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A New C-Type Lectin Similar to the Human Immunoreceptor DC-SIGN Mediates Symbiont Acquisition by a Marine Nematode†

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Although thiotrophic symbioses have been intensively studied for the last three decades, nothing is known about the molecular mechanisms of symbiont acquisition. We used the symbiosis between the marine nematode *Laxus oneistus* and sulfur-oxidizing bacteria to study this process. In this association a monolayer of symbionts covers the whole cuticle of the nematode, except its anterior-most region. Here, we identify a novel Ca^{2+}-dependent mannose-specific lectin that was exclusively secreted onto the posterior, bacterium-associated region of *L. oneistus* cuticle. A recombinant form of this lectin induced symbiont aggregation in seawater and was able to compete with the native lectin for symbiont binding in vivo. Surprisingly, the carbohydrate recognition domain of this mannose-binding protein was similar both structurally and functionally to a human dendritic cell-specific immunoreceptor. Our results provide a molecular link between bacterial symbionts and host-secreted mucus in a marine symbiosis and suggest conservation in the mechanisms of host-microbe interactions throughout the animal kingdom.

Stilbonematinae (Desmodoridae, Chromadoria) (27, 28) are especially abundant in tropical calcareous sands, where an oxidized surface layer overlies a reduced one. In the Belize Barrier Reef, two species, *Laxus oneistus* and *Stilbonema majum*, even dominate the nematode fauna of shallow sands. Stilbonematids repeatedly cross the boundary between oxidized and reduced sediment layers and thus represent an ideal substrate for bacteria that require both oxygen and sulfide. The worms, in turn, appear to obtain most of their nutrition by grazing on their symbionts (29).

Another peculiar feature of symbiotic marine nematodes is a conspicuous system of glandular sensory organs (GSOs) underlying their cuticle (see Fig. 2D). The GSOs produce a mucus envelope in which symbionts may be embedded. In each GSO the secretory granules accumulate in the canal and are released onto the cuticle surface through a hollow seta (18, 19).

There is no evidence of vertical transmission of the symbionts, but even very small juveniles carry a complete microbial coat. Colonization of recently hatched or molted stilbonematids must be a rapid process because field collections rarely yielded nonsymbiotic stilbonematids.

In *L. oneistus*, whose bacterial coat is composed of a single phylotype of rod-shaped γ-*Proteobacteria* (26, 31, 30), symbiont recruitment must be highly selective. The microbial coat starts with a sharp onset some distance behind the anterior end, and the bacterial rods are aligned perpendicularly to the worm's surface. The absence of symbionts on the anterior region does not correlate with fewer or smaller GSOs or with reduced mucus production.

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Incubation in D-mannose specifically led to symbiont detachment from nematodes belonging to the genus *Laxus* but not from *S. majum*. Furthermore, this monosaccharide was found on the surface of the symbionts, but not on *L. oneistus* cuticle (22). These data led to the hypothesis that *L. oneistus* binds its symbionts by secreting a mannose-binding protein(s) from their posterior GSOs onto their cuticle. We hypothesized that this putative mannose-binding protein belongs to a family of lectins whose carbohydrate recognition domain (CRD) may bind mannose in a Ca^{2+}-dependent manner and that is always extracellular (C-type lectins [CTLs]).

The present study sought *L. oneistus* CTLs in order to identify molecules that would mediate symbiont adhesion to the worm cuticle.

MATERIALS AND METHODS

Nematodes. *L. oneistus* and *S. majum* were collected at a 0.5-m depth from a back-reef sand bar at Carrie Bow Cay, Belize Barrier Reef, Caribbean Sea (25). The worms were extracted by shaking the sand and pouring the supernatant through a 63-μm-pore-size mesh screen. They were then picked by hand under a dissecting microscope and directly used for in vivo assays or stored deep-frozen in RNA*later* (Sigma) for mRNA extraction or in methanol for immunofluorescence experiments.

Homology cloning. mRNA was extracted with the QuickPrep Micro mRNA purification kit (Amersham Biosciences) from 500 *L. oneistus* and 500 *S. majum* individuals and ligated with an RNA Oligo at the 5′ (GeneRacer kit; Invitrogen), and cDNA was synthesized with the Ready-To-Go T-primed first-strand kit (Amersham Biosciences). A 375-bp cDNA fragment was amplified from *S. majum* cDNA by using the degenerate oligonucleotide primer against the WIGL motif, SB1A (5′-CAAAGCTTTGGGTXGGXTTX-3′), and the Ready-To-Go T-primed First-Strand Kit NotI-d(T)18 primer. The T_m for the first three PCR cycles was 45°C, and the T_m for the remaining 30 cycles was 57°C. Elongation was carried out for 90 s. The corresponding full-length *S. majum mermaid-3* clone was obtained by using the GeneRacer 5′ primer against the RNA Oligo and the SB27 primer in the 3′UTR (5′-CTAACAGTCACTGACTCTCAACGAATCC-3′). *L. oneistus mermaid-1*, *L. oneistus mermaid-2*, *S. majum mermaid-1*, and *S. majum mermaid-2* cDNA clones were obtained by using primer SB34 in the 5′ untranslated region (5′UTR; 5′-TTTTTTATTTCACAGCCATCGGTTTCC-3′)

and primer SB27 in the 3′UTR. *mermaid* mRNA sequences have been deposited at GenBank under the following accession numbers: *L. oneistus mermaid-1* (AY927369), *L. oneistus mermaid-2* (AY927370), *S. majum mermaid-1* (AY927371), *S. majum mermaid-2* (AY927372), and *S. majum mermaid-3* (AY927373).

Sequence analysis. Sequences were analyzed by pBLAST of the NCBI databases (http://www.ncbi.nlm.nih.gov/BLAST/), and alignments were created by using the CLUSTAL W facility in MacVector 7.2.3 (Accelerys, Inc.). Homology modeling was performed on SwissModel (http://www.swissmodel.expasy.org/), and the protein diagrams were made on Pymol. GenBank accession numbers of the aligned CTLs are as follows: *L. oneistus* Mermaid-1, AAX22004; CBG19888, CAE72672; AJL2, Q90WJ8; *H. sapiens* CLECSF6 (2), NP_919432; and *H. sapiens* DC-SIGN, AAK91855.

Expression and purification of recombinant mermaid. A PCR fragment corresponding to amino acids 20 to 161 of *S. majum* Mermaid-3 was NdeI/BamHI cloned into pET15b (Novagen). The resulting His-Mermaid fusion protein contained an N-terminal hexahistidine tag (His tag) and was expressed in the *Escherichia coli* strain BL21-AI (Invitrogen) essentially according to the manufacturer's instructions. Bacterial lysates containing His-Mermaid were applied to a nickel chelate resin (HisBind resin; Novagen) in binding buffer (8 M urea, 20 mM Na_2HPO_4-NaH_2PO_4, 0.5 M NaCl, 10 mM imidazole) and was eluted with the same buffer (except that the imidazole concentration was increased to 0.5 M). Eluted proteins were dialyzed against phosphate-buffered saline (PBS) containing decreasing urea concentrations to allow renaturation.

Antibodies and Western blots. According to standard procedures, we generated a rabbit polyclonal antibody against a peptide that is conserved in all Mermaid isoforms (amino acids 65 to 81), as well as a mouse polyclonal antibody against purified His-Mermaid.

Proteins were separated by reduced sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on NuPAGE 4 to 12% Bis-Tris precast gels (Invitrogen) and were transferred to Hybond ECL nitrocellulose membranes (Amersham Biosciences). Membranes were blocked 30 min in PBS containing 5% (wt/vol) nonfat milk at room temperature and probed overnight at 4°C with rabbit anti-peptide antibody (1:500), mouse anti-His-Mermaid antibody (1:100), or a monoclonal antibody anti-His tag (1:1,000; Amersham Biosciences) in PBS containing 5% nonfat milk, followed by a 30-min incubation at room temperature with a horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibody (1:4,000 and 1:2,000, respectively; Amersham Biosciences) in PBS containing 5% nonfat milk. Protein-antibody complexes were visualized by using ECL Plus detection reagents (Amersham Biosciences).

Immunofluorescence. *L. oneistus* individuals were fixed for 30 min in 8% (wt/vol) paraformaldehyde at room temperature, permeabilized by a 5-min incubation in methanol, and subsequently washed in methanol-acetone, acetone, and finally 0.1% Tween 20-PBS (washing solution) three times. Blocking of the worms was carried out for 1 h in 2% (wt/vol) bovine serum albumin and 0.1% Tween 20-PBS (blocking solution), and peptide antibody anti-Mermaid was diluted 1:500 in blocking solution and applied to the blocked worms overnight at 4°C under gentle agitation. Primary antibody was removed by three washes in washing solution, and Alexa 488-conjugated secondary antibody anti-rabbit (Molecular Probes) was then applied at a concentration of 1:500 in blocking solution for 1 h at room temperature. After three washes in washing solution, worms were mounted in Slow-Fade Antifade kit (Molecular Probes). All of the washes were carried out for 5 min. Images were recorded on a Leica TCS-NT confocal microscope.

Agglutination assays. A total of 500 freshly collected *L. oneistus* individuals were incubated in Ca^{2+}-free artificial seawater (see below), and dissociated symbionts were collected by 2 min of centrifugation at 6 krpm. Symbiont pellets were washed with and resuspended in 350 μl of filter-sterilized seawater. His-Mermaid sugar-binding sites were saturated with D-mannose prior to the assay by resuspending 0.5 μg of His-Mermaid in 1 M D-mannose–PBS, followed by incubation at room temperature for 30 min. This yielded 0.2 M D-mannose during the actual agglutination assay. His-Mermaid heat inactivation was performed at 95°C for 10 min, whereas His-Mermaid was immunologically inactivated prior to the assay by preincubation with a 100-fold-higher concentration of mouse antibody anti-His-Mermaid. Then, 0.5 μg of His-Mermaid, D-mannose preincubated His-Mermaid, TES70-His (11), and heat-inactivated, as well as immunologically inactivated, His-Mermaid were each added to separate 50-μl aliquots of *L. oneistus* symbionts. The experiment was performed in triplicate. Agglutination, which started in a few minutes, was carried out in tubes on the bench, and after 24 h the symbionts were gently transferred to a microtiter plate for photographic documentation.

Dissociation assays. To test the Ca^{2+} dependence of host-symbiont adhesion, three separate batches of *L. oneistus* individuals were incubated in Ca^{2+}-free artificial seawater, Mg^{2+}-free artificial seawater, and artificial seawater (400 mM NaCl, 20 mM $MgSO_4 \cdot 7H_2O$, 10 mM $CaCl_2 \cdot H_2O$, 1.7 mM KBr, 10 mM KCl, and 20 mM $MgCl_2 \cdot 6H_2O$). In both Ca^{2+}- and Mg^{2+}-free artificial seawater the concentration of NaCl was adjusted to avoid variations in osmolarity among the three different solutions. The assay was carried out at room temperature under gentle agitation, and the number of symbiont-free worms was scored after 42 h. The experiment was performed in triplicate, and the numbers of symbiont-free worms in the three assays were 10, 10, and 8. The mean percentage ± the standard error of symbiont-free worms was calculated on arc sin-transformed values.

TABLE 1. His-Mermaid blocks *E. coli* CS180 phagocytosis by HeLa-DC-SIGN but not by HeLa-CEACAM3[a]

E. coli strain	HeLa-DC-SIGN		HeLa-CEACAM3	
	I	II	I	II
pGEM	184	194	0	0
pGEM + 10 μg/ml His-Mermaid	10	9	0	0
pEXI	220	175	102	95
pEXI + 10 μg/ml His-Mermaid	12	22	121	98

[a] I, experiment number 1; II, experiment number 2. Values are expressed as numbers of phagocytized bacteria per well times 10^{-3}.

To investigate His-Mermaid induced symbiont dissociation, five separate batches of 50 freshly collected *L. oneistus* were placed in tubes, each containing 300 μl of filter-sterilized seawater. Then, 0.5 μg of His-Mermaid, D-mannose preincubated His-Mermaid, concanavalin agglutinin (ConA; Sigma), and TES70-His were added to each batch, respectively. The dissociation assay was carried out at room temperature under gentle agitation. His-Mermaid sugar binding sites were saturated with D-mannose as described above. The experiment was performed in triplicate, and worms were transferred to petri dishes for photographic documentation.

Culture cell lines and phagocytosis assays. *E. coli* K-12 strain CS180 contains the core lipopolysaccharide (LPS) but lacks O antigen (33). pGEM and pEXI are *E. coli* K-12 strains containing the empty vector pGEM3Z and expressing the phase-variable opacity protein I (OpaI), respectively (2). An immortalized Henrietta Lacks (HeLa) cell line stably expressing human dendritic-cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN) and a HeLa cell line expressing a transmembrane protein of the carcinoembryonic antigen family (CEACAM3; formerly known as CGM1a) were constructed by transfecting HeLa cells with the corresponding cDNAs and selected for surface antigen expression (21, 34, 17). HeLa-DC-SIGN cells were shown to phagocytize *E. coli* CS180 (10), whereas HeLa-CEACAM3 cells were shown to internalize pEXI (2). The phagocytosis assays were performed as described previously (10) four times for *E. coli* CS180 and two times each for pGEM and pEXI (indicated as I and II in Table 1).

RESULTS

Symbiont binding to the host is Ca^{2+} dependent. Because the activity of all known mannose-binding lectins is Ca^{2+} dependent, we tested whether incubation of living *L. oneistus* in Ca^{2+}-free seawater would cause symbiont dissociation. After 42 h, 97.6% ± 2.4% of these worms completely lost their bacterial coat, whereas both Mg^{2+}-free artificial seawater and artificial seawater, which contains Ca^{2+}, did not induce symbiont dissociation. This indicates that Ca^{2+} is indeed required for symbiont adhesion to the host.

Cloning of mermaid cDNA. We searched for CTLs by homology, using a set of degenerate primers against the highly conserved WIGL motif in the CRD of previously identified sugar-binding CTLs (37). Here, we identify two *L. oneistus* cDNAs, *mermaid-1* and *mermaid-2*, whose corresponding protein products differ only at amino acids 108, 109, and 151, depending on the occurrence of the amino acids Asp, Ala, and Asn or Asn, Tyr, and Ser, respectively, at these positions. The same protein variants are also present in *S. majum*, together

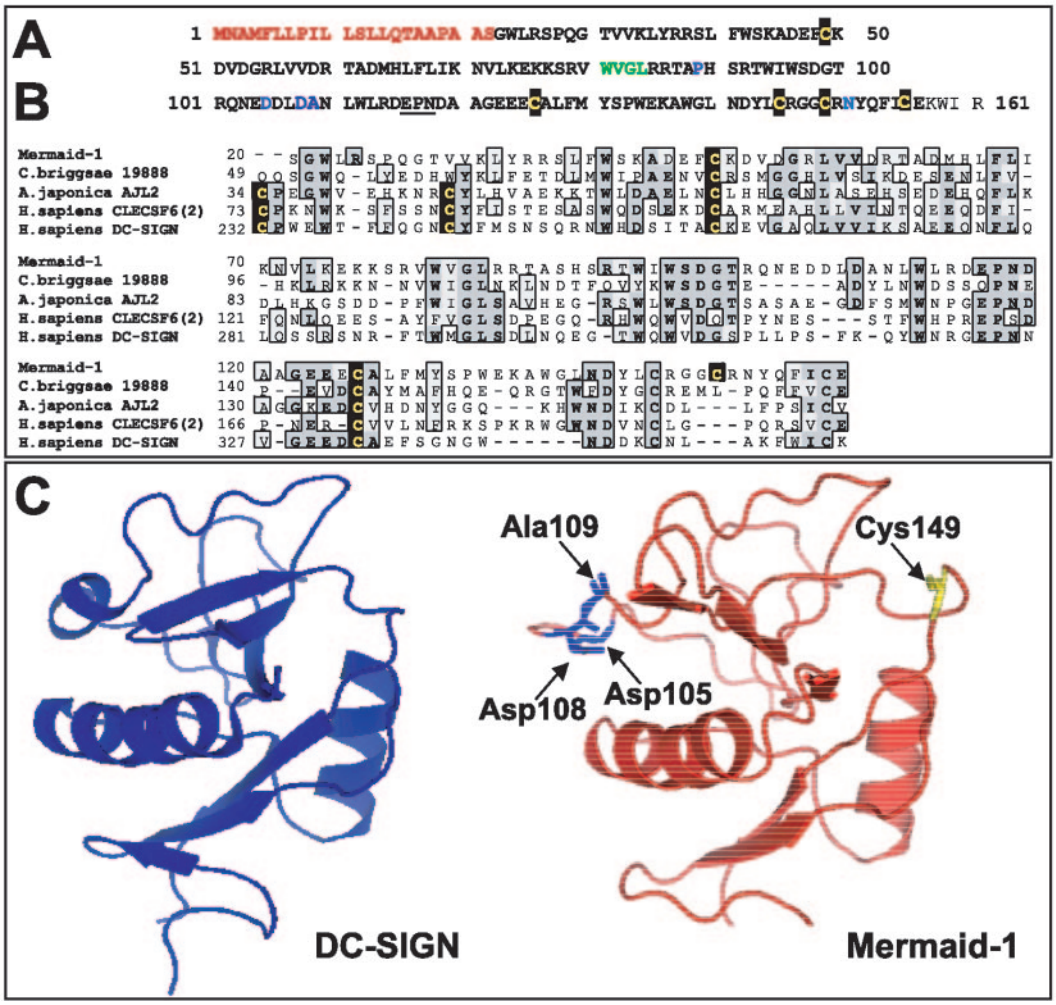

FIG. 1. Domains, alignment of CTL CRDs, and a three-dimensional model of Mermaid CRD. (A) Amino acid sequence of *L. oneistus* Mermaid-1 showing the signal peptide (red), the CTL CRD (black), and the WIGL motif (green). Cysteine residues are yellow on a black background, variable amino acids are blue, and the carbohydrate-binding motif is underlined. (B) Alignment of CTL CRDs. Cysteine residues are yellow on a black background. Residues that are identical between three or more of the selected CTLs are on a dark gray background; similar amino acids are on a light gray background. (C) X-ray crystal structure of DC-SIGN CRD (blue) and a model of Mermaid-1 CRD (red). The side chain of the supernumerary cysteine at position 149 is yellow, and the side chains of the variable amino acids at positions 105, 108, and 109 are blue.

with a third one, *S. majum* Mermaid-3, whose CRD differs from *L. oneistus* Mermaid-1 CRD at two more positions (a Ser instead of a Pro at position 89 and a Gly instead of an Asp at position 105). The name *mermaid* refers to the ability of the recombinant CTL to attract the bacterial symbionts in seawater (see below). *mermaid* cDNA is predicted to encode a 161-amino-acid protein, which includes a 22-amino-acid signal peptide for secretion (TargetP score of 0.888 [20]), and a CRD domain between positions 23 and 157 (NCBI Conserved Domain Search program [15], E-value = 6×10^{-11}). The predicted molecular mass of the protein, excluding the signal peptide and any posttranslational modifications, is 16.4 kDa. The presence of a Glu-Pro-Asn (EPN) motif in the CRD indicates specificity for mannose-containing carbohydrate structures (9) (Fig. 1A). In contrast to most CTLs, Mermaid has no potential N glycosylation site.

Database searches with the predicted peptide sequence revealed its closest match to be the *Anguilla japonica* divalent lectin AJL-2 (36) (33% [41 of 124] identity, 50% similarity), a homodimeric CTL purified from the skin mucus of the Japanese eel. Mermaid CRD also showed significant identity to human dendritic cell type II lectin-like immunoreceptors such as CLECSF6 (32) (31% [38 of 119] identity, 46% similarity) and DC-SIGN (16) (31% [34 of 108] identity, 45% similarity). Dendritic cells are localized in peripheral mucosal tissues throughout the human body, and they recognize specific carbohydrate structures on the cell wall of pathogens (1).

A three-dimensional model of Mermaid CRD was obtained by comparison with DC-SIGN CRD whose crystal structure has been resolved (5) (Fig. 1C). Based on this model the supernumerary cysteine at position 149 might allow polymerization. Moreover, the variable amino acid positions 105, 108,

and 109 are in an unconstrained loop, which would tolerate substitutions without functional change. Therefore, the different isoforms probably have the same overall conformation and sugar specificity.

Mermaid specifically localizes to the bacterium-associated region of *L. oneistus* cuticle. To test whether the bacteria distribution on the worm correlates with the CTL localization pattern, we generated a polyclonal rabbit peptide antibody and a polyclonal mouse anti-His-Mermaid antibody. Both antibodies specifically recognized a band of ~20 kDa in immunoblots of purified His-Mermaid, which has a predicted molecular mass of 19 kDa (Fig. 2A, lanes 2 and 5). This band corresponds to His-Mermaid because it migrates at the expected size, is absent after preincubation of the antibody with the peptide used for immunization (Fig. 2A, lane 1), and does not appear if the same blots are probed with a rabbit or a mouse preimmune serum (Fig. 2A, lanes 3 and 6, respectively). Since the peptide antibody gave a stronger signal than the antibody anti-His-Mermaid, we chose the former to analyze the CTL expression pattern.

In immunoblots of *L. oneistus* protein extracts, the peptide antibody specifically detected an ~28-kDa band (Fig. 2A, lanes 8 and 9), presumably corresponding to a Mermaid dimer resistant to dissociation into monomers upon boiling in SDS under reducing conditions.

When used in immunofluorescence experiments, the peptide antibody stained the symbiont monolayer and, in particular, the bacterial surface (Fig. 2B and C). The bacterium-free region of the *L. oneistus* cuticle, however, did not stain (Fig. 2B). At a higher magnification, the CTL appeared to accumulate in the canal of a posterior GSO and to be secreted through the corresponding seta (Fig. 2E and F). In contrast, the CTL was absent from the canal of anterior GSOs (Fig. 2G) and, accordingly, we could not detect any ~28-kDa band in the protein extracts of anterior moieties dissected off the worms (Fig. 2A, lane 7). Finally, all of the stainings described were not observed in worms probed with rabbit preimmune serum (data not shown).

The posterior GSO-specific expression pattern of Mermaid and its exclusive presence on the bacterium-coated region of the cuticle are consistent with a role in mediating symbiont attachment.

Recombinant Mermaid induces symbiont aggregation. Most lectins tend to be multivalent and may therefore clump cells together. We tested the ability of His-Mermaid to aggregate *L. oneistus* symbionts by so-called agglutination assays. Live symbionts were agglutinated by a concentration of recombinant CTL of at least 10 μg/ml in seawater (Fig. 3A), a phenomenon that could be strongly reduced by coincubation with 0.2 M D-mannose (Fig. 3B), as well as by heat inactivation (Fig. 3C) or preincubation with antibody anti-His-Mermaid (data not shown). TES70-His, a recombinant form of the *Toxocara canis* CTL TES70 (11) (Fig. 3D), caused minimal aggregation, whereas no agglutination at all occurred after treatment with 10 μg of Mermaid/ml in Ca^{2+}-free seawater (data not shown).

Taken together, these findings indicate that His-Mermaid forms multimers and that it mediates symbiont-symbiont adhesion through mannose-binding Ca^{2+}-dependent CRDs, whereas the His tag is apparently not responsible for this property.

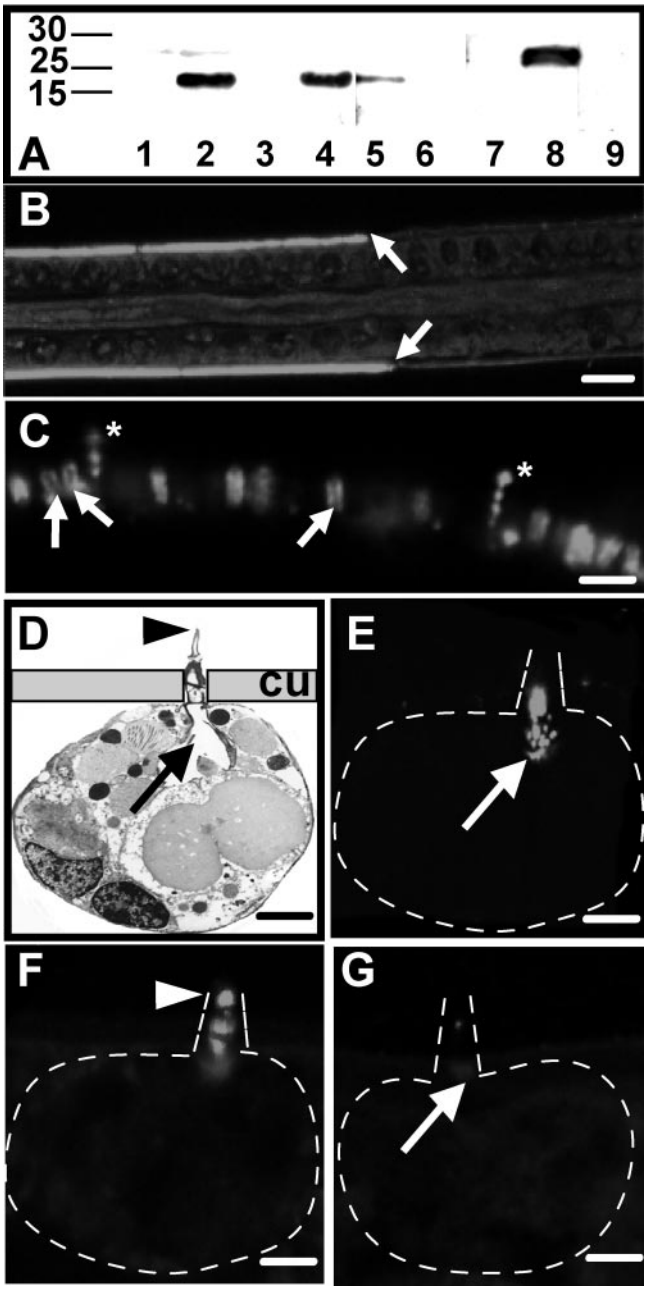

FIG. 2. Localization pattern of Mermaid. (A) A peptide antibody against Mermaid (lane 2), a mouse antibody against His-Mermaid (lane 5), and an anti-His-tag antibody (lane 4) all detect an ~20-kDa band on immunoblots of purified His-Mermaid. This band protein can be blocked by preincubation of peptide antibody with excess peptide (lane 1), and it is absent from blots of His-Mermaid probed with rabbit or mouse preimmune serum (lanes 3 and 6, respectively). (B to G) *L. oneistus* individuals were stained by immunofluorescence using the peptide antibody anti-Mermaid (green). (B to G) Optical longitudinal sections of *L. oneistus* (B), its symbiont coat (C), a posterior GSO (E and F, two sections taken at a 2-μm interval along the *z* axis), and an anterior GSO (G); a schematic adaptation of a TEM picture of a GSO (18) is also shown (D). cu, cuticle. In panels B and C, arrows point to the beginning of the bacterial coat and to single symbionts, respectively. Asterisks in panel C indicate two setae. In panels D to G, arrowheads points to the setae and arrows point to the canals. Scale bars: 20 μm in panel B, 2 μm in panels C to G.

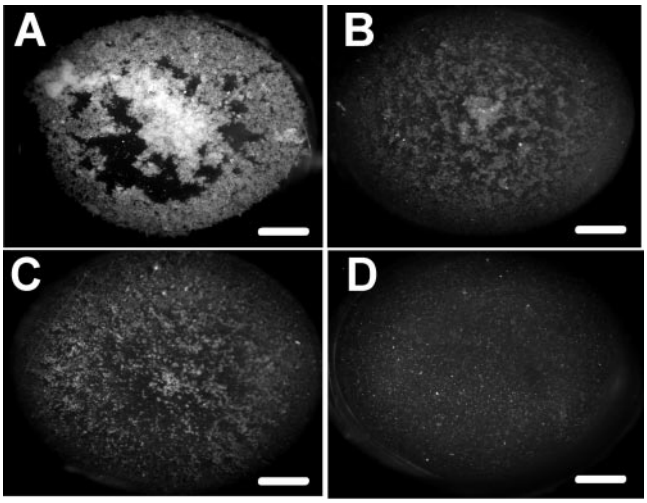

FIG. 3. His-Mermaid-induced symbiont agglutination. *L. oneistus* symbionts were incubated in seawater containing 10 μg of His-Mermaid/ml (A), 0.2 M D-mannose/10 μg of His-Mermaid/ml (B), and 10 μg of TES70-His/ml (D). (C) *L. oneistus* symbionts were incubated with 10 μg of heat-inactivated His-Mermaid/ml. Scale bar: 500 μm.

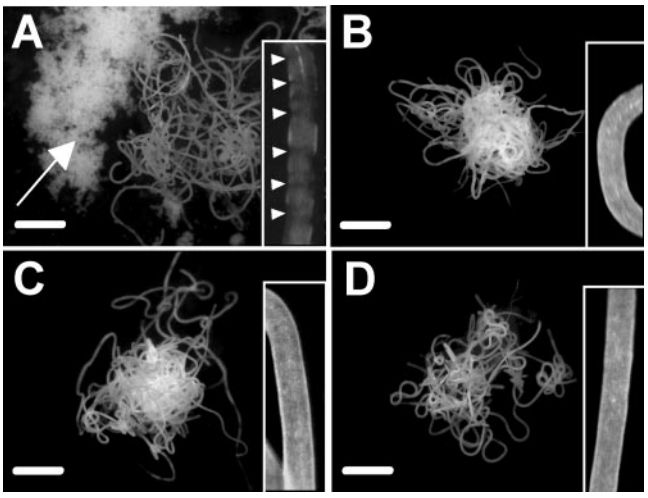

FIG. 4. His-Mermaid-induced host-symbiont dissociation. Symbiotic *L. oneistus* individuals were incubated in seawater containing 10 μg of His-Mermaid/ml (A), 10 μg of ConA/ml (B), 10 μg of TES70-His/ml (C), and 0.2 M D-mannose/10 μg of His-Mermaid/ml (D). Insets in panels A to D show higher-magnification images of the *L. oneistus* cuticle. Arrow and arrowheads in panel A point to dissociated symbionts and bacterium-free patches on the cuticle, respectively. Scale bar: 2 mm.

Recombinant Mermaid induces symbiont dissociation from the host in vivo. We also tested whether the recombinant CTL could compete with the native CTL for symbiont binding in vivo. For this purpose, batches of live *L. oneistus* were incubated in seawater containing His-Mermaid, TES70-His, ConA, or in seawater alone. ConA is a commercially available plant CTL with D-mannose and D-glucose specificity. After 1 h, bacterial symbionts dissociated from His-Mermaid incubated worms and formed large aggregates (>1 mm), so that symbiont-free patches were visible on the worm cuticle (Fig. 4A). In contrast, bacterial coats were not affected by ConA or TES70-His treatment (Fig. 4B and C) or by seawater alone (data not shown). Accordingly, His-Mermaid, but not other CTLs—whether native and with similar sugar specificity, such as ConA, or His tagged such as TES70-His—can compete specifically with the native Mermaid for symbiont binding in vivo. Worms treated with D-mannose-saturated His-Mermaid showed no bacterial dissociation after 1 h (Fig. 4D), further indicating that this CTL recognizes and binds mannose residues on the symbiont surface. After 48 h, however, symbiont-free patches appeared on the worms, although the detached bacteria did not form visible aggregates in the surrounding media (data not shown). This may reflect the presence of free D-mannose, which is known to cause symbiont detachment, although with a much slower dynamic (i.e., dissociation starts only after 16 h and goes to completion after 90 h (22).

Because His-Mermaid may compete with the native CTL for symbiont binding in vivo, we conclude that the presence of Mermaid in the mucus mediates symbiont attachment to the worm.

Mermaid and DC-SIGN share similar antigen specificity. A strain of immortalized tumor cells expressing human DC-SIGN (HeLa-DC-SIGN) was shown to bind and phagocytize a strain of *E. coli* lacking O antigen (CS180); accordingly, the core LPS of *E. coli* was found to be the ligand of human DC-SIGN (10, 38). Because of the similarity between Mermaid and DC-SIGN CRDs at the level of primary and tertiary structure, we investigated whether the recombinant worm CTL could compete with DC-SIGN for the binding to *E. coli* CS180 and, therefore, block phagocytosis of this bacterial strain by HeLa-DC-SIGN. We show that 10 μg of His-Mermaid/ml reduced the average number of phagocytized bacteria/well $\times 10^{-3}$ from 235.5 ± 39.7 (standard deviation) to 28 ± 6.8 ($n = 4$). Notably, only a 100-fold higher concentration of mannan could cause such a dramatic reduction (data not shown). His-Mermaid, however, could not prevent the phagocytosis of an *E. coli* CS180 strain expressing the OpaI protein (pEXI) by HeLa-CEACAM3 (Table 1). This indicates that the worm recombinant CTL blocks phagocytosis by specifically competing with DC-SIGN for the binding to *E. coli*, but not with other proteins on the surface of *E. coli* or HeLa cells.

We conclude that a marine nematode CTL and the human immunoreceptor DC-SIGN have similar sugar specificities and are able to recognize similar microbial antigens.

DISCUSSION

This study provides evidence that a soluble, secreted, mannose-specific CTL mediates symbiont acquisition from the environment by *L. oneistus*.

The significance of the CTL protein sequence polymorphism remains unclear. Based on homology modeling, all Mermaid isoforms are expected to share the same sugar specificity. Nevertheless, we cannot exclude that different isoforms, either alone or in combinations, display different affinities for the same sugar residues.

Although the predicted MW of secreted Mermaid is 16.4 kDa, this CTL migrated at ~28 kDa in Western blots of worm protein extracts separated under reducing conditions, probably due to dimerization. Alternatively, the CTL might form a sta-

ble, SDS- and dithiothreitol-resistant complex with large carbohydrates on the symbiont surface.

Mermaid is significantly similar to the Japanese eel AJL2, a divalent CTL that agglutinated *E. coli* and suppressed its growth. The agglutination of pathogens in the skin mucus by AJL2 might hinder their penetration into the eel body, allowing eels to wash them off by shedding new mucus (36). Although some parasitic nematode CTLs also have strong similarities with mammalian lectins (11–13), this has not been observed for free-living nematode CTLs other than Mermaid (4).

The posterior GSO-specific Mermaid expression pattern, resulting from either posterior specific gene transcription or anterior specific degradation of *mermaid* mRNA, contradicts a direct role of this CTL in *L. oneistus*'s response to pathogens. This contrasts with what has been observed with *Caenorhabditis elegans*, where increases in CTL expression in the gut have been observed upon infection (14). An indirect role of Mermaid in immune defense, however, cannot be excluded: a functional symbiotic coat appears to protect stilbonematids from being fouled by deleterious microbes.

Since we could not amplify *mermaid* gene fragments from symbiont genomic DNA by PCR with specific primers, symbiont staining by anti-peptide antibody cannot be attributed to bacterial endogenous expression (data not shown). We suggest, therefore, that once secreted onto the worm surface, CTL polymers bind mannose residues on the bacterial surface and are thereby retained on the worm rather than diffusing into the environment. This implies that, before symbiosis establishment, mucus must be viscous enough to prevent the diffusion of the CTL polymers out of the mucus layer.

The presence of Mermaid in the mucus overlying the posterior region of the nematode could facilitate the recruitment of specific symbionts from the environment by mediating their aggregation. In the association between the squid *Euprymna scolopes* and *Vibrio fischeri*, both gram-positive and gram-negative bacteria can induce shedding of host mucus during light organ development. Nevertheless, only gram-negative bacteria form aggregates therein, and only *V. fischeri* ultimately colonizes the mature light organ (23).

Recombinant Mermaid competed the symbionts off *L. oneistus* in vivo, indicating that bacteria are immobilized on the cuticle by CTL-containing mucus. The preferential adherence of microbes to host mucus is crucial in intestinal symbioses (35) and in the *E. scolopes* association, where *V. fischeri* must establish its dominance in the developing light organ. Although *N*-acetylneuraminic acid and *N*-acetylgalactosamine are present in squid exudates (24) and *V. fischeri* chemotaxis was shown toward *N*-acetylneuraminic acid (3), it remains unknown how these or other as-yet-unidentified molecules might mediate *V. fischeri* adhesion to the host mucus.

Although the recombinant CTL induced symbiont loss in all *L. oneistus* individuals, dissociation was not complete on any worm. Therefore, additional molecules might help establish this highly specific association. It also remains unclear whether D-mannose binds to Mermaid as an homopolymer or forms a specific motif with other sugar residues. The fact that ConA (a D-mannose-binding CTL) does not induce symbiont dissociation and that His-Mermaid induces it much faster than free D-mannose suggests that the latter is only part of the lock-and-key mechanism.

A better characterization of Mermaid sugar specificity, as well as of the LPS composition of both *L. oneistus* and *S. majum* symbionts, will help elucidate the role played by this *CTL* expression in the latter stilbonematid. Although *S. majum* symbionts belong to at least five different phylotypes of cocci only distantly related to *L. oneistus* symbionts, His-Mermaid induced their agglutination (K. Vanura and S. Bulgheresi, unpublished data). Therefore, we currently cannot exclude a CTL-mediated acquisition of bacteria other than *L. oneistus*-specific symbionts. In this case, the high sulfide concentration in *L. oneistus*'s microenvironment could still select *S. majum* symbionts, along with other "unwanted" microbes, off the surface of *L. oneistus*. In fact, symbiotic *L. oneistus* tolerates much higher concentrations of thiols than does *S. majum* (8).

In the shallow-water nematode *L. oneistus*, Mermaid is exclusively secreted onto the bacterium-associated moiety of the worm cuticle. Since this mannose-specific lectin aggregates and attaches the symbionts on the host surface, our results provide a missing link between host-secreted mucus and beneficial microbes in a marine environment.

The surprising ability of Mermaid to compete with human DC-SIGN in binding microbial antigens further confirms how blurred the border between symbiosis and pathogenesis may be. Since pathogens such as *Mycobacterium tuberculosis* and human immunodeficiency virus type 1 may subvert several functions of dendritic cells by interacting with DC-SIGN (6, 7), it will be very exciting to test the ability of His-Mermaid to block these interactions.

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