag processing (16).

Large expansions of Vγ2Vδ2$^+$ T cells occur during some bacterial and parasitic infections (reviewed in Ref. 1). In some patients, these $\gamma\delta$ T cell expansions can be extremely large such that almost all (in erhlichiosis) or half (in salmonellosis and tularemia) of peripheral blood T cells are $\gamma\delta$ T cells. The $\gamma\delta$ T cell expansion can occur rapidly after infection (as early as 7 days; Ref. 19) and can persist for up to a year (20). Vγ2Vδ2$^+$ T cells recognize cells infected with bacteria (21–23) and can reduce bacterial viability through the release of granulysin (6, 24). Vγ2Vδ2$^+$ T cells also produce large amounts of the inflammatory TNF-α and IFN-γ cytokines when stimulated by nonpeptide Ags (25). Moreover, Vγ2Vδ2$^+$ T cells activated by nonpeptide Ags protect SCID mice from in vivo bacterial infections by reducing bacterial numbers (26). These results suggest that human $\gamma\delta$ T cells have important roles in immunity to these pathogens similar to and perhaps greater than the roles played by $\gamma\delta$ T cells in mice.

Determining the importance of $\gamma\delta$ T cells in human immunity has been hampered by the lack of an experimental animal model system to study this T cell subset. Few foreign Ags have been discovered for $\gamma\delta$ T cells from other species. Other nonprimate animals, including mice and rats, do not have V genes homologous to Vγ2 and Vδ2 genes and do not respond to nonpeptide Ags that stimulate human Vγ2Vδ2$^+$ T cells. Recently, we demonstrated that Vγ2Vδ2$^+$ T cells in rhesus monkeys can expand in response to infection with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG)[4] (27). Monkey Vγ2Vδ2$^+$ T cells mounted adaptive (memory) responses because reinfection of the monkeys with BCG resulted in earlier and larger $\gamma\delta$ T cell expansions. Importantly, this capacity to rapidly expand coincided with a clearance of BCG

*Division of Rheumatology, Department of Internal Medicine and Interdisciplinary Group in Immunology, University of Iowa College of Medicine, Iowa City, IA 52442; †Division of Rheumatology, Immunology, and Allergy, Department of Medicine, Brigham and Women's Hospital, and ‡Division of Viral Pathogenesis, Beth Israel Hospital, Harvard Medical School, Boston, MA 02115; §Structural and Cell Biology Program, Department of Biomedical Sciences, Wadsworth Center, New York State Department of Health, Albany, NY 12201; and ¶Center for Advanced Research in Biotechnology, University of Maryland Biotechnology Institute, Rockville, MD 20850

Received for publication November 26, 2002. Accepted for publication January 31, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants from the National Institutes of Health (to C.T.M., Z.W.C., J.F.B., and R.A.M.), the Arthritis Foundation (to C.T.M. and J.F.B.), the American College of Rheumatology (to C.T.M.), the National Multiple Sclerosis Society (to R.A.M.), and the Howard Hughes Medical Institute Research Resources Award (to C.T.M.).

[2] Current address: Laboratory of Development and Differentiation, Division of Functional Biology, Korea Research Institute of Bioscience and Biotechnology, Yusong, Daejeon, Korea.

[3] Address correspondence and reprint requests to Dr. Craig T. Morita, Division of Rheumatology, Department of Internal Medicine and the Interdisciplinary Group in Immunology, University of Iowa College of Medicine, John W. Eckstein Medical Research Building 340F, Iowa City, IA 52242. E-mail address: Craig-Morita@uiowa.edu

[4] Abbreviations used in this paper: BCG, bacillus Calmette-Guérin; SEA, staphylococcal enterotoxin A; CDR, complementarity-determining region; MEP, monoethyl phosphate; IPP, isopentenyl pyrophosphate; HMBPP, (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate; EPP, ethyl pyrophosphate.

0022-1767/03/$02.00

bacteremia and immunity to fatal tuberculosis in BCG-vaccinated rhesus monkeys.

To extend our study of rhesus monkey γδ T cells, we have analyzed rhesus monkey γδ T cell responses to the described nonpeptide Ags and superantigens. Like human γδ T cells, rhesus monkey γδ T cells expanded when exposed to prenyl pyrophosphates and other phosphoantigens and to alkylamine Ags. These expansions were limited to γδ T cells expressing Vγ2Vδ2 TCRs. Rhesus monkey $V\gamma2V\delta2^+$ T cells also responded to the superantigen, staphylococcal enterotoxin A (SEA), and to an unknown Ag expressed by the B cell lymphoma, RPMI 8226. The amino acid sequence of the rhesus monkey Vγ2Vδ2 TCR has strong homology to human Vγ2Vδ2 TCR. A molecular model of the rhesus monkey Vγ2Vδ2 TCR shows a basic region in the complementarity-determining region (CDR)3 binding groove that is similar to that seen in the human Vγ2Vδ2 TCR. These data demonstrate that recognition of nonpeptide prenyl pyrophosphate and alkylamines is conserved in primates providing an animal model for human γδ T cell responses.

Materials and Methods

Nonpeptide Ags and superantigens

Prenyl pyrophosphates and alkylamines were purchased from Sigma-Aldrich (St. Louis, MO). Phosphoantigens including monoethyl phosphate (MEP) and nucleotide-conjugated compounds were synthesized as described (10, 28). The SEA superantigen was produced as a recombinant protein as described (29).

Expansion of γδ T cells by exposure to nonpeptide Ags

Mononuclear cells were prepared from heparinized rhesus monkey blood by centrifugation over density gradients of Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ). PBMC were incubated with media, isopentenyl pyrophosphate (IPP) (50 μM), or partially purified (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP) (1/1000 dilution of a concentrated, <1 kDa *Mycobacterium fortuitum* supernatant). IL-2 was added to 1 nM on day 3. The cells were harvested on days 7–10 and stained with the indicated mAbs for flow cytometric analysis as detailed below. For the alkylamines, 1 million PBMC in 1 ml of medium were cultured for 10 days with 400 μM sec- or iso-butylamine, followed by double-staining with anti-Vδ2/Jδ2 (15D) and anti-Vγ2 (7A5) mAbs and analysis by flow cytometry. Four monkeys were tested.

Derivation and maintenance of T cell lines and clones

Rhesus monkey γδ T cell clones were derived from the blood of a normal rhesus monkey. PBMC were stimulated with a mycobacterial supernatant from *M. fortuitum* (8). After 2 wk, γδ T cells comprised 96% of T cells. γδ T cells were cloned by limiting dilution at 5 cells/well. T cell clones were maintained by periodic restimulation with PHA-P (30).

T cell proliferation assay

For proliferation assays, T cells were plated in triplicate in round-bottom 96-well plates at $5–10 \times 10^4$ T cells per well with 1×10^5 irradiated (7000 rad) human PBMC feeder cells, the EBV-transformed B cell line, DG.EBV, PBMC, or glutaraldehyde-fixed Va-2 tumor cells with various Ags. Prenyl pyrophosphates in ammonium hydroxide and methanol were dried by N_2 gas and dissolved in media by sonicating in an ultrasonic water bath for 5 min. The cultures were pulsed with 1 μCi [^{3}H]thymidine (2 Ci/mmol) on day 1 and harvested 16–18 h later.

Monoclonal Abs

mAbs reactive with human γδ TCR were tested to identify those that cross-react with rhesus monkey γδ T cells using either rhesus monkey PBMC or rhesus monkey Vγ2Vδ2 T cell lines. The cross reactivity for mAbs for γδ TCR are detailed in Table I. Based on these results, the following anti-TCR mAbs were used: anti-TCRδ1 (pan anti-TCRγδ), δTCS1 or TS8 (anti-Vδ1/Jδ1/2), 15D (anti-Vδ2/Jδ2/3), 7A5 (anti-Vγ2 (Vγ2 is also called Vγ9)), 23D12 (anti-Vγ1.2, 1.3, and 1.4), and 4A11 (anti-Vγ1.4). mAb to surface molecules were FN18 (anti-monkey CD3), OKT4 (anti-CD4), OKT8 (anti-CD8α), and OKT11 (anti-CD2). Isotype-matched control mAbs were used as controls.

Table I. *Cross-reactivity of anti-human TCR Abs to rhesus monkey γδ T cells*

Specificity	mAb Designation	Rhesus Monkey Reactivity	Reference
Cδ	anti-TCRδ1	+	71
Cγ	TCR γ/δ-1	−	72
Cδ	510	−	73
γδ	B1	+	60
γδ non-Vδ1	515	−	73
Vδ1	A13	−	74, 75
Vδ1/Jδ1/2	δTCS1	+	76
Vδ1	LL112	−	77
Vδ1	LL113	−	77
Vδ1	TS8–1E12	+	77
Vδ1	TS9–3C10	+	77
Vδ1	R9.12.6.2	+	78
Vδ1	3/62	−	77
Vδ2/Jδ2/3	15D	+	38
Vδ2	BB3	−	79
Vδ2	4G6	−	80
Vδ2/Jδ1	7A8	−	80
Vδ2	389	−	73
Vδ2	B6	−	81
Vδ2	6.2	−	82
Vδ2	G1	−	74
Vδ2	anti-TiVδ2	−	75
Vδ3	P11.5B	+	78
Vγ1.2, 1.3, 1.4	23D12	±?[b]	82
Vγ1.4	4A11	+	81
Vγ1.4	94	+	78
Vγ1.5 (Vγ1.3)	56.3	±?[b]	83
Vγ1.8	B10.11.17	+	78
Vγ1.8	B18	−	84
Vγ2	TiγA	−	85
Vγ2	7A5	+	86
Vγ2	360	+	73
Vγ2	4D7	+	80
Vγ2	B3	+	81

[a] Rhesus monkey PBMC or rhesus monkey $V\gamma2V\delta2^+$ T cell clones were stained with the indicated mAbs and analyzed by one-color flow cytometry.
[b] Unable to definitively verify reactivity.

Flow cytometric analysis

Mononuclear cells were prepared from heparinized rhesus monkey blood by centrifugation over density gradients of Ficoll-Hypaque (Pharmacia Fine Chemicals). Isolated mononuclear cells, T cell lines, and T cell clones were analyzed by one- or two-color immunofluorescence after staining with the appropriate mAb as described (31). Cells were incubated with mouse mAbs on ice for 30 min, washed, and stained with FITC-conjugated F(ab′)2 goat anti-mouse IgG and IgM antisera (Tago Scientific, Burlingame, CA) for an additional 30 min on ice. After washing, the cells were resuspended in propidium iodide and analyzed by flow cytometry. To prevent background staining of rhesus monkey B cells by anti-mouse Ig antisera, 2% monkey serum was included in the staining buffer. Flow cytometry was performed with a FACScan flow cytometer using CellQuest software (BD Biosciences, Palo Alto, CA).

Sequencing of rhesus monkey Vγ2Vδ2 TCR

RNA was isolated from rhesus monkey T cell lines (Micro RNA isolation kit; Stratagene, La Jolla, CA) followed by cDNA synthesis using SuperScript II reverse transcriptase and random hexamers (SuperScript first-strand synthesis system for RT-PCR; Life Technologies, Gaithersburg, MD). PCR was done with Platinum *Taq* High Fidelity DNA polymerase (Life Technologies). PCR primers used to derive full-length Vγ2Cγ and Vδ2Cδ chains were as described previously (6) except that for the Vδ2Cδ chain the following primer was used to introduce a *Kpn*I restriction site into the 5′ region of the Vδ2Cδ2 chain for cloning: 5′-gggggtaccCAGGCA GAAGGTGGTTGAGAG-3′ Vδ2 5′ untranslated region. The Vγ2Cγ and Vδ2Cδ PCR products were cloned into pREP7 and pREP9 vectors through *Kpn*I-*Xho*I and *Kpn*I-*Bam*HI sites (Invitrogen, Carlsbad, CA), respectively. Sequencing was done using an automated sequencer using the pREP forward and reverse primers along with the following reverse primers: Cγ, 3′UT ATGGCCTCCTTGTGCCACCG; Cγ internal, TGTGTCGTTA

GTCTTCATGG; Cδ, 3′UT GGAGTGTAGCTTCCTCATGC; and Cδ internal, GACAATAGCAGGATCAAACT. Nucleotide sequences have been deposited into GenBank under the accession numbers of AY190025 (RM.2.32γ), AY190026 (RM.2.32δ), AY190027 (RM.2.14γ), and AY190028 (RM.2.14δ).

Derivation of human/rhesus monkey Vγ2Vδ2 TCR transfectants

Chimeric rhesus monkey Vγ2/human Vδ2 TCR transfectants were derived by electroporation of the Jurkat mutant, J.RT3-T3.5 with the rhesus monkey RM2.14 TCR-γ chain cDNA (cloned in the pREP-7 vector) and the human DG.SF68 TCR-δ chain cDNA (cloned in the pREP-9 vector) as described previously (32). Chimeric human Vγ2/rhesus monkey Vδ2 TCR transfectants were derived using a similar strategy with human DG.SF13 TCR-γ chain cDNA and rhesus monkey RM2.32 TCR-δ chain cDNA except that human Cδ was substituted for rhesus monkey Cδ (see below). Human Vγ2 and Vδ2 TCR cDNAs were inserted into pREP-7 and pREP-9, respectively (15). In initial experiments, neither rhesus monkey Vγ2Vδ2 TCRs nor chimeric human Vγ2/rhesus monkey Vδ2 TCRs were expressed when transfected into J.RT3-T3.5, presumably due to the inability of rhesus monkey Cδ to associate with the human CD3 complex. To allow expression of a chimeric receptor, human Cδ was substituted for rhesus monkey Cδ. A rhesus monkey Vδ2 fragment from the RM2.32 T cell clone and a human Cδ fragment were produced by PCR using Vδ2 forward with rhesus monkey Vδ2 5′ UT reverse primers and human Cδ forward with Cδ 3′ UT reverse primers, respectively. PCR primers were as above except for: rhesus monkey Vδ2, 5′ UT reverse, CAGTCACACGGGTCCCTTTTCC AAAGATG; human Cδ forward, CATCTTTGGAAAAGGAACCCGTGT GACTG.

A complete cDNA from the fragments was then produced by annealing PCR under the following conditions: a 5-min denaturation step at 94°C followed by five cycles of annealing (94°C for 1 min, 50°C for 4 min, 72°C for 1.5 min), 40 cycles of amplification (94°C for 30 s, 55°C for 1 min, 72°C for 1 min), and a final extension at 72°C for 10 min. The rhesus monkey Vδ2/human Cδ cDNA was then cloned into pREP-9.

IL-2 release and assay

Stimulation of TCR transfectants for IL-2 release was performed as described (16). Briefly, 1×10^5 transfectants were cultured in triplicate with the indicated Ag in the presence of 1×10^5 glutaraldehyde-fixed Va-2 cells and 10 ng/ml PMA. After 24 h, supernatants were harvested, frozen, thawed, and used at a 1/8 dilution to stimulate the proliferation of the IL-2-dependent cell line, HT-2.

Modeling of the rhesus monkey Vγ2Vδ2 TCR

A model of the rhesus monkey Vγ2Vδ2TCR was built using homology modeling as described for the Vγ2 chain (29) using the coordinates for the human Vγ2Vδ2TCR (kindly provided by Dr. D. Garboczi, National Institutes of Health, Bethesda, MD). The sequences of monkey and human Vγ2Vδ2 TCRs were aligned using a GeneMine package (Molecular Applications Group, Palo Alto, CA). Because they show >85% sequence identity, the CARA module of LOOK homology modeling package (33) was used to generate the model of the monkey Vγ2Vδ2 TCR using the coordinates of the crystal structure of human Vγ2Vδ2 TCR as the initial template. There are two insertions in the TCR CDR3. To model this loop, the SEGMOD module of GeneMine (34) was used. The figures were generated by Molscript (35) and Raster3D (36) (see Fig. 7, *A–C*) and GRASP (see Fig. 7*D*).

Results

γδ T cell repertoire of rhesus monkey blood resembles human neonatal blood

γδ T cells in humans at birth are present at low levels with a predominance of $V\delta1^+$ cells. Between the ages of 3 and 10 years, $V\gamma2V\delta2^+$ T cells expand in response to environmental factors resulting in the predominance of $V\gamma2V\delta2^+$ T cells that is found in most adults. To determine whether rhesus monkeys maintained in closed, specific pathogen-free colonies also exhibit a predominance of $V\gamma2V\delta2^+$ T cells, PBMC from rhesus monkeys were analyzed for V gene segment expression by flow cytometry. Unlike most human adults, adolescent and adult rhesus monkeys exhibited a predominance of $V\delta1^+$ T cells (Table II and Fig. 1). This pattern of V gene expression is seen in human newborns and in human infants <3–10 years of age (31, 37). Because the change in the proportion of $V\delta1^+$ to $V\delta2^+$ T cells in humans is due to an environmental influence, this suggests that rhesus monkeys maintained in closed colonies may not be exposed to the same pathogens or natural flora as humans.

Nonpeptide phosphoantigens and alkylamine Ags stimulate the expansion of rhesus monkey $V\gamma2V\delta2^+$ T cells

To determine whether γδ T cells from rhesus monkeys respond to nonpeptide Ags, PBMC from rhesus monkeys were stimulated with the major bacterial phosphoantigen, HMBPP, from *M. fortuitum* and the alkylamines, *sec*-butylamine and *iso*-butylamine. Exposure of monkey PBMC to HMBPP resulted in an expansion of $V\gamma2V\delta2^+$ T cells to between 20 and 97% of $CD3^+$ T cells (Fig. 2*A*). Similarly, exposure of PBMC from other rhesus monkeys to *sec*-butylamine and *iso*-butylamine resulted in $V\gamma2V\delta2^+$ T cell expansions of between 10 and 43% and 32 and 46% of $CD3^+$ T cells, respectively (Fig. 2*B*). Note that one monkey did not respond to *sec*-butylamine and a second different monkey did not respond to *iso*-butylamine. A similar lack of response to nonpeptide Ags has

Table II. *γδ T cells expressing Vδ1 predominate in rhesus monkey blood*[a]

	Age (y)	γδ TCR	Vδ1/Jδ1	Vδ2/Jδ2/3	Vδ3	Vγ1.4	Vγ2
RM3	ND	6.7	1.7	1.4	ND	0.3	1.3
RM4	5	7.0	2.3	4.2	ND	1.0	6.5
RM5	10	3.8	0.6	2.2	ND	0.3	2.4
RM6	11	5.0	2.2	1.2	ND	0.2	0.8
RM7	4	4.8	1.3	1.1	ND	0.1	1.0
RM8	1	3.3	1.4	0.5	0.4	0.4	1.0
RM9	6	3.9	2.0	0.1	0.7	0.4	0.5
RM10	1	6.6	2.7	0.2	1.5	0.3	0.8
RM11	1	4.3	1.2	0.4	1.0	0.6	1.1
RM12	1	2.8	0.4	0.1	0.4	0.2	0.1
Rhesus blood mean ± SD (n = 6)		4.8 ± 1.5	1.6 ± 0.7	1.1 ± 1.3	0.8 ± 0.5	0.4 ± 0.3	1.6 ± 1.8
% of γδ TCR^+			33	24	17	8	32
Human blood[b] mean ± SD (n = 36)		4.8 ± 4.2	0.7 ± 1.2	2.6 ± 2.5	ND	ND	ND
% of γδ TCR^+[c]			15	54			

[a] Rhesus blood PBMC were stained with the indicated mAbs and analyzed by one-color flow cytometry. Values are expressed as the percent of $CD3^+$ cells. Background staining has been subtracted.

[b] Values are from Fig. 1, Morita et al. (37) and are included for comparison.

[c] Values do not add up to 100% due to background staining.

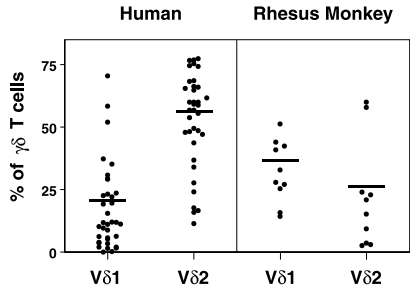

FIGURE 1. γδ V gene repertoire in rhesus monkeys. PBMC were isolated from 10 monkeys and stained with anti-Vγ2 (7A5), anti-Vδ1/Jδ1 (TS8), or anti-Vδ2/Jδ2/3 (15D). The stained cells were analyzed by one-color flow cytometry and the representation of each V gene was determined as the percentage of total γδ T cells. Each point represents one monkey. The arithmetic mean for each V gene is given by the line. These data are presented in Table II. The human data are from Fig. 1, Morita et al. (37) and are included for comparison.

been observed in ~10–20% of human adults (17). Thus, rhesus monkey Vγ2Vδ2$^+$ cells, like human Vγ2Vδ2$^+$ T cells, proliferate in response to nonpeptide phosphoantigens and alkylamine Ags.

Conservation of nonpeptide Ag and superantigen responses by Vγ2Vδ2$^+$ T cell clones

Human Vγ2Vδ2$^+$ T cells respond to nonpeptide phosphoantigens and the superantigen, SEA. To determine the response of rhesus monkey Vγ2Vδ2$^+$ T cells at the clonal level, rhesus monkey Vγ2Vδ2$^+$ T cell clones were derived and tested for recognition of the MEP prenyl pyrophosphate analog and the SEA superantigen. A rhesus monkey Vγ2Vδ2$^+$ T cell line was derived by stimulating PBMC with HMBPP followed by limited dilution cloning. γδ T cells expressed CD8αα homodimers (CD8α staining was 88% whereas CD8β was 0.9%) and CD2 (99%) (data not shown). Ninety-four percent (51 of 54 clones) of the monkey Vγ2Vδ2$^+$ T cell clones derived from this line responded to the prenyl pyrophosphate analog, MEP. Like human γδ T cells (29), the rhesus monkey Vγ2Vδ2$^+$ T cell clones also responded to the superantigen, SEA (representative examples are shown in Fig. 3).

Rhesus monkey Vγ2CγVδ2Cδ TCR sequence

To determine whether the conservation of nonpeptide Ag reactivity reflects conservation of the Vγ2Vδ2 TCR sequence, we cloned and sequenced full-length Vδ2 and Vγ2 cDNA from several rhesus monkey clones. The Vδ2 gene segment was highly conserved showing 88% similarity to the human Vδ2 gene segment (Fig. 4*A*). Few amino acid changes were noted in CDR1 and CDR2. The Jδ1 and Jδ2 segments were highly conserved with no amino acid differences noted. Some Vγ2Vδ2$^+$ T cell clones did not react with the 15D mAb that is specific for Vδ2 in humans (data not shown and Ref. 38). Sequencing the Vδ2 chain from these clones revealed that clones with weak or absent reactivity with the 15D mAb expressed the Vδ2 gene segment in conjunction with the Jδ1 junctional segment (Fig. 4*B*). Vγ2Vδ2$^+$ T cell clones strongly reactive with the 15D mAb expressed the Vδ2 gene segment in conjunction with the Jδ2 junctional segment. This suggests that the 15D mAb has a restricted reactivity with rhesus monkey γδ T cells preferentially reacting with Vδ2Jδ2 and possibly Jδ3 chains. Other anti-human Vδ2 mAbs lack reactivity to the rhesus monkey Vδ2 gene segment (eight of nine Vδ2-specific mAbs; Table I) suggesting that they may react with the same epitope(s) that are lost in rhesus monkeys.

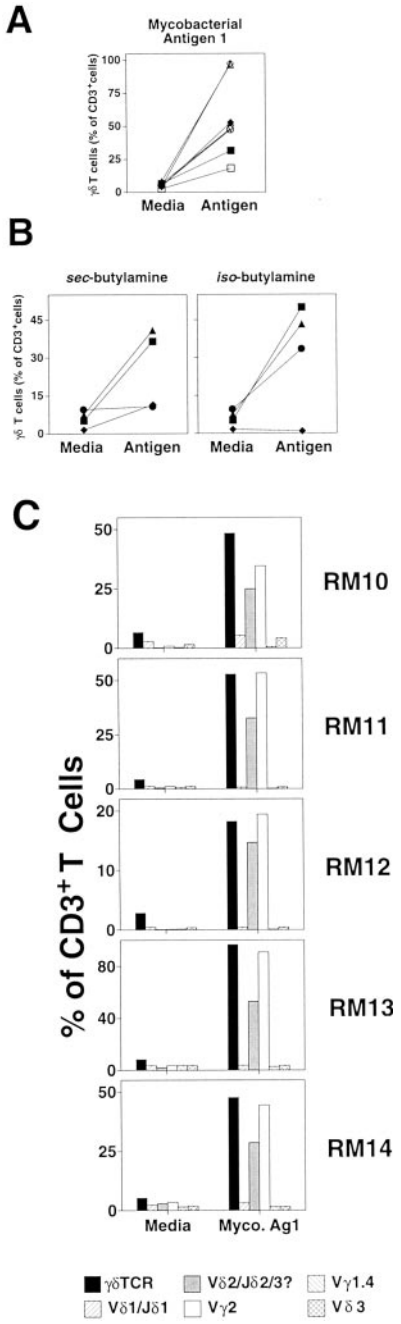

FIGURE 2. Expansion of Vγ2Vδ2$^+$ T cells by exposure to nonpeptide prenyl pyrophosphate and alkylamine Ags. Exposure to HMBPP from *M. fortuitum* (*A*) or the alkylamines, *iso*-butylamine or *sec*-butylamine (*B*), stimulated γδ T cell proliferation. Rhesus monkey PBMC were cultured in vitro with media, HMBPP from *M. fortuitum*, or the alkylamines, *iso*-butylamine or *sec*-butylamine. On day 3, 0.5 nM IL-2 was added to the cultures. On day 8, the cultures were harvested and the cells analyzed by flow cytometry. *C*, Expansion of rhesus monkey γδ T cells was primarily restricted to Vγ2Vδ2$^+$ T cells. Rhesus monkey PBMC were cultured in vitro with media or *M. fortuitum* HMBPP as described in *A*. On day 8, the cells were harvested, stained with anti-Cδ mAb (anti-TCRδ1), anti-Vδ1/Jδ1 mAb (TS8), anti-Vδ2/Jδ2/3 mAb (15D), anti-Vγ2 mAb (7A5), anti-Vγ1.4 mAb (4A11), anti-Vδ3 mAb (P11.5B), and control IgG mAbs in the presence of rhesus monkey plasma followed by rabbit anti-mouse immunoglobulin, and then analyzed by one color flow cytometry. Note that the expanded γδ T cell population exclusively or predominantly expressed Vγ2Vδ2 TCRs. Staining of Vδ2$^+$ T cells with the 15D mAb always gave a lower percentage staining when compared with Vγ2 staining due to the more restricted specificity of the 15D mAb for Vδ2 recombined with Jδ2/3 but not Jδ1.

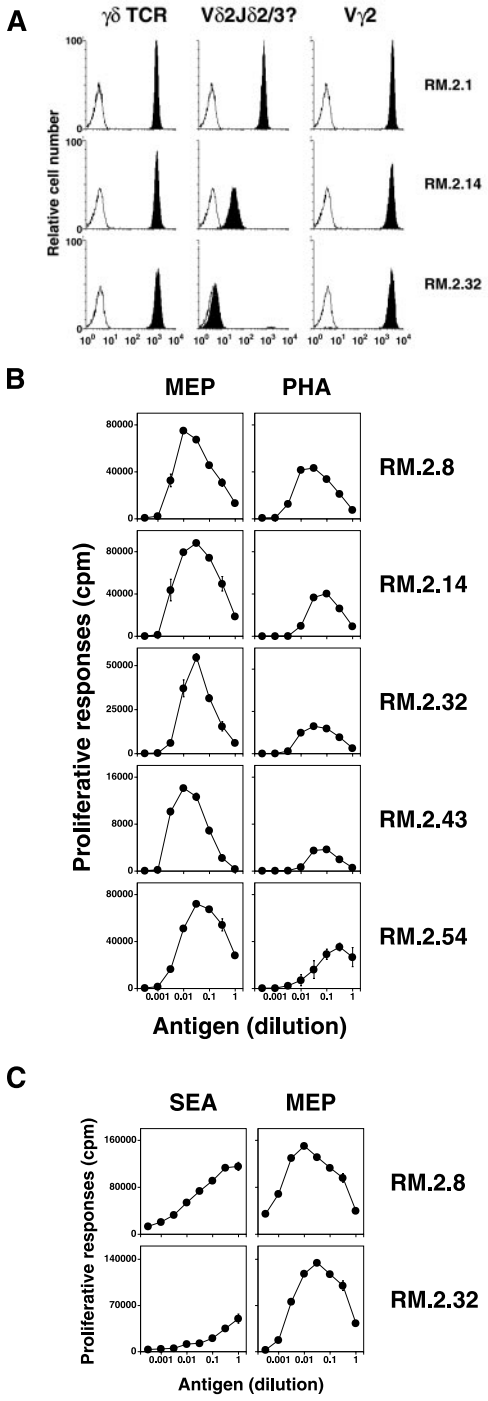

FIGURE 3. Rhesus monkey $V\gamma2V\delta2^+$ T cell clones respond to nonpeptide prenyl pyrophosphate Ags and the superantigen, SEA. *A*, Expression of $V\gamma2V\delta2$ TCR by T cell clones. T cells were stained with mAbs to the γδ TCR (mAb anti-TCRδ1), the Vδ2 domain (mAb 15D), and the Vγ2 domain (mAb 7A5). Note that some clones stained poorly with the 15D mAb due to the restricted specificity of the 15D mAb for Vδ2 recombined with Jδ2/3 but not Jδ1 (see Fig. 4*B*). *B*, $V\gamma2V\delta2^+$ T cell clones respond to the nonpeptide prenyl pyrophosphate Ag analog, MEP. Human EBV-transformed B cells (DG.EBV line) were fixed with glutaraldehyde and used as APCs for γδ T cell clones in the presence of MEP (*left panels*) or the mitogen, PHA-P (*right panels*). rIL-2 was added to 0.03 nM. Initial concentrations were a 1/100 dilution of crude MEP or a 1/100 dilution of a 10 mg/ml solution of PHA-P. On day 1, each culture was pulsed with [^{3}H]thymidine, and harvested 18 h later. *C*, $V\gamma2V\delta2^+$ T cell clones respond to the nonpeptide prenyl pyrophosphate Ag, MEP, and the superantigen, SEA. Culture conditions were as in (*B*) except that the initial concentration of SEA was 1 μg/ml.

Comparison of the amino acid sequence of the rhesus monkey, *Aotus* monkey, chimpanzee, and the human Vγ2 gene segment revealed 91% similarity between the rhesus monkey and human sequence (Fig. 4*C*). The CDR1 and CDR2 of Vγ2 had three and two amino acid differences, respectively, that were mostly conservative amino acid changes (Fig. 4*E*). The CDR3 region showed at least four amino acid differences in the Jγ1.2 segment that were all conservative changes, including an arginine for lysine difference (Fig. 4*D*). We and others have proposed that basic residues in the Jγ1.2 segment may constitute contact residues for pyrophosphate binding (28, 39). The rhesus monkey Vγ2 TCR also showed conservation in Vγ2 CDR3 length with all three Vγ2 CDR3s within two amino acids in length (Fig. 4*D*). This is similar to human Vγ2 TCR where 98% of adult $V\gamma2V\delta2$ TCR have Vγ2 CDR3 lengths of ±1 of a modal value (40).

The Cγ and Cδ gene segments were also highly conserved. The Cδ segment showed 87% similarity with the human Cδ segment (Fig. 4*F*) and the Cγ segment showed 93% similarity with the human Cγ segment (Fig. 4*G*). Thus, rhesus monkey and human $V\gamma2V\delta2$ TCRs are highly conserved in amino acid sequence with only minor differences noted in CDRs.

Fine phosphoantigen specificity of rhesus monkey $V\gamma2V\delta2^+$ T cells is similar to human $V\gamma2V\delta2^+$ T cells

Because human and monkey $V\gamma2V\delta2$ TCR differ in their CDRs (Fig. 4), we sought to determine whether these changes affect the fine Ag specificity of rhesus monkey $V\gamma2V\delta2^+$ T cells. A variety of different prenyl pyrophosphate Ags and analogs were used to stimulate human and rhesus monkey $V\gamma2V\delta2^+$ T cell clones. Despite the CDR differences between human and rhesus monkey $V\gamma2V\delta2$ TCR, the relative potency of different analogs was identical such that the hierarchy of phosphoantigen bioactivity did not change (Fig. 5). For example, similar strong reactivity to IPP and ethyl pyrophosphate (EPP) and low reactivity to phenylethyl- and *iso*-amyl pyrophosphate was noted for both monkey and human $V\gamma2V\delta2^+$ T cells (Fig. 5). The rhesus monkey $V\gamma2V\delta2^+$ T cell clones shown were more sensitive to Ag stimulation as compared with human $V\gamma2V\delta2^+$ T cell clones but this was not a consistent finding with all monkey clones (such variation is also seen with human clones). Thus, despite differences in CDR sequence between human and monkey $V\gamma2V\delta2^+$ T cells, fine phosphoantigen specificity was unchanged suggesting that these differences do not affect nonpeptide Ag recognition.

Rhesus monkey/human chimeric TCR transfectants maintain specificity to nonpeptide Ag

To further study the conservation in recognition of nonpeptide Ag between rhesus monkey and human $V\gamma2V\delta2$ TCR, human/monkey chimeric γδ TCR transfectants were derived by cotransfection and tested for their response to nonpeptide Ags by IL-2 release. TCR transfectants expressing either monkey Vγ2 paired with human Vδ2 or human Vγ2 paired with monkey Vδ2 were found to respond to HMBPP, EPP, and the bisphosphonate, risedronate, in a similar manner (Fig. 6*A*). Detailed testing of the TCR transfectant expressing human Vγ2 paired with monkey Vδ2 revealed responsiveness to all of the major Ags defined for human $V\gamma2V\delta2^+$ T cells including prenyl pyrophosphate Ags and analogs, alkylamines, bisphosphonates, the bacterial phosphoantigen, HMBPP, and the B cell lymphoma, RPMI 8226 (Fig. 6*B*). Moreover, the rank order of the response to the different prenyl pyrophosphate Ags and analogs was similar (compare Fig. 6*B*, *top panel*, with Fig. 5). Thus, the rhesus monkey and human Vγ2 and Vδ2 gene segments can be interchanged without loss or major alteration in

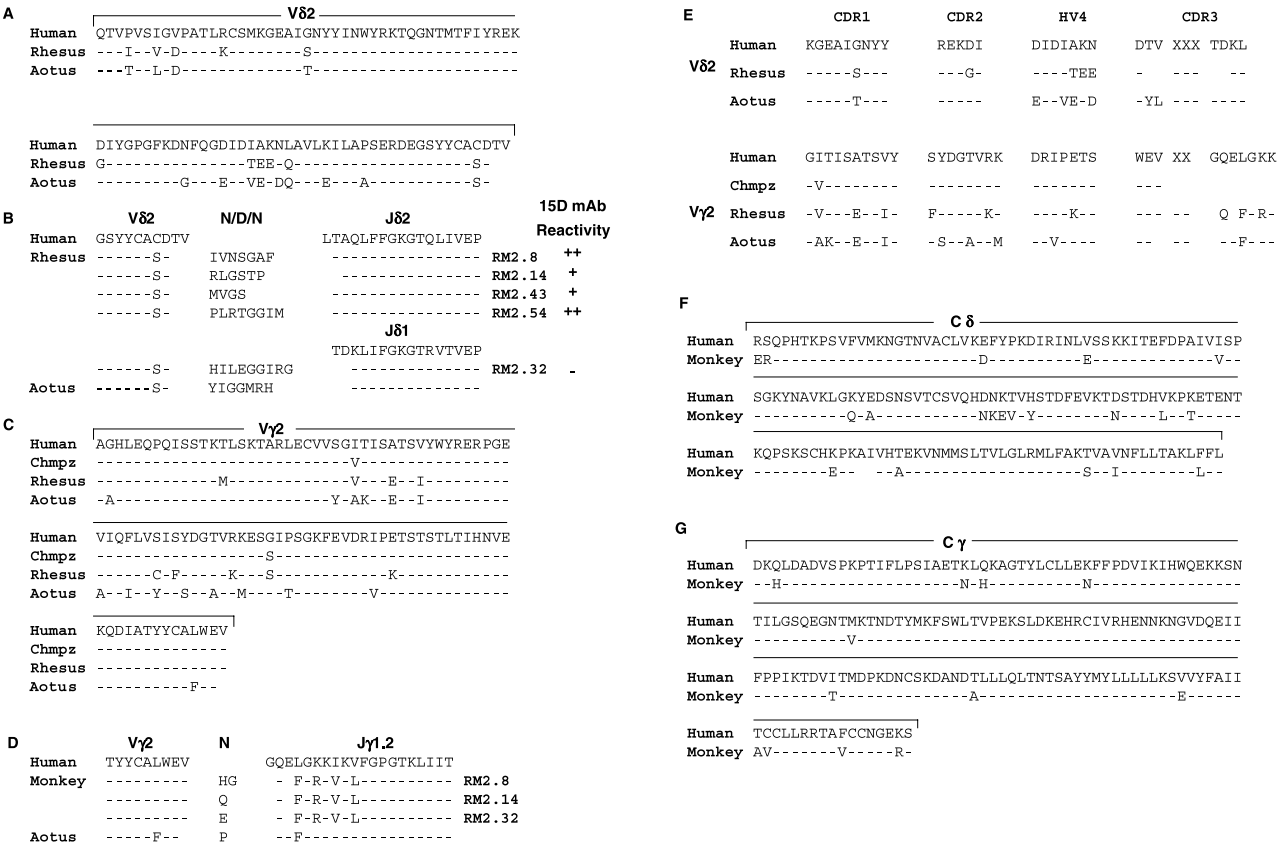

FIGURE 4. Sequence of rhesus monkey γδ TCR segments. *A*, Amino acid comparison of the rhesus monkey, *Aotus* monkey, and human Vδ2 segments. The rhesus monkey Vδ2 segment shares 88% homology with human Vδ2. *B*, CDR3 sequences of Vδ2 chains of rhesus monkey T cell clones. Note that the lack of 15D reactivity correlates with the presence of the Jδ1 segment by the RM2.32 clone. *C*, Amino acid comparison of the rhesus monkey, *Aotus* monkey, chimpanzee, and human Vγ2 segments. Rhesus monkey Vγ2 shared 91% homology with human Vγ2. *D*, CDR3 sequences of Vγ2 chains of rhesus monkey T cell clones. Note the four conservative replacements in the Jγ1.2 sequence. *E*, CDR1, CDR2, and CDR3 comparison for rhesus monkey and human Vδ2 and Vγ2 segments. *F* and *G*, Amino acid comparison of the Cδ (*F*) and Cγ (*G*) segments. The amino acid similarity was 87% for the Cδ segment and 93% for the Cγ segment. The nucleotide and deduced amino acid sequences have been deposited into the GenBank database.

reactivity to any of the known nonpeptide Ags recognized by the Vγ2Vδ2 TCR.

Comparison between rhesus monkey Vγ2Vδ2 TCR and the human Vγ2Vδ2 TCR

Because there was close sequence similarity between the rhesus monkey and the human Vγ2Vδ2 TCR (Fig. 4), we made a homology model of the rhesus monkey Vγ2Vδ2 TCR based on the crystal structure of the human Vγ2Vδ2 TCR (39). Consistent with their close sequence similarities, there were few differences between the two TCRs when the carbon backbone was superimposed (Fig. 7, *A* and *B*). Despite the differences in the sequence of the CDRs, the position of the CDR loops showed little variation. The amino acid differences (colored red) in the CDR1 and CDR2 of the monkey and human Vγ2 chain are located in areas that are not the highest points on the CDR loops (Fig. 7*C*). Moreover, when the surface potential of the two receptors was compared, both have similar basic (positively charged) regions in the CDR3 groove encoded by a lysine (K109) from the Jγ1.2 region of the CDR3 of Vγ2 and an arginine (R51) from the CDR2 loop of Vδ2. These two basic residues are conserved between rhesus monkeys, humans, and *Aotus* monkeys and are postulated to form a potential binding site for the pyrophosphate residues of phosphoantigens (28, 39). Consistent with this hypothesis, this region is required for Ag recognition because alterations in the CDR3 of the Vγ2 chain or direct mutation of the K109 lysine results in the loss of Ag recognition (41, 42). Another basic lysine residue, K108, that is required for phosphoantigen recognition (our unpublished observation and Ref. 42), exhibits a conservative change because the corresponding residue is an arginine in the rhesus monkey TCR. Thus, close similarities are noted in the structure of the rhesus monkey and human Vγ2Vδ2 TCRs with conservation of the CDR loop topology and in a basic region in the CDR3 groove.

Discussion

In this study, we demonstrate that recognition of nonpeptide Ags, superantigens, and B cell lymphomas is conserved in rhesus monkey γδ T cells. Like in humans, phosphoantigens, bisphosphonates, and alkylamines stimulate the rhesus monkey Vγ2Vδ2$^+$ T cell subset. Moreover, even the fine specificity for phosphoantigens exhibited by rhesus monkey Vγ2Vδ2$^+$ T cells is identical to that of human Vγ2Vδ2$^+$ T cells. Comparison of the primary amino acid sequence of rhesus monkey Vγ2 and Vδ2 gene segments revealed close similarities with amino acid conservation in all of the CDRs. This sequence similarity was sufficient to allow the expression of chimeric human/monkey Vγ2Vδ2 TCRs that recognized phosphoantigens identically to human Vγ2Vδ2 TCRs. Modeling of the rhesus monkey Vγ2Vδ2 TCR, revealed a similar topology to the CDR loops with identical amino acids in the highest points of the loops. These results suggest that the conservation of γδ T cell recognition extends to lower primates allowing their use as an animal model for human γδ T cells responses.

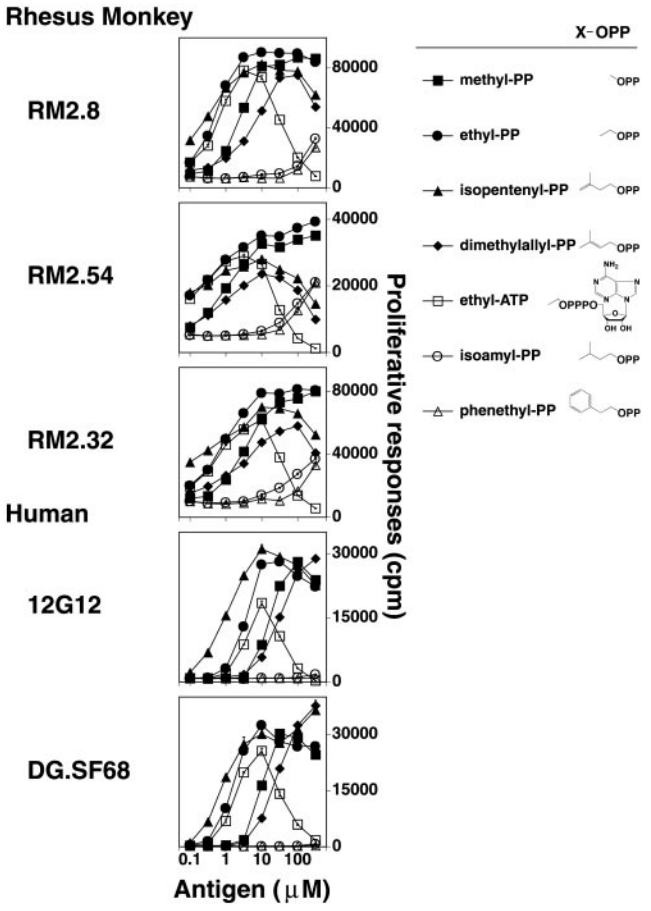

FIGURE 5. Phosphoantigen fine specificity of rhesus monkey $V\gamma2V\delta2^+$ T cells is similar to human $V\gamma2V\delta2^+$ T cells. To determine whether rhesus monkey and human $V\gamma2V\delta2^+$ T cell clones show similar fine specificity for Ags despite their differences in CDR sequences, a panel of phosphoantigens was used to stimulate four rhesus monkey and two human $V\gamma2V\delta2^+$ T cell clones. Phosphorylated compounds were incubated with rhesus monkey (RM2.8, RM2.54, and RM2.32) or human (12G12 or DG. SF68) $V\gamma2V\delta2^+$ T cell clones and irradiated Va2 cells for 2 days. Note that although the Ag concentration required for half-maximal stimulation varied between rhesus monkey and human $V\gamma2V\delta2^+$ T cells, the relative potency of the different compounds was similar such that the hierarchy of phosphoantigen bioactivity did not change.

Rhesus monkey $V\gamma2V\delta2^+$ T cells showed complete conservation of nonpeptide Ag, lymphoma, and superantigen reactivity. Thus, responses were noted to prenyl pyrophosphates and analogs including IPP, EPP, and HMBPP. Moreover, there were minimal differences in the relative potency of the different analogs such that the hierarchy of reactivity did not significantly differ between rhesus monkey and human $V\gamma2V\delta2^+$ T cell clones. Similarly, reactivity to the other nonpeptide Ags, the alkylamines and the bisphosphonates (Figs. 2*B* and 6, *A* and *B*) was conserved as was reactivity to the superantigen, SEA (Fig. 3). The conservation of Ag and superantigen reactivity probably reflects the conservation of the amino acid sequence of the Vγ2 CDR1, CDR2, and HV4 regions (Fig. 4*E*) where there are few changes in the predicted critical contact residues (29). Reactivity to the uncharacterized Ag on the B cell lymphomas, Daudi (43), and RPMI 8226 (Fig. 6*B*), was also conserved. Thus, reactivity to all of the known Ags for $V\gamma2V\delta2^+$ T cells was conserved in rhesus monkeys.

The Vγ2 and Vδ2 variable regions are highly conserved between the rhesus monkey and humans (91 and 88%, respectively). Vβ genes show a similar level of amino acid similarity because 17 different Vβ genes averaged 88% similarity to their human counterparts (44). The amino acid differences noted in the CDR1 and CDR2 of Vγ2 and Vδ2 variable regions are mainly conservative changes that are localized to less exposed regions of the TCR on our structural model (Fig. 7*C*). Thus, the basic arginine residue (residue 43) from the CDR2 of the Vδ2 gene and the lysine residue from the Jγ1.2 region that makes up the basic region located in the groove of the $V\gamma2V\delta2$ TCR are conserved in the three primates (Fig. 4*E*). Rhesus (Fig. 4*B* and Ref. 45) and *Aotus* monkeys (46) also express a hydrophobic residue at codon 109 in the CDR3 of the Vδ2 gene that is found in most human $V\gamma2V\delta2^+$ T cells (47).

Despite the importance of the CDR3 of the Vγ2 gene in determining reactivity to nonpeptide Ags (41, 42), the rhesus monkey Jγ1.2 gene differs at a number of amino acid residue from human and *Aotus* Jγ1.2 genes (Fig. 4 and Ref. 45). The first basic residue in the rhesus monkey Jγ1.2 gene segment is an arginine rather than the lysine found in humans and *Aotus* monkeys. This residue is located near the basic region composed of the second lysine residue of the Jγ1.2 gene with a basic residue from the CDR2 of Vδ2 (28, 39). Both of the basic residues in the human Jγ1.2 gene segment are critical for nonpeptide Ag recognition (42) and thus have been conserved in rhesus monkeys. Three other residues exhibit conservative replacements whereas a fourth residue, the aspartic acid found in the third position, is not present in rhesus monkeys. Although differing in amino acid sequence, rhesus monkey $V\gamma2V\delta2^+$ T cells are similar to human $V\gamma2V\delta2^+$ T cells (40, 47) in that they conserve the length of the CDR3 (Fig. 4*D* and Refs. 45, 48). Thus, the length of the CDR3 may be more important than certain CDR3 amino acid residues.

The similarities in rhesus monkey and human $V\gamma2V\delta2$ TCRs also extend to their fine specificity to phosphoantigens. Different analogs of IPP showed similar or identical hierarchies of potencies when different rhesus monkey $V\gamma2V\delta2$ clones were compared with human $V\gamma2V\delta2$ clones. Therefore, γδ T cell recognition of phosphoantigens differs from αβ T cell recognition of nonpeptide haptens and drugs presented by MHC class I and class II molecules. These αβ T cells commonly show differences in fine specificity for Ag between clones (49, 50) as well as specificity to the peptide conjugated to the hapten (51). In contrast, the differences in the CDR1, CDR2, and CDR3 seen between rhesus monkey and human $V\gamma2V\delta2$ TCRs do not affect the fine Ag specificity. We speculate that if an Ag-presenting element for prenyl pyrophosphate Ags exists, it is relatively nonpolymorphic and may bind to the $V\gamma2V\delta2$ TCR such that the Ag contacts a relatively small portion of a germline encoded region of the TCR. In this way, amino acid differences in the CDR3 and in other CDRs would not affect fine Ag specificity.

The conservation of nonpeptide Ag recognition in primates suggests that this type of recognition plays an important role in primate immune systems. Like rhesus monkeys, reactivity to phosphoantigens is conserved in *Aotus* monkeys (46) and recognition of the B cell lymphoma, Daudi, is conserved in chimpanzees (52). Thus, both Old World (*Catarrhini*) and New World (*Platyrrhini*) monkeys that separated ~60 million years ago, as well as higher primates (chimpanzees and humans), are able to recognize nonpeptide Ags.

We recently demonstrated that, unlike innate immune cells, $V\gamma2V\delta2^+$ T cells can mount adaptive immune responses in response to infection with *M. bovis* BCG (27). We now show that the majority of γδ T cells from adolescent and adult rhesus monkeys from the New England Primate Center (Southborough, MA) express TCRs using Vδ1 (Fig. 1 and Table II). A similar predominance of Vδ1 T cells is seen in children before an environmentally driven expansion of $V\gamma2V\delta2^+$ T cells that occurs in most people between ages 3 and 10 (31). This expansion is presumably due to

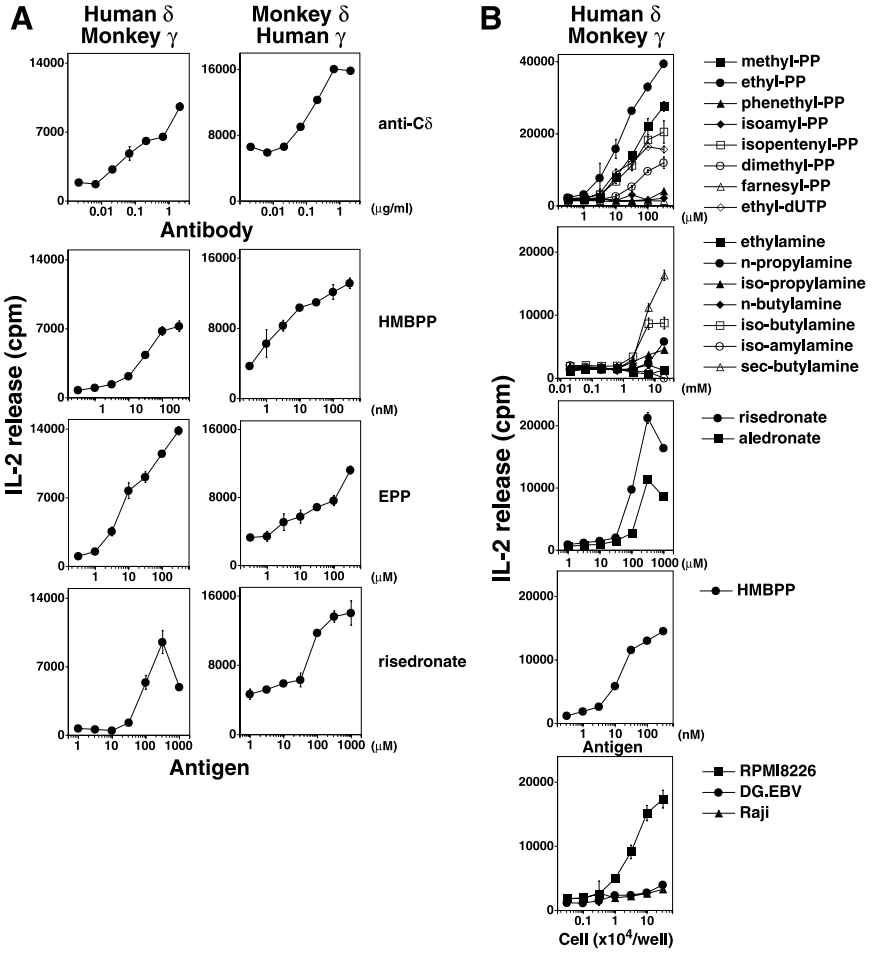

FIGURE 6. Chimeric rhesus monkey/human γδ TCR transfectants share similar nonpeptide Ag specificity. *A*, Chimeric monkey/human γδ TCR transfectant responses to nonpeptide Ags. Chimeric human Vδ2/rhesus monkey Vγ2 or rhesus monkey Vδ2/human Vγ2 TCR transfectants were made by cotransfection of a rhesus monkey γ- (from RM2.14) or δ-chain (from RM2.32) with a human γ from DG.SF13 or δ-chain from DG.SF68 into J.RT3-3.5 cells followed by G418/hygromycin selection. The resulting T cell transfectant lines or clones were cultured with HMBPP, EPP, or risedronate with Va2 presenter cells. The supernatants were harvested at 24 h and used to stimulate the proliferation of the IL-2-dependent cell line, HT.2. *B*, Chimeric human Vδ2/rhesus monkey Vγ2 TCR transfectant responded to all Ags for Vγ2Vδ2 T cells. The human Vδ2/rhesus monkey Vγ2 TCR transfectant was further tested for IL-2 release to a panel of nonpeptide Ags including (*top* to *bottom*) prenyl pyrophosphates/phosphoantigens, alkylamines, bisphosphonates, HMBPP, and the B cell tumor, RPMI 8226, as described in *A*.

a common bacterial infection(s) that occurs in humans but that does not occur in the closed monkey colony. When the rhesus monkeys were infected with BCG, $V\gamma2V\delta2^+$ T cells expanded peaking at days 20–30 post infection. Secondary infection with BCG lead to a rapid expansion (as early as 5 days) that peaked earlier and with higher numbers of cells (up to 25–35% of all peripheral blood T cells). Accompanying this expansion of γδ T cells in the blood were higher levels of $V\gamma2V\delta2^+$ T cells in the pulmonary alveoli and intestinal mucosa and higher levels of expansion in vitro to IPP and mycobacterial prenyl pyrophosphate Ags. Importantly, the expansion of $V\gamma2V\delta2^+$ T cells correlated with the clearance of BCG organisms from the blood. Moreover, when monkeys were vaccinated with BCG, they could survive aerosol infection with *Mycobacterium tuberculosis* that rapidly killed unvaccinated monkeys. Protection was associated with earlier increases in alveolar $V\gamma2V\delta2^+$ T cells as compared with naive animals.

The rapid expansion of $V\gamma2V\delta2^+$ T cells upon infection of rhesus monkeys is identical to that seen in many human bacterial and protozoal parasitic infections (reviewed in Ref. 1). This broad reactivity of $V\gamma2V\delta2^+$ T cells is likely due to their recognition of a common metabolic intermediate, HMBPP. HMBPP is an intermediate in the deoxyxylulose synthetic pathway for IPP (our unpublished data and Ref. 53) that is highly active. This pathway is found only in Eubacteria, Apicomplexan parasites, and the chloroplasts of plants. Thus, the presence of this Ag in the blood or tissue is a strong indication of an infection. Because almost all adult $V\gamma2V\delta2^+$ T cells respond to nonpeptide Ags, a remarkably high precursor frequency of 1 T cell in 20 to 1 in 50 can respond to the prenyl pyrophosphates and alkylamines produced by bacteria or parasites. This type of recognition shares great similarities with innate immune recognition (54, 55) and suggest that γδ T cells are using their Ag receptors like pattern recognition receptors (1).

The advantage of this innate T cell recognition is the ability to focus T cell responses earlier in immune responses and more broadly than would be normally possible. Although the function of $V\gamma2V\delta2^+$ T cells is difficult to assess, $V\gamma2V\delta2^+$ T cells can kill infected cells and bacteria (22, 23) and secrete large amounts of TNF-α and IFN-γ, two important type 1 cytokines (25, 56). Human $V\gamma2V\delta2^+$ T cells stimulated with nonpeptide Ags protect "humanized" SCID mice from several species of bacteria including *Staphylococcus aureus*, *Morganella morganii*, and *Escherichia coli* (26). $V\gamma2V\delta2^+$ T cells can recognize a potential nonpeptide Ag in B cell lymphomas (57, 58) suggesting a role for these cells in tumor immunity. $V\gamma2V\delta2^+$ T cells also express NK receptors (59, 60), including NKG-2D (61), that may allow $V\gamma2V\delta2^+$ T cells to play a similar role to NK cells in antitumor and antiviral immunity including immunity to HIV/SIV (43, 62, 63). Furthermore, although few Ags for murine γδ T cells have been discovered, murine γδ T cells are required for optimal protection from infection with several bacterial species including *Klebsiella pneumoniae* (64), *M. tuberculosis* (65), *Listeria monocytogenes* (66), and *Nocardia asteroides* (67). Murine γδ T cells also decrease the severity of several autoimmune diseases (5) and inflammatory responses to infections (68, 69). Therefore, these studies suggest a role for γδ T cells in immunity to infection, tumors, and in autoimmunity.

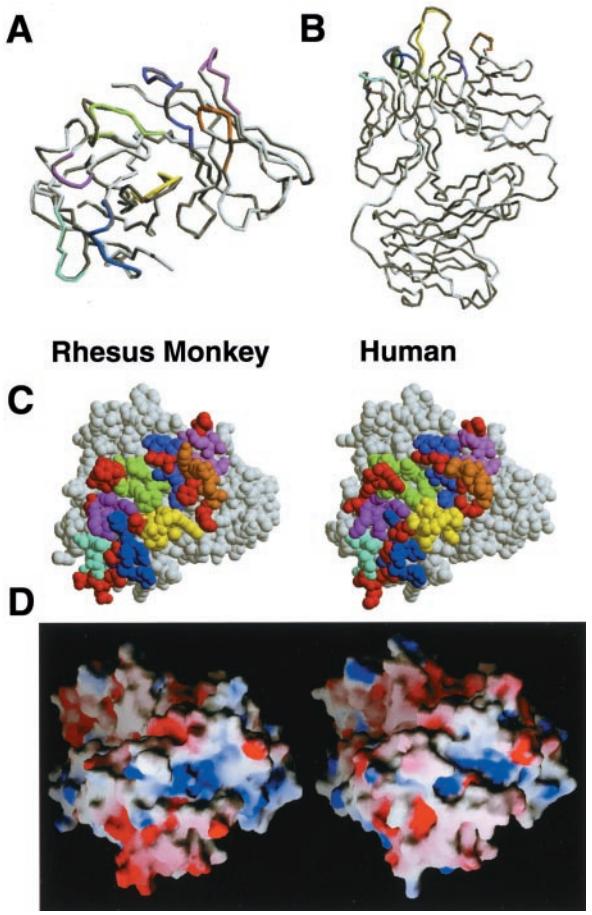

FIGURE 7. Comparison of a model of the rhesus monkey Vγ2Vδ2 TCR with the crystal structure of the human Vγ2Vδ2 TCR. A model of the rhesus monkey Vγ2Vδ2 TCR from RM.2.32 was constructed based on its strong homology with the human Vγ2Vδ2 TCR. *A*, Top view of the superposition of monkey and human Vγ2Vδ2 TCRs showing the CDR loops. The monkey model is colored light gray, except for CDR1-purple, CDR2-orange, CDR3-green, and HV4-violet for the γ-chain and CDR1-blue, CDR2-magenta, CDR3-yellow, HV4-cyan for the δ-chain. The human TCR backbone is colored dark gray. *B*, Side view of the superposition of monkey and human Vγ2Vδ2 TCRs. Colors for each molecule and CDR are the same as in *A*. *C*, Polymorphic residues between monkey and human Vγ2Vδ2 TCRs. The model of monkey Vγ2Vδ2 TCR is shown on the *left*, while human TCR is shown on the *right*. Colors for CDR residues are the same as in *A*. Residues that differ between the two species are colored red. Differences in the N and D encoded regions of CDR3 are not colored. *D*, Surface potential comparison between monkey and human Vγ2Vδ2 TCRs. *Left*, Monkey Vγ2Vδ2 TCR; *right*, human Vγ2Vδ2 TCR. The orientation of the molecules is similar to *C*. Surface potential was calculated using the GRASP program. Basic (positively charged) areas are colored blue whereas acidic (negatively charged) areas are colored red. Note the basic area in the CDR3s of both monkey and human Vγ2Vδ2 TCR due to lysine residue 109 from the Jγ1.2 segment and arginine residue 51 from the CDR1 of the Vδ2 segment.

The development of an animal model for $V\gamma2V\delta2^+$ T cells would greatly help the development of nonpeptide Ag vaccines that target these cells for their broad antibacterial and antiparasitic effects. Mice, rats, rabbits, guinea pigs, and ruminants do not appear to recognize known nonpeptide Ags because there is no reactivity seen in direct testing and because none of these animal species have V genes homologous to primate Vγ2 or Vδ2 genes. Our results show that rhesus monkeys can serve as animal models to study all facets of nonpeptide Ag recognition and $V\gamma2V\delta2^+$ T cell function in vivo. Indeed, i.v. infusion of rhesus monkeys with the weak phosphoantigen diphosphoglycerate is reported to increase in vitro expansions and production of IFN-γ and TNF-α by $V\gamma2V\delta2^+$ T cells upon IPP stimulation although this effect only persisted for 60 days (70). A more potent nonpeptide Ag vaccine that stimulates an adaptive $V\gamma2V\delta2^+$ T cell response could play an important role in a multicomponent vaccine for tuberculosis, leprosy, or anthrax infections or for protection from drug-resistant bacteria.

Acknowledgments

We thank Dr. Kia-Joo Puan and Chenggang Jin for critical reading of this manuscript. We thank Pamela Bruellman and Zhimei Fang for technical assistance. We thank Drs. Marc Bonneville, Genaro de Libero, Klaus Pfeffer, Simon Carding, Dieter Kabelitz, Frederick Treibel, and Lorenzo Moretta for the providing mAbs used in this report. We thank Dr. David Garboczi for providing the crystal coordinates for human Vγ2Vδ2 TCR, G115.

References

1. Morita, C. T., R. A. Mariuzza, and M. B. Brenner. 2000. Antigen recognition by human γδ T cells: pattern recognition by the adaptive immune system. *Springer Semin. Immunopathol. 22:191.*
2. Penninger, J. M., T. Wen, E. Timms, J. Potter, V. A. Wallace, T. Matsuyama, D. Ferrick, B. Sydora, M. Kronenberg, and T. W. Mak. 1995. Spontaneous resistance to acute T-cell leukaemias in TCR Vγ1.1Jγ4Cγ4 transgenic mice. *Nature 375:241.*
3. Jameson, J., K. Ugarte, N. Chen, P. Yachi, E. Fuchs, R. Boismenu, and W. L. Havran. 2002. A role for skin γδ T cells in wound repair. *Science 296:747.*
4. Born, W., C. Cady, J. Jones-Carson, A. Mukasa, M. Lahn, and R. O'Brien. 1999. Immunoregulatory functions of γδ T cells. *Adv. Immunol. 71:77.*
5. Peng, S. L., M. P. Madaio, A. C. Hayday, and J. Craft. 1996. Propagation and regulation of systemic autoimmunity by γδ T cells. *J. Immunol. 157:5689.*
6. Spada, F. M., E. P. Grant, P. J. Peters, M. Sugita, A. Melián, D. S. Leslie, H. K. Lee, E. van Donselaar, D. A. Hanson, A. M. Krensky, et al. 2000. Self recognition of CD1 by γδ T cells: implications for innate immunity. *J. Exp. Med. 191:937.*
7. Groh, V., A. Steinle, S. Bauer, and T. Spies. 1998. Recognition of stress-induced MHC molecules by intestinal epithelial γδ T cells. *Science 279:1737.*
8. Tanaka, Y., S. Sano, E. Nieves, G. De Libero, D. Roca, R. L. Modlin, M. B. Brenner, B. R. Bloom, and C. T. Morita. 1994. Nonpeptide ligands for human γδ T cells. *Proc. Natl. Acad. Sci. USA 91:8175.*
9. Constant, P., F. Davodeau, M.-A. Peyrat, Y. Poquet, G. Puzo, M. Bonneville, and J.-J. Fournié. 1994. Stimulation of human γδ T cells by nonpeptidic mycobacterial ligands. *Science 264:267.*
10. Tanaka, Y., C. T. Morita, Y. Tanaka, E. Nieves, M. B. Brenner, and B. R. Bloom. 1995. Natural and synthetic non-peptide antigens recognized by human γδ T cells. *Nature 375:155.*
11. Kunzmann, V., E. Bauer, and M. Wilhelm. 1999. γ/δ T-cell stimulation by pamidronate. *N. Engl. J. Med. 340:737.*
12. Kunzmann, V., E. Bauer, J. Feurle, F. Weissinger, H. P. Tony, and M. Wilhelm. 2000. Stimulation of γδ T cells by aminobisphosphonates and induction of antiplasma cell activity in multiple myeloma. *Blood 96:384.*
13. Das, H., L. Wang, A. Kamath, and J. F. Bukowski. 2001. Vγ2Vδ2 T-cell receptor-mediated recognition of aminobisphosphonates. *Blood 98:1616.*
14. Bukowski, J. F., C. T. Morita, and M. B. Brenner. 1999. Human γδ T cells recognize alkylamines derived from microbes, edible plants, and tea: implications for innate immunity. *Immunity 11:57.*
15. Bukowski, J. F., C. T. Morita, Y. Tanaka, B. R. Bloom, M. B. Brenner, and H. Band. 1995. Vγ2Vδ2 TCR-dependent recognition of non-peptide antigens and Daudi cells analyzed by TCR gene transfer. *J. Immunol. 154:998.*
16. Morita, C. T., E. M. Beckman, J. F. Bukowski, Y. Tanaka, H. Band, B. R. Bloom, D. E. Golan, and M. B. Brenner. 1995. Direct presentation of nonpeptide prenyl pyrophosphate antigens to human γδ T cells. *Immunity 3:495.*
17. Panchamoorthy, G., J. McLean, R. L. Modlin, C. T. Morita, S. Ishikawa, M. B. Brenner, and H. Band. 1991. A predominance of the T cell receptor Vγ2/Vδ2 subset in human mycobacteria-responsive T cells suggests germline gene encoded recognition. *J. Immunol. 147:3360.*
18. Ohmen, J. D., P. F. Barnes, K. Uyemura, S. Z. Lu, C. L. Grisso, and R. L. Modlin. 1991. The T cell receptors of human γδ T cells reactive to *Mycobacterium tuberculosis* are encoded by specific V genes but diverse V-J junctions. *J. Immunol. 147:3353.*
19. Poquet, Y., M. Kroca, F. Halary, S. Stenmark, M.-A. Peyrat, M. Bonneville, J. J. Fournié, and A. Sjöstedt. 1998. Expansion of Vγ9Vδ2 T cells is triggered by *Francisella tularensis*-derived phosphoantigens in tularemia but not after tularemia vaccination. *Infect. Immun. 66:2107.*
20. Kroca, M., A. Tärnvik, and A. Sjöstedt. 2000. The proportion of circulating γδ T cells increases after the first week of onset of tularaemia and remains elevated for more than a year. *Clin. Exp. Immunol. 120:280.*
21. Havlir, D. V., J. J. Ellner, K. A. Chervenak, and W. H. Boom. 1991. Selective expansion of human γδ T cells by monocytes infected with live *Mycobacterium tuberculosis. J. Clin. Invest. 87:729.*

22. Dieli, F., M. Troye-Blomberg, J. Ivanyi, J.-J. Fournié, M. Bonneville, M. A. Peyrat, G. Sireci, and A. Salerno. 2000. Vγ9/Vδ2 T lymphocytes reduce the viability of intracellular *Mycobacterium tuberculosis*. *Eur. J. Immunol. 30: 1512.*
23. Ottones, F., J. Dornand, A. Naroeni, J. P. Liautard, and J. Favero. 2000. Vγ9Vδ2 T cells impair intracellular multiplication of *Brucella suis* in autologous monocytes through soluble factor release and contact-dependent cytotoxic effect. *J. Immunol. 165:7133.*
24. Dieli, F., M. Troye-Blomberg, J. Ivanyi, J. J. Fournie, A. M. Krensky, M. Bonneville, M. A. Peyrat, N. Caccamo, G. Sireci, and A. Salerno. 2001. Granulysin-dependent killing of intracellular and extracellular *Mycobacterium tuberculosis* by Vγ9/Vδ2 T lymphocytes. *J. Infect. Dis. 184:1082.*
25. García, V. E., P. A. Sieling, J.-H. Gong, P. F. Barnes, Y. Tanaka, B. R. Bloom, C. T. Morita, and R. L. Modlin. 1997. Single cell cytokine analysis of γδ T cell responses to nonpeptide mycobacterial antigens. *J. Immunol. 159:1328.*
26. Wang, L., A. Kamath, H. Das, L. Li, and J. F. Bukowski. 2001. Antibacterial effect of human Vγ2Vδ2 T cells in vivo. *J. Clin. Invest. 108:1349.*
27. Shen, Y., D. Zhou, L. Qiu, X. Lai, M. Simon, L. Shen, Z. Kou, Q. Wang, L. Jiang, J. Estep, et al. 2002. Adaptive immune response of $V\gamma2V\delta2^+$ T cells during mycobacterial infections. *Science 295:2255.*
28. Morita, C. T., H. K. Lee, H. Wang, H. Li, R. A. Mariuzza, and Y. Tanaka. 2001. Structural features of nonpeptide prenyl pyrophosphates that determine their antigenicity for human γδ T cells. *J. Immunol. 167:36.*
29. Morita, C. T., H. Li, J. G. Lamphear, R. R. Rich, J. D. Fraser, R. A. Mariuzza, and H. K. Lee. 2001. Superantigen recognition by γδ T cells: SEA recognition site for human Vγ2 T cell receptors. *Immunity 14:331.*
30. Morita, C. T., S. Verma, P. Aparicio, C. Martinez-A, H. Spits, and M. B. Brenner. 1991. Functionally distinct subsets of human γ/δ T cells. *Eur. J. Immunol. 21: 2999.*
31. Parker, C. M., V. Groh, H. Band, S. A. Porcelli, C. Morita, M. Fabbi, D. Glass, J. L. Strominger, and M. B. Brenner. 1990. Evidence for extrathymic changes in the T cell receptor γ/δ repertoire. *J. Exp. Med. 171:1597.*
32. Grant, E. P., M. Degano, J. P. Rosat, S. Stenger, R. L. Modlin, I. A. Wilson, S. A. Porcelli, and M. B. Brenner. 1999. Molecular recognition of lipid antigens by T cell receptors. *J. Exp. Med. 189:195.*
33. Lee, C., and M. Levitt. 1991. Accurate prediction of the stability and activity effects of site-directed mutagenesis on a protein core. *Nature 352:448.*
34. Levitt, M. 1992. Accurate modeling of protein conformation by automatic segment matching. *J. Mol. Biol. 226:507.*
35. Kraulis, P. J. 1991. MOLSCRIPT: a program to produce both detailed and schematic plots of protein structures. *J. Appl. Crystallogr. 24:946.*
36. Merritt, E. A., and M. E. P. Murphy. 1994. Raster3D: photorealistic molecular graphics. *Acta Crystallogr. D 50:869.*
37. Morita, C. T., C. M. Parker, M. B. Brenner, and H. Band. 1994. T cell receptor usage and functional capabilities of human γδ T cells at birth. *J. Immunol. 153: 3979.*
38. Li, B., M. D. Rossman, T. Imir, A. F. Oner-Eyuboglu, C. W. Lee, R. Biancaniello, and S. R. Carding. 1996. Disease-specific changes in γδ T cell repertoire and function in patients with pulmonary tuberculosis. *J. Immunol. 157:4222.*
39. Allison, T. J., C. C. Winter, J. J. Fournie, M. Bonneville, and D. N. Garboczi. 2001. Structure of a human γδ T-cell antigen receptor. *Nature 411:820.*
40. Delfau, M.-H., A. J. Hance, D. Lecossier, E. Vilmer, and B. Grandchamp. 1992. Restricted diversity of Vγ9-JP rearrangements in unstimulated human γ/δ T lymphocytes. *Eur. J. Immunol. 22:2437.*
41. Bukowski, J. F., C. T. Morita, H. Band, and M. B. Brenner. 1998. Crucial role of TCRγ chain junctional region in prenyl pyrophosphate antigen recognition by γδ T cells. *J. Immunol. 161:286.*
42. Miyagawa, F., Y. Tanaka, S. Yamashita, B. Mikami, K. Danno, M. Uehara, and N. Minato. 2001. Essential contribution of germline-encoded lysine residues in Jγ1.2 segment to the recognition of nonpeptide antigens by human γδ T cells. *J. Immunol. 167:6773.*
43. Gan, Y. H., C. D. Pauza, and M. Malkovsky. 1995. γδ T cells in rhesus monkeys and their response to simian immunodeficiency virus (SIV) infection. *Clin. Exp. Immunol. 102:251.*
44. Levinson, G., A. L. Hughes, and N. L. Letvin. 1992. Sequence and diversity of rhesus monkey T-cell receptor β chain genes. *Immunogenetics 35:75.*
45. MacDougall, A. V., P. Enders, G. Hatfield, D. C. Pauza, and E. Rakasz. 2001. Vγ2 TCR repertoire overlap in different anatomical compartments of healthy, unrelated rhesus macaques. *J. Immunol. 166:2296.*
46. Daubenberger, C. A., M. Salomon, W. Vecino, B. Hubner, H. Troll, R. Rodriques, M. E. Patarroyo, and G. Pluschke. 2001. Functional and structural similarity of Vγ9Vδ2 T cells in humans and Aotus monkeys, a primate infection model for *Plasmodium falciparum* malaria. *J. Immunol. 167:6421.*
47. Davodeau, F., M. A. Peyrat, M. M. Hallet, I. Houde, H. Vie, and M. Bonneville. 1993. Peripheral selection of antigen receptor junctional features in a major human γδ subset. *Eur. J. Immunol. 23:804.*
48. Rakasz, E., A. V. MacDougall, M. T. Zayas, J. L. Helgelund, T. J. Ruckward, G. Hatfield, M. Dykhuizen, J. L. Mitchen, P. S. Evans, and C. D. Pauza. 2000. γδ T cell receptor repertoire in blood and colonic mucosa of rhesus macaques. *J. Med. Primatol. 29:387.*
49. Morita, C. T., W. L. Godfrey, J. W. Goodman, and G. K. Lewis. 1986. Arsonate-specific murine T cell clones. III. Correlation between clonotype expression and fine specificity for analogs of L-tyrosine-*p*-azobenzenearsonate. *J. Immunol. 137: 2139.*
50. Mauri-Hellweg, D., M. Zanni, E. Frei, F. Bettens, C. Brander, D. Mauri, E. Padovan, H. U. Weltzien, and W. J. Pichler. 1996. Cross-reactivity of T cell lines and clones to β-lactam antibiotics. *J. Immunol. 157:1071.*
51. von Bonin, A., B. Ortmann, S. Martin, and H. U. Weltzien. 1992. Peptide-conjugated hapten groups are the major antigenic determinants for trinitrophenyl-specific cytotoxic T cells. *Int. Immunol. 4:869.*
52. Sturm, E., R. E. Bontrop, R. J. Vreugdenhil, N. Otting, and R. L. Bolhuis. 1992. T-cell receptor γ/δ: comparison of gene configurations and function between humans and chimpanzees. *Immunogenetics 36:294.*
53. Hintz, M., A. Reichenberg, B. Altincicek, U. Bahr, R. M. Gschwind, A. K. Kollas, E. Beck, J. Wiesner, M. Eberl, and H. Jomaa. 2001. Identification of (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate as a major activator for human γδ T cells in *Escherichia coli*. *FEBS Lett. 509:317.*
54. Fearon, D. T., and R. M. Locksley. 1996. The instructive role of innate immunity in the acquired immune response. *Science 272:50.*
55. Janeway, C. A., Jr. 1998. The road less traveled by: the role of innate immunity in the adaptive immune response. *J. Immunol. 161:539.*
56. Lang, F., M. A. Peyrat, P. Constant, F. Davodeau, J. David-Ameline, Y. Poquet, H. Vié, J. J. Fournié, and M. Bonneville. 1995. Early activation of human Vγ9Vδ2 T cell broad cytotoxicity and TNF production by nonpeptidic mycobacterial ligands. *J. Immunol. 154:5986.*
57. Fisch, P., M. Malkovsky, S. Kovats, E. Sturm, E. Braakman, B. S. Klein, S. D. Voss, L. W. Morrissey, R. DeMars, W. J. Welch, et al. 1990. Recognition by human Vγ9/Vδ2 T cells of a GroEL homolog on Daudi Burkitt's lymphoma cells. *Science 250:1269.*
58. Selin, L. K., S. Stewart, C. Shen, H. Q. Mao, and J. A. Wilkins. 1992. Reactivity of γδ T cells induced by the tumour cell line RPMI 8226: functional heterogeneity of clonal populations and role of GroEL heat shock proteins. *Scand. J. Immunol. 36:107.*
59. Battistini, L., G. Borsellino, G. Sawicki, F. Poccia, M. Salvetti, G. Ristori, and C. F. Brosnan. 1997. Phenotypic and cytokine analysis of human peripheral blood γδ T cells expressing NK cell receptors. *J. Immunol. 159:3723.*
60. Carena, I., A. Shamshiev, A. Donda, M. Colonna, and G. De Libero. 1997. Major histocompatibility complex class I molecules modulate activation threshold and early signaling of T cell antigen receptor-γ/δ stimulated by nonpeptidic ligands. *J. Exp. Med. 186:1769.*
61. Das, H., V. Groh, C. Kuijl, M. Sugita, C. T. Morita, T. Spies, and J. F. Bukowski. 2001. MICA engagement by human Vγ2Vδ2 T cells enhances their antigen-dependent effector function. *Immunity 15:83.*
62. Poccia, F., L. Battistini, B. Cipriani, G. Mancino, F. Martini, M. L. Gougeon, and V. Colizzi. 1999. Phosphoantigen-reactive Vγ9Vδ2 T lymphocytes suppress in vitro human immunodeficiency virus type 1 replication by cell-released antiviral factors including CC chemokines. *J. Infect. Dis. 180:858.*
63. Lehner, T., E. Mitchell, L. Bergmeier, M. Singh, R. Spallek, M. Cranage, G. Hall, M. Dennis, F. Villinger, and Y. Wang. 2000. The role of γδ T cells in generating antiviral factors and β-chemokines in protection against mucosal simian immunodeficiency virus infection. *Eur. J. Immunol. 30:2245.*
64. Moore, T. A., B. B. Moore, M. W. Newstead, and T. J. Standiford. 2000. γδ-T cells are critical for survival and early proinflammatory cytokine gene expression during murine *Klebsiella pneumonia*. *J. Immunol. 165:2643.*
65. Ladel, C. H., C. Blum, A. Dreher, K. Reifenberg, and S. H. E. Kaufmann. 1995. Protective role of γ/δ T cells and α/β T cells in tuberculosis. *Eur. J. Immunol. 25:2877.*
66. Mombaerts, P., J. Arnoldi, F. Russ, S. Tonegawa, and S. H. E. Kaufmann. 1993. Different roles of αβ and γδ T cells in immunity against an intracellular bacterial pathogen. *Nature 365:53.*
67. King, D. P., D. M. Hyde, K. A. Jackson, D. M. Novosad, T. N. Ellis, L. Putney, M. Y. Stovall, L. S. Van Winkle, B. L. Beaman, and D. A. Ferrick. 1999. Protective response to pulmonary injury requires γδ T lymphocytes. *J. Immunol. 162:5033.*
68. Egan, P. J., and S. R. Carding. 2000. Downmodulation of the inflammatory response to bacterial infection by γδ T cells cytotoxic for activated macrophages. *J. Exp. Med. 191:2145.*
69. Skeen, M. J., E. P. Rix, M. M. Freeman, and H. K. Ziegler. 2001. Exaggerated proinflammatory and Th1 responses in the absence of γ/δ T cells after infection with *Listeria monocytogenes*. *Infect. Immun. 69:7213.*
70. Poccia, F., M. Malkovsky, A. Pollak, V. Colizzi, G. Sireci, A. Salerno, and F. Dieli. 1999. In vivo γδ T cell priming to mycobacterial antigens by primary *Mycobacterium tuberculosis* infection and exposure to nonpeptidic ligands. *Mol. Med. 5:471.*
71. Band, H., F. Hochstenbach, J. McLean, S. Hata, M. S. Krangel, and M. B. Brenner. 1987. Immunochemical proof that a novel rearranging gene encodes the T cell receptor δ subunit. *Science 238:682.*
72. Borst, J., J. J. M. van Dongen, R. L. H. Bolhuis, P. J. Peters, D. A. Hafler, E. de Vries, and R. J. van de Griend. 1988. Distinct molecular forms of human T cell receptor γ/δ detected on viable T cells by a monoclonal antibody. *J. Exp. Med. 167:1625.*
73. Davodeau, F., I. Houde, G. Boulot, F. Romagné, A. Necker, N. Canavo, M.-A. Peyrat, M.-M. Hallet, H. Vié, Y. Jacques, R. Mariuzza, and M. Bonneville. 1993. Secretion of disulfide-linked human T-cell receptor γδ heterodimers. *J. Biol. Chem. 268:15455.*
74. Ferrini, S., I. Prigione, C. Bottino, E. Ciccone, G. Tambussi, S. Mammoliti, L. Moretta, and A. Moretta. 1989. Monoclonal antibodies which react with the T cell receptor γ/δ recognize different subsets of $CD3^+WT31^-$ T lymphocytes. *Eur. J. Immunol. 19:57.*
75. Miossec, C., F. Faure, L. Ferradini, S. Roman-Roman, S. Jitsukawa, S. Ferrini, A. Moretta, F. Triebel, and T. Hercend. 1990. Further analysis of the T cell receptor γ/δ^+ peripheral lymphocyte subset: the Vδ1 gene segment is expressed with either Cα or Cδ. *J. Exp. Med. 171:1171.*

76. Wu, Y. J., W. T. Tian, R. M. Snider, C. Rittershaus, P. Rogers, L. LaManna, and S. H. Ip. 1988. Signal transduction of γ/δ T cell antigen receptor with a novel mitogenic anti-δ antibody. *J. Immunol. 141:1476.*

77. Porcelli, S., M. B. Brenner, and H. Band. 1991. Biology of the human γδ T-cell receptor. *Immunol. Rev. 120:137.*

78. Romagné, F., M. A. Peyrat, C. Leget, F. Davodeau, I. Houde, A. Necker, M. M. Hallet, H. Vié, and M. Bonneville. 1996. Structural analysis of γδ TCR using a novel set of TCR γ and δ chain-specific monoclonal antibodies generated against soluble γδ TCR: evidence for a specific conformation adopted by the Jδ2 region and for a Vδ1 polymorphism. *J. Immunol. Methods 189:25.*

79. Ciccone, E., S. Ferrini, C. Bottino, O. Viale, I. Prigione, G. Pantaleo, G. Tambussi, A. Moretta, and L. Moretta. 1988. A monoclonal antibody specific for a common determinant of the human T cell receptor γ/δ directly activates $CD3^+WT31^-$ lymphocytes to express their functional program(s). *J. Exp. Med. 168:1.*

80. Deusch, K., F. Luling, K. Reich, M. Classen, H. Wagner, and K. Pfeffer. 1991. A major fraction of human intraepithelial lymphocytes simultaneously expresses the γ/δ T cell receptor, the CD8 accessory molecule and preferentially uses the Vδ1 gene segment. *Eur. J. Immunol. 21:1053.*

81. De Libero, G., G. Casorati, C. Giachino, C. Carbonara, N. Migone, P. Matzinger, and A. Lanzavecchia. 1991. Selection by two powerful antigens may account for the presence of the major population of human peripheral γ/δ T cells. *J. Exp. Med. 173:1311.*

82. Kabelitz, D., T. Ackermann, T. Hinz, F. Davodeau, H. Band, M. Bonneville, O. Janssen, B. Arden, and S. Schondelmaier. 1994. A new monoclonal antibody (23D12) recognizing three different Vγ elements of the human γδ T cell receptor: $23D12^+$ cells comprise a major subpopulation of γδ T cells in postnatal thymus. *J. Immunol. 152:3128.*

83. Hinz, T., D. Wesch, F. Halary, S. Marx, A. Choudhary, B. Arden, O. Janssen, M. Bonneville, and D. Kabelitz. 1997. Identification of the complete expressed human TCR Vγ repertoire by flow cytometry. *Int. Immunol. 9:1065.*

84. Söderström, K., A. Bucht, E. Halapi, C. Lundqvist, A. Grönberg, E. Nilsson, D. L. M. Orsini, Y. van de Wal, F. Koning, M.-L. Hammarström, and R. Kiessling. 1994. High expression of Vγ8 is a shared feature of human γδ T cells in the epithelium of the gut and in the inflamed synovial tissue. *J. Immunol. 152:6017.*

85. Jitsukawa, S., F. Faure, M. Lipinski, F. Triebel, and T. Hercend. 1987. A novel subset of human lymphocytes with a T cell receptor-γ complex. *J. Exp. Med. 166:1192.*

86. Janssen, O., S. Wesselborg, B. Heckl-Östreicher, K. Pechhold, A. Bender, S. Schondelmaier, G. Moldenhauer, and D. Kabelitz. 1991. T cell receptor/CD3-signaling induces death by apoptosis in human T cell receptor $γδ^+$ T cells. *J. Immunol. 146:35.*