Emergence and Disappearance of an Immune Molecule, an Antimicrobial Lectin, in Basal Metazoa

A TACHYLECTIN-RELATED PROTEIN IN THE SPONGE SUBERITES DOMUNCULA

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Sponges (phylum Porifera) represent the evolutionarily oldest metazoans that comprise already a complex immune system and are related to the crown taxa of the protostomians and the deuterostomians. Here, we demonstrate the existence of a tachylectin-related protein in the demosponge Suberites domuncula, termed Suberites lectin. The MAPK pathway was activated in response to lipopolysaccharide treatment of the three-dimensional cell aggregates, the primmorphs; this process was abolished by the monosaccharide α-D-GlcNAc. The cDNA encoding the S. domuncula lectin was identified and cloned; it comprises 238 amino acids (26 kDa) in the open reading frame. The deduced protein has one putative transmembrane region, three characteristic Cys residues, and six internal tandem repeats; it shares the highest sequence similarity with lectins from the horseshoe crab Tachypleus tridentatus. The steady-state level of expression of the Suberites lectin rises in primmorphs in response to lipopolysaccharide, an effect that was prevented by co-incubation with α-D-GlcNAc. The natural sponge lectin was purified by affinity chromatography; it has a size of 27 kDa and displays antibacterial activity against the Gram-negative bacteria Escherichia coli and the Gram-positive bacteria Staphylococcus aureus. The putative protein, deduced from the cloned gene, is identical/similar to the purified natural protein, as demonstrated by immunological cross-reactivity with specific antibodies. We conclude that the S. domuncula lectin acts as an antibacterial molecule involved in immune defense against bacterial invaders.

Sponges have developed several strategies to cope with these threats. First, they possess an efficient chemical defense system to inhibit bacterial growth (see Ref. 4). Triggered by ecological constraints, Porifera developed a large variety of biologically highly active compounds (5) that were shaped during the last 1 billion years for optimal specificity and activity. Second, sponges are provided with efficient humoral and cellular defense/immune mechanisms that share, at the sequence level, surprisingly high similarity with the immune molecules found in humans (6). Third, a mechanism in these animals eliminates microorganisms by engulfing them through phagocytosis (reviewed in Ref. 7). An early illustration and functional analysis of phagocytosis in sponges were given by Metchnikoff (8). Molecular data indicate that receptors present on the cell surface might have the capacity to discriminate between self-self and non-self (6); examples are receptors that comprise both SrcR (scavenger receptor comprising cysteine-rich) group A domains (9) and/or SrcR group B domains (10). In addition, sponges are very rich in lysosomal enzymes, e.g. cathepsins (11).

Lately, our research has focused on those molecules that are involved in the recognition of bacteria and in the intracellular signaling pathway(s) activated after interaction of sponge cells with microorganisms. The search for the kinase pathways that are activated in response to bacterial infection was already successful. It has been demonstrated that, after bacterial infections or after addition of the model molecule for bacterial infection (lipopolysaccharide (LPS)1), the phosphorylation of the two MAPKs p38 and JNK strongly increases (12). Hence, it can be deduced that sponges react upon ligation to LPS/bacteria with activation of p38 and JNK MAPKs.

One goal of this study was to identify the potential receptor for bacteria on the surface of the sponge cells. The model system for bacterial infection in sponge, LPS-induced activation of the p38 kinase in primmorphs, was used. For this study, the demosponge Suberites domuncula was selected because a cell culture system has been established in this species (13, 14). Primmorphs are a special form of three-dimensional cell aggregates that contain both proliferating and differentiating cells. The spherical aggregates comprise, in the center, non-differentiated archaeocytes and, on the surface, the pinacoderm, a layer of differentiated cells, the pinacocytes. Based on the functional analogy to the host defense lectins from the horseshoe crab Tachypleus tridentatus, we screened for a related molecule

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in S. domuncula. In T. tridentatus, a series of non-self-recognizing lectins have been described that were termed tachylectins or Tachypleus plasma lectins (see Ref. 15). A related molecule was cloned from the sponge cDNA library. The deduced protein shares high sequence similarity with the crab polypeptides, especially with the galactose-binding protein (GBP) from T. tridentatus (16); the S. domuncula molecule was named LEC_SUBDO. Based on the functional studies described here, we proposed that the S. domuncula lectin is also involved in non-self recognition.

MATERIALS AND METHODS

Chemicals and Enzymes—The sources of chemicals and enzymes used were given previously (17, 18). LPS from Escherichia coli O55:B5 and the sugars were from Sigma (Deisenhofen, Germany). The antibody directed against phospho-p38 MAPK (which recognizes the phosphorylated Thr180 and Tyr182 residues) and that against p38 MAPK (which recognizes the total p38 kinase) were obtained from New England Biolabs Inc. (Beverly, MA).

Sponges and Primmorphs—Live specimens of S. domuncula (Porifera, Demospongiae, Hadromeridae) were collected near Rovinj, Croatia. The sponges were kept in Mainz, Germany, for >4 months prior to the experiments. Where indicated, specimens were analyzed immediately after collection.

The procedure for the formation of primmorphs from single cells was applied as described previously (13, 14). Starting from single cells, primmorphs of 3–7 nm are formed after 5 days. For the experiments, 7-day-old primmorphs were used. They were cultivated in natural seawater supplemented with 0.2% RPMI 1640 medium.

Competition Experiments with LPS and GalNAc—The primmorphs were treated with 10 μg/ml LPS for 12 h in the absence or presence of monosaccharides. After incubation, the primmorphs were immediately processed for Western blot analysis.

Samples were homogenized in lysis buffer (1× Tris-buffered saline (pH 7.5), 1 mM EDTA, 1% Nonidet P-40, 10 mM NaF, protease inhibitor mixture (one tablet/10 ml; Roche Applied Science, Mannheim, Germany), and 1 mM sodium orthovanadate) and centrifuged, and the supernatants were subjected to SDS-PAGE. The protein concentration in the samples was determined (19) and adjusted to 2 mg/ml. Total cell extracts (10 μg/lane) were subjected to electrophoresis on 12% polyacrylamide gels containing 0.1% SDS according to Laemmli (27, 28).

Preparation and Expression Studies in Intact Specimens—RNA was extracted from liquid nitrogen-pulverized primmorphs with TRIzol reagent (Invitrogen). Reverse transcription-PCR was then performed as follows using commercial components (Invitrogen). cDNA was prepared using 10× PCR buffer, MgCl₂, dNTP mixture, dithiothreitol, SuperScript reverse transcriptase, and oligo(dT) as described in the Invitrogen protocol. RNA was digested with RNaseA, and a cDNA segment was amplified (initial denaturation at 95 °C for 3 min, followed by 35 amplification cycles at 95 °C for 30 s, 54 °C for 45 s, and 61 °C for 1.5 min and a final extension step at 74 °C for 10 min) with the following two primer pairs: first, with fe1 (5′-GGCGAATTTCAACAGATAAGC-3′, nucleotides 63–84) and re1 (5′-TATTCACATGTAGCTGCCTCAG-3′, nucleotides 853 to 829); second, with fe2 (5′-GTTCTGCCGACACTGGAATGT-3′, nucleotides 163–182) and re2 (5′-GGCCCTCATCCTGTCACCCATT-3′, nucleotides 649–627). The size of the product obtained with primers fe1 and re1 was 791 bp, and the size of the product obtained with primers fe2 and re2 was 487 bp. The products were size-separated on agarose, followed by staining with ethidium bromide.
20 mM EDTA, and 100 mM n-GlcNAc. Fractions of 2 ml were collected, and the pooled fractions (fractions 66–68) were dialyzed against Ca^{2+}- and Mg^{2+}-free artificial seawater.

**Antibacterial Activity**—The assay was performed as described (30). *E. coli* strain BL21 was grown in LB broth overnight. 20 µl of bacterial suspension was added to 80 µl of lecin (either the recombinant or the natural protein) and incubated for 60 min at room temperature. The lectin samples were dialyzed against Ca^{2+}- and Mg^{2+}-free artificial seawater. Antibacterial activity was assayed, and the reduction of colony-forming units was determined after serial dilution of the assay samples and overnight incubation. The percent inhibition was calculated as follows: (number of control and test colonies/number of control colonies) × 100 (n = 5). In one series of experiments, the lectin samples (100 µg/ml) were preincubated with LPS (10 µg/ml) for 12 h at 4 °C and then applied.

**Antibodies against LEC_SUBDO**—Polyclonal antibodies were raised against the recombinant lectin in New Zealand White female rabbits as described (31). In 4-weeks intervals, three boosts (10 µg of protein each) were injected. The serum was collected, and the polyclonal antibodies were termed pAb-LEC_SUBDO. In control experiments, 100 µl of pAb-LEC_SUBDO was adsorbed to 20 µg of recombinant lectin (30 min, 4 °C) prior to use.

**Inhibition of LEC_SUBDO-LPS Interaction Using an Enzyme-linked Immunosorbent Assay System**—Enzyme-linked immunosorbent assays were performed at 20 °C as described (32). 96-well polystyrene plates were coated with LPS (10 µg/ml) and incubated for 5 h. Excess protein-binding sites on the microtiter plates were blocked with 3% bovine serum albumin and washed three times. The wells were incubated with increasing concentrations of n-GlcNAc together with 10 µg/ml natural *S. domuncula* LEC_SUBDO. After an additional wash, the wells were incubated with pAb-LEC_SUBDO (1:500 dilution) for 30 min. The immunocomplexes were visualized using horseradish peroxidase-coupled secondary antibodies (1:1000) under application of o-phenylenediamine as substrate. The plates were read at 492 nm. The minimal inhibitory concentration of n-GlcNAc was determined.

**Western Blotting**—Gel electrophoresis of the protein extracts was performed on 10 or 15% polyacrylamide gels containing 0.1% SDS according to Laemmli (28). Protein samples were subjected to gel electrophoresis in the presence of 2-mercaptoethanol and stained with Coomassie Brilliant Blue. Semidry electrotransfer was performed according to Kyhse-Andersen (33) onto Immobilon membranes (polyvinylidene difluoride). Membranes were processed (34) and incubated with pAb-LEC_SUBDO (1:500 dilution) for 30 min. The immunocomplexes were visualized with a peroxidase-coupled secondary antibody, followed by the chemiluminescence procedure. The immunocomplexes were visualized with a peroxidase-coupled secondary antibody, followed by the chemiluminescence procedure.

**RESULTS**

**Competitive Effect of n-GlcNAc on p38 Kinase Phosphorylation**—In a previous study, it was documented that the p38 kinase undergoes phosphorylation after incubation of sponge cells (in the primmorph system) in the presence of LPS (12). Consequently, the primmorphs were incubated also in this study in the absence or presence of 10 µg/ml LPS. Where indicated, the following monosaccharides were added to the assays at 10 µg/ml: n-Gal, n-GalNAc, and n-GlcNAc. The extract was prepared, and equal amounts were separated by SDS-PAGE. The proteins were blotted-transferred and reacted either with anti-p38 MAPK antibody (p38) or anti-phospho-p38 MAPK antibody (pp38). The immunocomplexes were visualized with a peroxidase-coupled secondary antibody, followed by the chemiluminescence procedure.

**Cloning of the cDNA Encoding the S. domuncula Lectin**—The *S. domuncula* lectin protein (LEC_SUBDO) was obtained from the cDNA library by PCR cloning as described under “Materials and Methods.” The cDNA obtained (SDLEC) comprises an open reading frame that ranges from nucleotides 15/17 to 729/731 (stop codon). The deduced protein, termed LEC_SUBDO, comprises 238 amino acids residues with a calculated size of 25,908 Da. A potential transmembrane region was identified (35) ranging from amino acids 1 to 18 (Fig. 2A).

Three disulfide linkages have been assigned for the most closely related protein, *T. tridentatus* lectin L6 (36). The respective Cys residues exist in the sponge molecule (LEC_SUBDO) at identical positions; the first disulfide bridge is located between Cys^{49} and Cys^{53}, the second between Cys^{124} and Cys^{128}, and the third between Cys^{200} and Cys^{204}. As for *T. tridentatus* lectin L6, the characteristic feature of the six internal tandem repeats (36) is also found in the sponge molecule. They range in *S. domuncula* LEC_SUBDO from amino acids 19 to 55 (repeat 1), 56 to 90 (repeat 2), 91 to 129 (repeat 3), 130 to 166 (repeat 4), 167 to 205 (repeat 5), and 206 to 236 (repeat 6) (Fig. 2A).

**Phylogenetic Analysis**—The sponge LEC_SUBDO polypeptide shares the highest sequence similarity with the lectins from the horseshoe crab *T. tridentatus*. These lectins (tachylectin-P, lectin L6, and tachylectin-1) show ~50% amino acid identity and 65% amino acid similarity (with respect to the physicochemical properties) to sponge LEC_SUBDO. The similarity is lower to the deuterostomian sequences from the fish *Carassius auratus* putative membrane protein and the potential GBP from *Danio rerio* (22% identity and 40% similarity) as well as to the two tectonins from the Mycetozoa *Physarum polycephalum* (20% identity and 38% similarity). There is no considerable similarity to the protostomian/nematode putative proteins in the data base.

This relationship is also reflected by the phylogenetic tree, which was rooted with one predicted open reading frame from *Saccharomyces cerevisiae*. This tree shows that the *S. domuncula* protein clusters with the tachylectins (see Ref. 37) in one branch, from which the vertebrate lectins (38) and the tectonin sequences (39) are separated (Fig. 2B). The tachylectins are located extracellularly (37), whereas the *P. polycephalum* tec-
tonins are present in the nucleus (39). No (potential) function is known for the vertebrate molecules (38).

Hydropathicity plot analysis was performed to narrow down the relationship of the molecules mentioned above. The plots show that, in all proteins from metazoans, one N-terminal transmembrane region could be predicted according to the described method (35). In the *S. domuncula* sequence, the transmembrane helix was predicted to be between amino acids 1 and 18 (Figs. 2A and 3); in the *C. auratus* sequence, this region is found at amino acids 2–21; and in the GBP from *T. tridentatus*, it is between amino acids 1 and 24. In contrast, such a region is absent in *P. polycephalum* tectonin-1 (Fig. 3). The metazoan molecules comprise the six characteristic hydrophilic regions, which correspond to the six tandem repeats; they are absent in tectonin.

Recombinant LEC_SUBDO—Starting with the cDNA encoding sponges LEC_SUBDO, the recombinant protein was synthesized from amino acid 19 to the end of the deduced protein at amino acid 238; hence, the transmembrane segment was omitted. The fusion protein was obtained in *E. coli* BL21 and contained, in addition to the sponge protein (24,059 Da), the bacterial GST tag (26 kDa), the oligohistidine tag (0.9 kDa), and the S tag (S-peptide fragment of RNase A) (1.8 kDa) (Fig. 4, lanes a and b). The fusion protein (termed recombinant LEC_SUBDO) was extracted and purified sequentially using the His tag and subsequently the GST tag affinity purification procedure; the apparent size was ~60 kDa (Fig. 4, lanes b and c). The fusion protein was cleaved with the enterokinase, and the recombinant LEC_SUBDO molecule (tag-free, 24 kDa) was purified as described under “Materials and Methods” (data not shown).
Gene Expression of the Lectin in Different Specimens—To clarify whether different specimens express the SDLEC gene, *S. domuncula* specimens were collected from different areas around Rovinj at distances of 15–20 km. RNA was prepared immediately after collection and subjected to reverse transcription-PCR. As described under “Materials and Methods,” two primer sets were applied: primers fe1 and re1, resulting in 791-bp products; and primers fe2 and re2, resulting in 487-bp products. The reactions were performed with 10 specimens; all of them expressed the SDLEC gene as seen by the formation of the two PCR products; the expression patterns from animals 1–6 are shown in Fig. 5.

Induction of the Lectin Gene in Response to LPS—To determine whether the steady-state level of expression of SDLEC changes in response to LPS, primmorphs were incubated with the endotoxin. The cells that were used for the preparation of the primmorphs came from animals that had been kept in an aquarium for 5 months. From such animals, it is known that they are exposed to only a minute level of bacteria in the artificial seawater. The protein patterns from SDS-PAGE analysis of the crude extracts from *S. domuncula* and of the purified fraction are shown (Fig. 8A, lanes a and b).

Antibacterial Activity—The antibacterial activity of sponge morphs were taken at time 0 and after 24 or 36 h of incubation. In one series of experiments, the assay with LPS contained the competitor sugar D-GlcNAc. It is obvious that, in the absence of LPS, the expression of the lectin gene remained at a very low level (Fig. 6, upper panel). In contrast, if LPS was added, a strong increase in expression was seen, which amounted to severalfold after an incubation period of 36 h (Fig. 6, middle panel). This strong induction caused by LPS could be reduced if the primmorphs were co-incubated with LPS and D-GlcNAc; under such conditions, only a low steady-state level of expression of the lectin gene was seen (Fig. 6, lower panel).

Purification of LEC_SUBDO from Sponge Tissue—The tissue was homogenized in buffer A, and the supernatant (1.5 mg/ml protein, 550 ml; Fraction I) was fractionated by ammonium sulfate precipitation as described under “Materials and Methods.” The precipitate (after 0.70 saturation) was dissolved (6.5 mg/ml protein, 30 ml; Fraction II) and subjected to affinity chromatography using an LPS-Sepharose CL-4B column (Fig. 7). After adsorption of the protein with buffer B, the lectin was eluted with buffer C. Fractions 66–68 were pooled (total of 7.5 mg; Fraction III) and dialyzed against Ca2+- and Mg2+-free artificial seawater. The protein patterns from SDS-PAGE analysis of the crude extracts from *S. domuncula* and of the purified fraction are shown (Fig. 8A, lanes a and b).

Fig. 3. Hydropathicity plots of *S. domuncula* LEC_SUBDO and related molecules. The calculation was performed according to the method of Kyte and Doolittle (47). The horizontal axes show the amino acid (aa) numbers along the protein versus the corresponding hydropathicity. The dotted lines at the −5 value divide hydrophobic regions (above) from hydrophilic regions (below). The following sequences are shown: the *S. domuncula* lectin, the *T. tridentatus* GBP, the *C. auratus* molecule, and the *P. polycephalum* tectonin-1. The transmembrane region (TM) in the *S. domuncula* sequence is marked together with the six tandem repeats (tan1–tan6).

Fig. 4. Expression of *S. domuncula* LEC_SUBDO in *E. coli*. Recombinant LEC_SUBDO was prepared and separated by 10% SDS-PAGE as described under “Materials and Methods.” The gel was stained with Coomassie Brilliant Blue. The following protein samples were subjected to the gel: lysate from bacteria 3 h after induction with isopropyl-1-thio-D-galactopyranoside (IPTG; lane a) and after a 6-h induction period (lane b) and the purified fusion protein (recombinant LEC_SUBDO) together with the bacterial GST tag, the oligohistidine tag, and the S tag (lane c). Lane M, molecular mass markers in kilodaltons.

Fig. 5. Expression of SDLEC in different specimens from *S. domuncula*. Animals were collected from distances of 15–20 km and immediately used for extraction of RNA. Reverse transcription-PCR was performed, and the products were separated by agarose gel electrophoresis, followed by ethidium bromide staining. The patterns obtained from animals 1–6 with PCR fragments of 791 and 487 bp are shown. Lane M, DNA size markers in bp.
LEC_SUBDO was against the Gram-negative bacteria *E. coli* and the Gram-positive bacteria *Staphylococcus aureus*. The lectin was added to the bacteria and incubated. The antibacterial activity was determined in colony-forming units and expressed in percent inhibition as described under “Materials and Methods.”

The results are summarized in Table I and show that the natural lectin caused, even at a low concentration of 10 µg/ml, a significant antibacterial activity (16%) against *E. coli*, which increased with increasing concentrations; at 300 µg/ml, the inhibition was 81%. The recombinant lectin was also effective; at 300 µg/ml, a 36% inhibition was recorded. The significant antibacterial activity was reduced for both the natural (reduced to 43% at 300 µg/ml) and recombinant (reduced to 15% at 300 µg/ml) lectins when they were preincubated with LPS.

The inhibitory effect of the lectin on the Gram-positive bacteria *S. aureus* was comparatively low. At a concentration of 300 µg/ml natural lectin, an inhibition of only 15% was seen; for the recombinant protein, the activity was even lower (8%).

**Immunological Cross-reactivity**—To demonstrate that recombinant LEC_SUBDO shares identical/similar epitopes with the natural protein, antibodies were raised against the recombinant protein. The purified natural as well as recombinant lectins were size-separated by SDS-PAGE and, after blot transfer, reacted with the polyclonal antibody. The immunocomplex has been visualized by a labeled secondary antibody. For further data, see “Materials and Methods.”

**Table I**

| Bacteria                           | Inhibition % |
|-----------------------------------|--------------|
| *E. coli*                          |              |
| Natural; 10 µg/ml                  | 16 ± 2       |
| Natural; 30 µg/ml                  | 32 ± 4       |
| Natural; 100 µg/ml                 | 55 ± 4       |
| Natural; 300 µg/ml                 | 81 ± 14      |
| Recombinant; 10 µg/ml              | 4 ± 2        |
| Recombinant; 30 µg/ml              | 17 ± 2       |
| Recombinant; 100 µg/ml             | 28 ± 4       |
| Recombinant; 300 µg/ml             | 36 ± 4       |
| Recombinant (LPS-adsorbed); 300 µg/ml | 43 ± 4   |
| Recombinant (LPS-adsorbed); 300 µg/ml | 15 ± 2  |

| *S. aureus*                        |              |
| Natural; 300 µg/ml                 | 15 ± 2       |
| Recombinant; 300 µg/ml             | 8 ± 2        |
| Natural (LPS-adsorbed); 300 µg/ml  | 5 ± 2        |
| Recombinant (LPS-adsorbed); 300 µg/ml | 5 ± 2  |
tion procedure (fractions 18–20) (Fig. 7) were analyzed and were found not to react with pAb-LEC_SUBDO on the Western blot (Fig. 8B, lane b).

Inhibition of LEC_SUBDO-LPS Interaction—An enzyme-linked immunosorbent assay system was used to determine the minimal inhibitor concentration of b-GlcNAc. Under the conditions used, concentrations as low as 3 μM b-GlcNAc resulted in inhibition of the binding between LEC_SUBDO and LPS. No effect could be seen with b-Glc, b-Gal, b-GalNAc, b-GlcNH₂, or b-GalNH₂ even at concentrations >10 mM.

DISCUSSION

One major prerequisite for a successful evolution to the first metazoan animals, Urmetazoan, the hypothetical common ancestor of all metazoan phyla, was the establishment of an efficient immune system (41). As described in the Introduction, Porifera comprise an unusually complex immune system, ranging from highly active secondary metabolites to cytokines and receptors, which are very reminiscent of those present in higher metazoan phyla. In this study, we used the primmorph system from the demosponge S. domuncula as a model cell system for infection and LPS as a model molecule.

Exposure of primmorphs to LPS results in strong phosphorylation of p38 MAPK (see also Ref. 2). This is an indication that an inflammation-related process occurs after addition of this toxin to these 3D cell aggregates (see Ref. 42). p38 MAPK is known to be phosphorylated in response to LPS in mammalian systems (43). To clarify whether lectins are also involved in the host defense in sponges, primmorphs were incubated with LPS in the absence or presence of different sugars; the monosaccharide b-GlcNAc turned out to be the most effective competitor molecule.

By application of the PCR cloning approach and screening a potential cDNA encoding a horseshoe crab-related lectin was identified. The horseshoe crab lectins have been demonstrated to be effective defense molecules (see Ref. 15). In S. domuncula, a cDNA was found whose deduced polypeptide has significant sequence similarity to the tachylectins. The most closely related proteins are the GBP from T. tridentatus (16), tachylectin-P (44), and lectin L6/tachylectin-1 (36). Like the crab molecules, sponge LEC_SUBDO comprises a predicted transmembrane region, suggesting that the lectin is cell surface-associated. In addition, six distinct hydrophobic regions are present in the sponge molecule, corresponding to six tandem repeats in the tachylectins: these are absent in tectonins, related molecules found in the Mycetozoa P. polycephalum (39).

To rule out the possibility that the cloned LEC_SUBDO is not of sponge origin and might have been introduced into the cDNA library from commensal animals, different S. domuncula specimens were analyzed by reverse transcription-PCR. In all cases examined, a strong expression of the SDLEC transcript could be identified.

The described sequence similarity suggests that the sponge lectin is also, as the horseshoe crab lectins, involved in the bacterial defense mechanism of S. domuncula. It has been proposed that the horseshoe crab lectins, molecules that are present already before infection and are of a relative specificity, are key elements of the innate/natural immunity (see Ref. 15).

In horseshoe crabs, the lectins are associated with specialized cells, the hemocytes, and/or the hemolymph; lectin L6/tachylectin-1 from T. tridentus is associated with the cell membrane of hemocytes (36), whereas the GBP from T. tridentus (16) and tachylectin-P from T. tridentus (44) exist in the hemolymph. Like the crab molecules, the sponge LEC_SUBDO comprises a predicted transmembrane region, suggesting that the lectin might be associated with immune cells. The most likely candidates in sponges, which are devoid of a circulation system, to act as immune cells are the archaeocytes and perhaps also the choanocytes (45, 46).

To approach the question of whether the lectin is involved in the host antibacterial defense in the sponge S. domuncula, its expression was determined. It was found that SDLEC expression was strongly up-regulated after exposure of primmorphs to LPS. The conclusion that this gene is under the control of LPS was supported by competition experiments using the monosaccharide b-GlcNAc; this sugar almost completely abolished the effect of LPS.

After determining that the SDLEC gene is modulated in response to exposure of primmorphs to LPS, it was pressing to determine the potential antibacterial effect of LEC_SUBDO. Therefore, the lectin was isolated from the tissue and prepared in a recombinant way. By application of specific antibodies raised against the recombinant protein, it was confirmed that both forms of protein comprise the same antigenic sites, suggesting that they are the same species of molecules. The antimicrobial assays confirmed that the sponge lectin displays a strong antibacterial effect against Gram-negative bacteria E. coli and, to a lower extent, also against the Gram-positive bacteria S. aureus. Also in this respect, the sponge molecule shares a striking functional similarity with T. tridentus lectin L6/tachylectin-1; this molecule also displays a strong effect against Gram-negative bacteria and a lower effect against Gram-positive bacteria (36).

Taken together, the results summarized in this work indicate that a natural immune system exists also in sponges, as determined here with LEC_SUBDO as an antibacterial molecule. This finding contributes not only an important aspect to the complexity of the immune competence in sponges in general, but also interesting facts on the evolution of the immune system in particular. LEC_SUBDO identified in S. domuncula indicates that these animals can defend themselves against bacterial invaders by a (probably cell membrane-associated) antibacterial lectin. In view of the fact that a series of antimicrobial lectins exist in horseshoe crabs (reviewed in Ref. 15), it might be predicted that such a diversity occurs also in sponges. Screening studies by PCR cloning using degenerate primers directed against the conserved regions are under way. Likewise, studies to localize the immune cells by in situ hybridization are in progress. Furthermore, the finding sheds new light on the origin of the immune system in Metazoa. In previous studies, the immune molecules have been documented to have emerged during the transition to Urmetazoa (6). The data presented indicate that the immune system in sponges comprises also basic molecules that are reminiscent of molecules that are present in more ancestral multicellular organisms such as the slime mold P. polycephalum (tectonins, representing nuclear matrix molecules). Moreover, we have demonstrated that some potential immune molecules exist in sponges up to horseshoe crabs, the tachylectins from Tachypleus and the molecule now found also in the sponge S. domuncula, that have been lost later in the evolution to the crown taxa of Deuterostomia (animals characterized by the process of the blastopore becoming the anus in the adult) and of Protostomia (animals characterized by the process of the mouth arising from the blastopore).

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