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WC1 Is a Hybrid γδ TCR Coreceptor and Pattern Recognition Receptor for Pathogenic Bacteria

Haoting Hsu,*† Chuang Chen,*† Ariel Nenninger,* Lauren Holz,* Cynthia L. Baldwin,*† and Janice C. Telfer*†

WC1 proteins are uniquely expressed on γδ T cells and belong to the scavenger receptor cysteine-rich (SRCR) superfamily. While present in variable, and sometimes high, numbers in the genomes of mammals and birds, in cattle there are 13 distinct genes (WC1-I to WC1-13). All bovine WC1 proteins can serve as coreceptors for the TCR in a tyrosine phosphorylation dependent manner, and some are required for the γδ T cell response to *Leptospira*. We hypothesized that individual WC1 receptors encode Ag specificity via coligation of bacteria with the γδ TCR. SRCR domain binding was directly correlated with γδ T cell response, as WC1-3 SRCR domains from *Leptospira*-responsive cells, but not WC1-4 SRCR domains from *Leptospira*-nonresponsive cells, bound to multiple serovars of two *Leptospira* species, *L. borgpetersenii*, and *L. interrogans*. Three to five of eleven WC1-3 SRCR domains, but none of the eleven WC1-4 SRCR domains, interacted with *Leptospira* spp. and *Borrelia burgdorferi*, but not with *Escherichia coli* or *Staphylococcus aureus*. Mutational analysis indicated that the active site for bacterial binding in one of the SRCR domains is composed of amino acids in three discontinuous regions. Recombinant WC1 SRCR domains with the ability to bind leptospires inhibited *Leptospira* growth. Our data suggest that WC1 gene arrays play a multifaceted role in the γδ T cell response to bacteria, including acting as hybrid pattern recognition receptors and TCR coreceptors, and they may function as antimicrobials. *The Journal of Immunology*, 2015, 194: 2280–2288.

Infection with Gram-negative (1, 2) or Gram-positive bacteria (3) is dealt with early and potently by γδ T cells. γδ T cells not only mediate effector functions via production of IFN-γ and TNF-α, but also show a memory response to *Leptospira* and *Mycobacteria* (4–7). Despite their important role in cellular immunity, it is still not well understood how γδ T cells are activated. The γδ TCR interacts variably and directly with ligand, unlike the αβ TCR, which interacts with peptide presented on MHC class I and II molecules (8). However, like the αβ TCR, the γδ TCR signals through the CD3 complex, which requires a ligand to be constrained in its movement (e.g., in a plasma membrane). In some cases, this occurs because the γδ TCR binds to cell surface nonclassical and MHC-related molecules (9–11). In another example, human Vγ2Vδ2 T cells are activated by prenyl pyrophosphate, which requires the interaction of the γδ T cell with other cells expressing the transmembrane receptor BTN3A1, which is modified by prenyl pyrophosphates (12–14). Unlike the αβ TCR, which interacts with variable peptide via the hypervariable recombination-determined CDR3s from both α and β TCR chains and the relatively conserved MHC molecule via the less variable germline-encoded CDR1s and CDR2s, the γδ TCR interacts with its ligands with the germline-encoded CDR1 and CDR2 or the CDR3, sometimes from only one TCR chain (9–11, 15).

Coreceptors are known to potentiate activation of T cells; for example, the αβ T cell CDS coreceptor and the αβ TCR both bind to MHC class I, with coreceptor dependence for T cell activation inversely correlated with TCR affinity for peptide plus MHC (16). We have previously shown that the γδ T cell specific WC1 transmembrane proteins, members of the ancient and conserved scavenger receptor cysteine rich (SRCR) receptor superfamily closely related to CD163 molecules (17), can act as coreceptors for γδ T cells. Ab-mediated crosslinking of WC1 with the TCR-CD3 complex potentiates T cell activation, whereas ligation of WC1 alone has no effect or is inhibitory (18, 19). This potentiation of TCR signaling requires the phosphorylation of a tyrosine in the cytoplasmic tails of WC1 (19, 20) and is modulated by an endocytic dileucine motif and membrane proximal serines (21). Following activation, WC1 colocalizes with the γδ TCR-CD3 complex in lipid rafts (22). Knockdown of some WC1 gene products significantly decreases γδ T cell responses to *Leptospira* (23), suggesting that individual WC1 molecules have the capacity to determine whether a restricted set of γ or δ TCR is activated by bacterial pathogens.

WC1+ γδ T cells are divided into subpopulations known as WC1.1+ and WC1.2+ based on expression of a specific set of WC1 genes (20). Each WC1 gene encodes an extracellular domain containing either 11 or 6 group B SRCR domains, a transmembrane domain, and a cytoplasmic domain. Each group B SRCR domain is characterized by six to eight cysteines with conserved spacing and disulfide bonds. The N-terminal SRCR a1 domain is the most variable between WC1 proteins. Expression of particular WC1 molecules correlates with responses to bacteria. Only WC1.1+ γδ T cells respond to *Leptospira* (24), whereas only WC1.2+ γδ T cells were isolated as *Anaplasm*-responsive (25) in recall responses following vaccination. The TCR is involved in Ag recognition in both cases because anti-TCR Abs block the responses.
Materials and Methods

Cell culture

HEK-293 cells were cultured in IMDM (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated FCS and 50 μg/mL gentamicin.

Bacteria culture

Escherichia coli (DH10B; Invitrogen) was cultured in Luria broth at 37˚C, 250 rpm. Borrelia burgdorferi 297 (gift of Dr. Juan Anguita, University of Virginia, Charlottesville, VA) was cultured in BS-K H-complete media at 30˚C (Sigma-Aldrich, St. Louis, MO). L. interrogans serovar Copenhageni, L. interrogans serovar Hardjo, and Leptospira borgpetersenii serovar Hardjo strains 818 and 1343 (National Veterinary Services Laboratory, Ames, IA) were cultured in enriched EMJH medium (Invitrogen, Carlsbad, CA) at 37˚C. Bacterial cultures were grown to the stationary phase before subculture, reaching a density of ~1 × 10^8 cells per mL. Leptospira spp. were grown with 1 nM recombinant WC1-3b2 or WC1-4b2 SRCR domains or the equivalent volume of PBS vehicle in enriched EMJH medium. Bacteria were counted under dark-field microscopy or stained with BacLight RedoxSensor CTC Vitality kit (Invitrogen) as directed. Bacteria analyzed by flow cytometry were counted with a microsphere standard (Invitrogen). Live Leptospira spp. were fixed with 8% formaldehyde at 37˚C for 1 h before the binding assay. E. coli DH10B used in the growth inhibition study were diluted 1:100 from an overnight culture to an OD of 0.02 in LB. Triplicate 400 μL cultures were incubated with 1 nM WC1-3b2 SRCR domain, an equivalent volume PBS, or 100 μg/mL ampicillin for 6 h. Cell count was determined by diluting samples of the culture 1:10 in LB, measuring the OD 600, and converting that via the algorithm 1.0 OD = 8 × 10^8 bacteria/mL.

Expression and purification of WC1 SRCR proteins

SRCR domains from WC1-3, WC1-13, and WC1-4 were cloned and transfected as described (47). Supernatant was collected at 24, 48, and 72 h after transfection and supplemented with 2 mM PMSF and 200 μM NTA beads (Qiagen, Gaithersburg, MD) per 10 mL supernatant. The beads were washed five times with wash buffer (50 mM NaH2PO4, 300 mM NaCl, 20 mM imidazole, 0.05% Tween 20 [pH 8.0]), and the expressed protein was eluted with 50 mM NaH2PO4 buffer containing 300 mM imidazole, 300 mM NaCl, and 10% glycerol. Purified protein was quantified by comparison with a standard curve of BSA resolved by SDS-PAGE and stained with Coomassie blue.

Bacterial binding assay and immunoblotting

Freshly grown cultures described above or bacteria L. borgpetersenii serovar Hardjo-bovis from Spirovac (Zoetis, Florham Park, NJ), L. interrogans serovar Hardjo-prajitno from Leptavoid (Schering Plough, Kenilworth, NJ), and Staphylococcus aureus (Pansorbin; EMD Millipore, Billerica, MA) were used. The concentration of killed bacterial cells was measured by OD at 600 nm based on the estimate that 1 OD unit = 5 × 10^9 cells per milliliter. Individual SRCR domains (5–10 ng, ~0.625–2.5 nM) were incubated with 5 × 10^5 bacteria in 400 μL of TBS-BSA buffer (20 mM Tris, 150 mM NaCl, and 5 mM CaCl2, plus 1% BSA), rotating at 4˚C for 1 h. The centrifuged bacterial pellet was washed three times with TBS-BSA or TBS buffer, suspended in SDS-PAGE sample buffer, and boiled for 5 min. After centrifugation at 16,000 × g, eluted proteins were resolved on 12–15% SDS-PAGE. WC1 SRCR domains were detected by hybridization with anti-Myc Ab (clone 9E10) at 4˚C overnight, followed by secondary HRP-conjugated goat anti-mouse Ab (Southern Biotech, Birmingham, AL) and detection by ECL Western blotting Detection reagents (GE Healthcare Life Sciences, Pittsburgh, PA).

Mutagenesis

Site-directed mutagenesis was carried out with the QuickChange II Site-Directed Mutagenesis Kit (Stratagene, Wilmington, DE, USA). WC1-13 SRCR a1 domain mutant 1 was generated by the forward primer 5'-GAGGGGAGAGTGGAAG-3' and its reverse complement; WC1-3 SRCR domain b2 mutant 1 was generated by the forward primer 5'-CCCTACACTTCCACTCTCGCAGTGTCATCTGGTGCAGAG-3' and its reverse complement; WC1-3 SRCR domain b2 mutant 2 by the forward primer 5'-CTTCTCAGAGTGGCTGCACAGTCCAGCG-3' and its reverse complement; WC1-3 SRCR domain b2 mutant 3 by the forward primer 5'-ACCACAGTGGGACGTCACAGGTTG-3' and its reverse complement.

Enzymatic treatment

Leptospira or Borrelia were treated at 2.5 × 10^5/mL with 50 μg/mL of proteinase K at 37˚C for 1 h followed by a wash and 5 mM PMSF at 37˚C for 0.5 h or 50 μg/mL polymyxin B at 37˚C for 1 h. Bacteria were fixed with 8% formaldehyde at 37˚C for 1 h.

Statistical analysis

Statistical analysis by two-tailed Student’s test (Gaussian distribution, equal SD) was performed using Prism 6 (GraphPad).

Results

WC1-3 SRCR domains, but not WC1-4 SRCR domains, directly bind to Leptospira

We have previously shown that knockdown of WC1-3, which is expressed by γδ T cells responding in recall responses to Leptospira following vaccination, diminishes the γδ T cell response to Leptospira (23). To address the hypothesis that γδ T cell responses
to Leptospira are determined by coligation of WC1 molecules with the TCR by pathogen-associated molecular patterns on Leptospira, recombinant WC1 SRCR domains were used in a bacterial binding and precipitation assay. The Leptospira-responsive γδ T cells are within the subpopulation defined by reactivity with mAb BAG25A and designated as WC1.1\(^+\) cells (24). BAG25A reacts with the N-terminal SRCR a1 domain in the extracellular domains of WC1-2, WC1-3, and WC1-11 (47). WC1 proteins have the SRCR organization a1-[b2-c3-d4-e5-d6]-[b7-c8-d9-e10-d’11], with equivalent alphabet letters clustering together in the same clade as those of related SRCR molecules across species. The unique SRCR a1 domain is diagnostic for WC1 in multiple species (17). WC1.1\(^+\) cells also express WC1-6, WC1-8, WC1-10, WC1-12, and WC1-13 (20). In contrast, mAb CACTB32A defines the WC1.2\(^+\) Leptospira-unresponsive subpopulation and reacts with the SRCR a1 domain of WC1-4 (47). The WC1 molecules expressed by WC1.1\(^+\) cells are highly similar regarding of whether they react with the defining mAb. For example, WC1.1-type WC1-3a1 and WC1-13a1 SRCR domains are 87.5% identical whereas, in contrast, WC1-3a1 SRCR domain is only 61.5% identical to the WC1.2-type WC1-4a1 SRCR domain (Fig. 1A). In this study, we compared bacterial binding of WC1.1-type WC1-3 and WC1-13 to WC1.2-type WC1-4.

Testing the entire 11-SRCR domain extracellular region of WC1-3 as well as the WC1-13a1 SRCR domain showed that they bind Leptospira but not Gram-negative E. coli or Gram-positive S. aureus (Fig. 1B, C). This was inhibited by 2 mM EDTA (Fig. 1C, lane 3), suggesting that Ca\(^{2+}\) contributes to binding. We next compared the Leptospira-binding capacity of the eleven SRCR domains from WC1-3 and WC1-4 to two species and serovars of Leptospira. Five SRCR domains of WC1-3 (a1, b2, d6, e10, and e11) weak binding to e5; Fig. 2A, 2B), but none of the SRCR domains from WC1-4 (Fig. 2C, 2D), bind strongly and reproducibly to L. borgdorferi serovar Hardjo-bovis (Fig. 2A, 2C) and L. interrogans serovar Hardjo-prajitno (Fig. 2B, 2D) from vaccine preparations. The induction of a memory response in WC1-3\(^+\) γδ T cells, but not WC1-4\(^+\) γδ T cells, by vaccination with these strains (24) is correlated with direct binding of multiple WC1-3, but not WC1-4, SRCR domains to Leptospira spp. from the vaccine preparations.

We also tested freshly grown Leptospira spp. for SRCR domain binding and found that three WC1-3 SRCR domains (a1, b2, and d6; Fig. 2E), but no WC1-4 SRCR domains (Fig. 2F), bound L. interrogans serovars Copenhageni and Hardjo. The more membrane proximal WC1-3 SRCR domains c8 and e10, which bound to Leptospira from vaccine, did not bind to freshly grown L. interrogans (Fig. 2E). We also tested the five SRCR domains bound by bacteria from the vaccine preparation of L. borgdorferi serovar Hardjo-bovis using freshly-grown strains 818 and 1343 and found that they bound the same three WC1-3 SRCR domains as freshly grown L. interrogans serovars Copenhageni and Hardjo. No WC1-4 SRCR domains bound to freshly grown L. borgdorferi (Fig. 2G, 2H).

Multiple WC1.1 type SRCR a1 domains bind to Leptospira spp.

Because the SRCR a1 domains are the most variable among WC1 SRCR domains (40), we tested all 13 WC1 SRCR a1 domains for bacterial binding. Consistent with the lack of proliferation and IFN-γ production by WC1.2\(^+\) γδ T cells to Leptospira spp. (24), the SRCR a1 domains of WC1-2-type molecules WC1-4, WC1-7, and WC1-9 did not bind to Leptospira spp. In contrast, the SRCR a1 domains of WC1.1 molecules WC1-6, WC1-8, and WC1-10 and the previously tested WC1-3 and WC1-13 SRCR a1 domains bound Leptospira spp. (Fig. 3A). Surprisingly, the SRCR a1 domains of WC1.1-type molecules WC1-1, WC1-2, WC1-5, WC1-11, and WC1-12 did not bind to Leptospira spp., despite their sequence similarity to SRCR a1 domains with binding activity (Fig. 3B).

Potential WC1 ligands

To examine whether the three WC1 SRCR domains that bind to freshly grown Leptospira spp. bind to proteins, we pretreated Leptospira spp. with proteinase K, washed them, and then incubated them with the protease inhibitor PMSF to protect the recombinant SRCR domain from residual protease K activity. Protein digestion was confirmed by SDS-PAGE analysis. Bacterial binding was not diminished with undigested bacteria, indicating that these SRCR domains do not bind to protein (Fig. 4, lane 2 versus lane 3). To test whether leptospiral LPS is the ligand for WC1 SRCR domains, we pretreated Leptospira spp. with polymyxin B, a molecule used to block LPS signaling through TLR, and tested the related bacteria Borrelia burgdorferi for binding. Although Borrelia and Leptospira are both members of the order Spirochaetales, B. burgdorferi does not have any LPS on its surface, because it lacks the enzymes required for its manufacture (48). The binding of WC1-13a1, WC1-3b2, or WC1-3d6 SRCR domains to L. borgdorferi is not blocked by polymyxin B (Fig. 4, lane 4). These SRCR domains bind to untreated and to proteinase K–treated B. burgdorferi, suggesting that the ligands are not proteinaceous or LPS (Fig. 4, lanes 6 and 7).

WC1 binding motif

DMBT1 SRCR domains bind to poly-phosphorylated or polysulfated molecules via a VEVLXXXXX motif similar to one in...
WC1, which is predicted to be located in an outside loop (23, 34). The VEVLXXXXW motif in DMBT1 binds to poly-phosphorylated ligands like LPS as a peptide (32, 34). We mutated VEVKHQGGEW in WC1-13a1 to VEAKYKGEW, the equivalent sequence in the nonbinding WC1-4a1 SRCR domain (Figs. 3, 5E). This mutation did not reduce WC1-13a1 SRCR domain binding to Leptospira spp., indicating that it does not bind to ligand in the same way as DMBT1 (Fig. 5A). This is consistent with our finding that several WC1a SRCR domains had the same sequence as WC1-13a1 (VEVKHQ GEW), but did not bind Leptospira spp. (Fig. 3). WC1a SRCR domains are the most variable of the WC1 SRCR domains, but it is not possible to identify any amino acids at a single position in Leptospira-binding WC1a1 SRCR domains that are different in all Leptospira-nonbinding WC1a1 SRCR domains. It is possible that the Leptospira-binding motif in WC1a1 SRCR domains is instead composed of multiple variable amino acids located upstream of the third cysteine, between the fourth and fifth cysteines and upstream of the eighth cysteine (Figs. 3B, 5E). WC1b2 and b7 SRCR domains are much more conserved than WC1a1 SRCR domains are, at 81–87% identity. We compared the sequence of the Leptospira-binding (WC1-3b2) to that of Leptospira-nonbinding SRCR b domains (WC1-3b7, WC1-4b2, and WC1-4b7; Figs. 2, 5E). There are only five amino acids in three areas that are unique to the binding WC1-3b2 domain; amino acids at the same positions in the nonbinding SRCR WC1-3b7, WC1-4b2, and WC1-4b7 domains have a shared identity. The equivalent five amino acids in WC1a1 SRCR domains do not show the same correlation (Fig. 3B, asterisks). We made four mutants in which we converted amino acids in the binding WC1-3b2 domain to those shared at the same position in the nonbinding SRCR b domains: mutation 1 (A33P/I36V), mutation 2 (S60D/A61G), mutation 3 (S95A), and mutation 2+3 (S60D/A61G/S95A; Fig. 5E). The mutated amino acids normally occur in the context of the nonbinding WC1 SRCR b domains (i.e., WC1-3b7, WC1-4b2, and WC1-4b7); therefore, it is unlikely that they compromise the overall conformation of the SRCR domain. Mutations 1, 2, and 3 all significantly diminished WC1-3b2 SRCR domain binding to Leptospira spp. (Fig. 5B, 5C), with the combined mutation 2+3 diminishing binding further (Fig. 5D). These mutations are located in loops, and an α helix at the edge of a hydrophobic core in the predicted structure (Fig. 5E). This indicates, that unlike the previously characterized linear bacterial binding motif of DMBT1, the WC1b2 SRCR domain contacts its bacterial ligand via non-continuous amino acids.

**SRCR domain inhibits Leptospira spp. growth in a binding-dependent manner**

The WC1 relative DMBT1 is a soluble molecule consisting of multiple SRCR domains that inhibits the cytoviaison of *Salmonella enterica* (49). WC1 transcripts are extensively alternatively spliced, including the splicing out of the exon encoding the transmembrane domain, which would be predicted to result in a soluble secreted molecule like DMBT1 (40). We observed toxic effects of some WC1 SRCR domains on *E. coli* during cloning, presumably from low-level expression inside the bacteria, and we investigated whether incubation with WC1 SRCR domains could affect the growth of Leptospira spp. *Leptospira* spp. were cultured in a single dose of 1 nM *Leptospira*-binding WC1-3b2, non-binding WC1-4b2, or an equivalent volume of PBS. Bacterial cell counts over a period of 6–14 d showed a statistically significant growth inhibition by the *Leptospira*-binding WC1-3b2, but not for its nonbinding counterpart WC1-4b2 or for the vehicle control PBS (Fig. 6A–D). Metabolically active bacteria detected by the redox dye CTC were even more reduced by a single treatment of WC1-3b2 relative to WC1-4b2, to 60-fold less at 7 d after the single-dose treatment (Fig. 6E). The local concentration of TCR, which is expressed at a level comparable to WC1, is 7 nM in a 10-μm region surrounding the cell (50). The local concentrations of secreted cytokines IL-2 and IFN-γ at 6 h postactivation are 23 and 12 nM, respectively (51). Thus, the 1 nM concentration used in these experiments is physiologically relevant to action over short
distances by transmembrane or soluble WC1. We also compared the growth kinetics of *E. coli* incubated with 1 nM recombinant WC1-3b2 SRCR domain to control *E. coli* cultures or *E. coli* incubated with 100 μg/ml ampicillin as a control for inhibition. WC1-3b2 SRCR domain does not bind to *E. coli* and incubation of *E. coli* with WC1-3b2 SRCR domain does not inhibit bacterial growth compared with control (Fig. 6F). Combined with the correlation of *Leptospira*-binding with the WC1b2 growth inhibition, this indicates that WC1 SRCR domain binding to the outside of the bacteria is necessary for growth inhibition.

## Discussion

Whereas we previously showed a correlation between the ability of a *Leptospira* vaccine to protect against infection with serovar Hardjo strains with its ability to engage WC1.1 + γδ T cell immune responses (24, 44), in this study we extend that correlation showing the WC1 molecules expressed by the responding γδ T cells bind *Leptospira* directly. This supports a model in which coligation of a WC1 coreceptor and the γδ TCR by bacterial ligands is required to reach a threshold for cellular activation. Five WC1-3 SRCR domains (a1, b2, d6, c8, and e10) bound to *L. interrogans* serovar Hardjo isolated from the protective commercial vaccine preparations, whereas no SRCR domains from the WC1 molecule found on nonresponsive cells (i.e., WC1-4) bound leptospires. Interestingly, only three of those five WC1-3 SRCR domains (a1, b2, and d6) bound to freshly grown *L. interrogans* serovars Copenhageni or Hardjo or *L. borgpetersenii* serovar Hardjo. This may be due to bacterial genetics, growth conditions, or processing differences. It remains to be determined whether the membrane proximal WC1-3 c8 and e10 SRCR domains binding *Leptospira* are critical to the
with that of the sequence of the mutant WC1-13a1 SRCR domain, with the variable amino acids underlined. SRCR domains (mut1, mut2, and mut3), with the variable amino acids underlined. The sequence of the wild type (WT) WC1-13a1 SRCR domain is aligned with the sequences of the mutated WC1-3b2 and WC1-4b2 SRCR domains (23). The sequence of the wild type (WT) WC1-3b2 SRCR domain is aligned with the sequences of the mutated WC1-3b2 and WC1-4b2 SRCR domains (23). The sequence of the wild type (WT) WC1-3b2 SRCR domain is aligned with the sequences of the mutated WC1-3b2 and WC1-4b2 SRCR domains (23).

Overall avidity of individual WC1 molecules for bacterial ligands in vivo, or to the development of a γδ T cell memory response following vaccination (46).

Treatment of Leptospira with various agents, in addition to the results of binding studies with other bacteria, led to the conclusion that the bacterial ligands are not protein, LTA, or LPS. Mutations of the linear bacteria-binding motif (VEVLXXXW) responsible for DMBT1 and CD163A binding to E. coli, S. aureus, and S. mutans (29, 34, 35), did not reduce binding of Leptospira to WC1-13a1, which is consistent with the lack of WC1 binding of E. coli and S. aureus. However, mutational analysis of five amino acids in the WC1b2 SRCR domain pinpointed critical amino acids and showed that the binding motif is noncontinuous. When mapped onto a predicted WC1 SRCR domain modeled on a CD5 SRCR domain crystal structure, these five amino acids are at the edge of a potential binding cavity with a hydrophobic core (23). These mutational studies predict that single nucleotide polymorphisms control bacterial binding by WC1 SRCR domains and helps explain why multiple closely related WC1 open reading frames have been maintained over millions of years of evolution. WC1-3 SRCR domains bound multiple species and strains of Leptospira, which could have a protective advantage over the serovar-specific Ab responses.

Of the 13 WC1 molecules in cattle, the most distal SRCR al domains from only five of them bound to Leptospira. This result suggests that the selective pressure maintaining the other eight WC1 reading frames derives from their binding to other pathogens, conveying a diverse pattern recognition response to the γδ TCR. Prime candidates for other pathogens bound by WC1 include bacteria that elicit a γδ T cell response, such as Anaplasma, Borrelia, Mycobacteria, and Salmonella (reviewed in Ref. 52). WC1 genes, often in multiple copy arrays, are present in most of the 13 WC1 molecules in cattle, the most distal SRCR al domains from only five of them bound to Leptospira. This result suggests that the selective pressure maintaining the other eight WC1 reading frames derives from their binding to other pathogens, conveying a diverse pattern recognition response to the γδ TCR. Prime candidates for other pathogens bound by WC1 include bacteria that elicit a γδ T cell response, such as Anaplasma, Borrelia, Mycobacteria, and Salmonella (reviewed in Ref. 52). WC1 genes, often in multiple copy arrays, are present in most of the 13 WC1 molecules in cattle, the most distal SRCR al domains from only five of them bound to Leptospira. This result suggests that the selective pressure maintaining the other eight WC1 reading frames derives from their binding to other pathogens, conveying a diverse pattern recognition response to the γδ TCR. Prime candidates for other pathogens bound by WC1 include bacteria that elicit a γδ T cell response, such as Anaplasma, Borrelia, Mycobacteria, and Salmonella (reviewed in Ref. 52). WC1 genes, often in multiple copy arrays, are present in most.
mammals and in birds, with the notable exception of mice and primates. However, CD163c-α/SCART proteins are the closest known homolog to WC1 (17, 53, 54). Human and bovine CD163c-α and murine SCART2 (one of two CD163c-α genes in mice) are expressed on γδ T cells (17, 54, 55), suggesting that they might have a role similar to that of WC1 in the immune response to pathogens.

WC1 transcripts are extensively alternatively spliced (40), which may be a mechanism to modulate ligation by bacteria. All the assays shown here used a 1 nM concentration of the individual SRCR domains, suggesting that the affinity of WC1 for its ligands is high, although this remains to be determined. A change in the number of SRCR domains in the extracellular region of WC1 molecules after splicing could affect avidity or the overall size of the extracellular domain and, as a consequence, affect WC1 localization in the immune synapse and WC1:TCR coligation. In addition, we have isolated multiple WC1 transcripts that have spliced out the transmembrane domain, which would be predicted to be secreted molecules (40). DMBT1 and Spα are secreted SRCR domain molecules, which aggregate bacteria and protect the host from bacterial invasion (reviewed in Ref. 56). Similarly, the WC1-3b2 SRCR domain significantly inhibited the growth of pathogens.

In conclusion, WC1 may function both as a cell-surface transmembrane receptor driving the activation of γδ T cells and as a soluble antimicrobial protein. Elucidation of WC1 and CD163c-α ligands could lead to vaccine designs that activate γδ T cell memory responses. It is also feasible that soluble WC1 SRCR domain molecules could be designed as therapeutics that interfere with bacterial growth or invasion.

Disclosures
The authors have no financial conflicts of interest.

References
14. Wang, H., O. Henry, M. D. Distefano, Y. C. Wang, J. Rääkkönen, J. M. Schornagel, R. P. Vloet, C. D. Dijkstra, and T. K. van den Berg. 2009. The molecular basis for the WC1-3b2 SRCR domain significantly inhibited the growth of Leptospira spp. in a bacteria-binding dependent manner. It will be of interest whether ligation by bacteria and cellular activation promotes splice variants, perhaps in a manner similar to affinity maturation of Abs.

In conclusion, WC1 may function both as a cell-surface transmembrane receptor driving the activation of γδ T cells and as a soluble antimicrobial protein. Elucidation of WC1 and CD163c-α ligands could lead to vaccine designs that activate γδ T cell memory responses. It is also feasible that soluble WC1 SRCR domain molecules could be designed as therapeutics that interfere with bacterial growth or invasion.


