We have purified a small size antimicrobial peptide, named gomesin, from the hemocytes of the unchallenged tarantula spider *Acanthoscurria gomesiana*. Gomesin has a molecular mass of 2270.4 Da, with 18 amino acids, including a pyroglutamic acid as the N terminus, a C-terminal arginine α-amide, and four cysteine residues forming two disulfide bridges. This peptide shows marked sequence similarities to antimicrobial peptides from other arthropods such as tachyplesin and polyphemusin from horseshoe crabs and androctonin from scorpions. Interestingly, it also shows sequence similarities to protegrins, antimicrobial peptides from porcine leukocytes. Gomesin strongly affects bacterial growth, as well as the development of filamentous fungi and yeast. In addition, we showed that gomesin affects the viability of the parasite *Leishmania amazonensis*.

Antimicrobial peptides are ubiquitously produced throughout the phylogenetic tree. The widespread occurrence of these antimicrobial substances suggests that they play a key role in innate immunity against microorganisms and other pathogens (1–3). Despite the diversity in their structure, most of these antimicrobial substances are small (less than 10 kDa) cationic and amphipathic peptides (for reviews see Refs. 4–6). Over the past 20 years several of these antimicrobial peptides were isolated and characterized from the hemolymph of invertebrates including two chelicerates: horseshoe crabs (merostomata) (for a review see Ref. 7) and scorpions (arachnida) (8, 9). In fact, the hemocytes of horseshoe crabs contain various cysteine-rich cationic peptides (tachypleisin, polyphemusin, tachycitin, tachystatin, and big defensin) with inhibitory effects on the growth of Gram-positive and Gram-negative bacteria and fungi. In addition, two non-cysteine-rich proteins, anti-lipopolysaccharide factor and factor D, with antimicrobial properties mainly against Gram-negative bacteria are produced in response to a microbial infection (7). The tachypleсин family of antimicrobial peptides, including polyphemusins, is located in the small granules of the hemocytes, as also are tachycitin and tachystatin, whereas the anti-lipopolysaccharide factor and factor D are found in the large granules and the big defensin in both types of granules. In addition to this group of chelicerates, several antimicrobial peptides were characterized in the hemolymph of the scorpions *Leiurus quinquestratus* (8) and *Androctonus australis* (9). Among these peptides, two are members of the widespread invertebrate defensin family (for a review see Ref. 10). These scorpion defensins are mainly active against Gram-positive bacteria. Although only a defensin type molecule was found in *L. quinquestratus* (7), three distinct antimicrobial peptides have been characterized from the hemolymph of unchallenged scorpions of the species *A. australis*: an insect defensin-like molecule (as mentioned above) and two additional cysteine-containing peptides, androctonin and buthinin (9). Androctonin is a 25-residue peptide with four cysteine residues, active against both Gram-positive and Gram-negative bacteria and fungi. Androctonin shows sequence similarities to tachyplesin and polyphemusin. In contrast, buthinin, a 34-amino acid peptide with three internal disulfide bridges, shows no sequence similarities with known antimicrobial peptides. Buthinin was found to be active only against bacteria.

Interestingly, in addition to strictly antimicrobial peptides found in chelicerates, additional molecules with antibacterial properties have been recently isolated from the venom of the wolf spider *Lycosa carolinensis*. In this spider, two toxins (lycotoxins I and II) containing 25–27 amino acids and deprived of cysteine residues exhibit antimicrobial properties. Surprisingly, they show marked sequence similarities to some frog antimicrobial peptides such as magainin, adenoregulin, and dermaseptins. In addition to having antimicrobial activity, they dissipate ion and voltage gradients across the membranes of excitable cells and probably contribute to paralysis of envenomed prey (11).

Nevertheless, no report of a strictly antimicrobial peptide from spiders is available in the literature. To understand whether spiders are able to mount an immune response through the production of antimicrobial substances as observed in insects, we started to investigate such possibility in the mygalomorph spider *Acanthoscurria gomesiana*. The characterization of antimicrobial peptides in this class of chelicerates would be of great value to understand the evolutionary aspects of innate immunity. On this basis, the use of mygalomorph spiders as an experimental model is very useful, because they
are one of the oldest species in the order Araneae.

In this paper, we report the isolation and full characterization of a small size antimicrobial peptide from hemocytes of unchallenged A. gomesiana. This peptide, named gomesin from the species gomesiana, has 18 amino acids and two disulfide bridges. This open-ended cyclic peptide shows marked sequence similarities to tachypleisin and polyphemusin from horseshoe crabs (12, 13) and to androctonin from the scorpion A. australis (9). More interestingly, gomesin shares also structural similarities with protegrins, antimicrobial peptides from porcine leukocytes (14). In addition to being effective against the growth of Gram-positive and -negative bacteria, synthetic gomesin was found to be strongly efficient against the growth of filamentous fungi and yeast and moderately active against the viability of the parasite Leishmania amazonensis.

EXPERIMENTAL PROCEDURES

Animals and Hemolymph Collection

The common spider (A. gomesiana) was obtained from the Arthropods Laboratory of the Butantan Institute (São Paulo, Brazil). A. gomesiana is a tarantula spider of the Theraphosidae family. The hemolymph was collected in the presence of sodium citrate buffer, pH 4.6 (2:1, v/v) (15). The hemocytes degranulation and coagulation, the hemolymph was collected from prechilled animals different stages of development was collected from prechilled animals by cardiac puncture with an aropgenic syringe. To avoid hemocyte degraulation and coagulation, the hemolymph was collected in the presence of sodium citrate buffer, pH 4.6 (2:1, v/v) (15). The hemocytes were removed from plasma by centrifugation at 800 for 10 min at 4 °C. The supernatant, obtained by centrifugation at 13,800 g for 30 min at 4 °C, was directly subjected to purification by solid phase extraction.

Peptide Extraction and Purification

After concentration, the hemocytes collected from the hemolymph (approximately 0.4 ml/spider) from animals of either sex at different stages of development was collected from prechilled animals by cardiac puncture with an aropgenic syringe. To avoid hemocyte degraulation and coagulation, the hemolymph was collected in the presence of sodium citrate buffer, pH 4.6 (2:1, v/v) (15). The hemocytes were removed from plasma by centrifugation at 800 for 10 min at 4 °C. The supernatant, obtained by centrifugation at 13,800 g for 30 min at 4 °C, was directly subjected to purification by solid phase extraction.

The combined cytosolic and organelle acid extracts were loaded onto serially linked classic Sep-Pak C18 cartridges (Waters Associates) equilibrated in acidified water (0.05% trifluoroacetic acid). Three stepwise elutions were successively performed with 5%, 40%, and 80% acetonitrile in acidified water. The 40% Sep-Pak fraction was concentrated in a vacuum centrifuge and lysed by concentration in a vacuum centrifuge, reconstituted with MilliQ water, and applied to a vacuum centrifuge, reconstituted with MilliQ water, and applied to a semipreparative Aquapore RP-300 C8 (10 cm × 0.46 cm, Brownlee™) using a linear gradient of 2–60% acetonitrile in acidified water over 120 min at a flow rate of 0.8 ml/min.

Synthetic amidated peptide (90 nmol) was subjected to reduction and S-pyridylethylated using a Finnigan LCQ™ Duo mass spectrometer (Thermoquest) at a mass to charge (m/z) range of 3000. The peptides dissolved in water/ acetonitrile (50:50, v/v) were introduced in the spectrometer by flow injection at 5 μl/min and analyzed in the positive mode. Calibration was performed using the following mixture of standard substances: caffeine (m/z 195.2), 1,2,3-trimethylipropyl-phenyllalanine-alanine acetate H2O (m/z 524.3), and Ultramark 1621 (m/z 1022, 1122, 1222, 1322, 1422, 1522, 1622, 1722, 1822, and 1922).

A liquid growth inhibition assay. The presence of antibacterial activity was determined by a liquid growth inhibition assay. The following additional fungal and bacterial strains were generous

Peptide Synthesis

Nonamidated and amidated peptides were synthesized according to a procedure previously described (9). Briefly, the peptides were synthesized according to classical Fmoc (N-9-fluorenyl)methoxy carbonyl) chemistry. At the end of the process, the peptides (25 μmol) were taken up to 2.5 liters in 0.1 m ammonium acetate buffer, pH 8.5, and allowed to renature by air oxidation overnight at room temperature under stirring. After renaturation, the pH was adjusted to 4 with acetic acid and the peptide solution loaded onto Sep-Pak Vac C18 cartridges (Waters Associates) equilibrated with 0.5% acetic acid. The peptides were eluted with 60% acetonitrile in acidified water, concentrated in a vacuum centrifuge and purified to homogeneity by reversed phase chromatography using a preparative column (Aquapore RP 300 C8, 150 × 10 mm, Brownlee™).

Biossays

The microbial strains were those used in previous studies (19–22). The following additional fungal and bacterial strains were generous
gels from different colleagues: Enterococcus faecalis, Staphylococcus epidermidis, S. hemolyticus (H. Monteil, Institute of Bacteriology, University of Strasbourg, France), Nocardia asteroides (Y. Piémont, Institute of Bacteriology, University of Strasbourg, France), Serratia marcescens Db11 (H.G. Roman, Department of Microbiology, University of Stockholm, Stockholm, Sweden), Beauveria bassiana (INRA, St-Christol-les-Ales, France), and Tricophyton mentagrophytes and Candida tropicalis (H. Koenig, Laboratory of Mycology, University of Medicine, Strasbourg, France).

During the purification procedure, antibacterial activity was monitored by a liquid growth inhibition assay against Micrococcus luteus and Escherichia coli SBS363 as described in Ref. 25. The MIC of the synthetic peptides was determined against fungi and bacterial strains using a procedure described previously (9). When the antifungal activity assay was performed in the presence of salt, the culture medium was prepared in phosphate-buffered saline, 137 mM NaCl. MICs are expressed as the [a] – [b] interval of concentrations, where [a] is the highest concentration tested at which the microorganisms are growing and [b] is the lowest concentration that causes 100% growth inhibition.

The bactericidal assay was monitored against M. luteus and E. coli D22 as described in Ref. 9. The final concentration of amidated gomesin tested was 10 μM. For the fungicidal assay, spores of Neurospora crassa were incubated in the presence of 10 μM amidated gomesin. After 0.5, 1, 2, 4, and 8 h, the culture medium containing the peptide was removed and replaced with fresh medium. After 48 h the cultures were examined microscopically and spectrophotometrically.

The hemolytic activity assay was monitored against human erythrocytes. Two times serial dilutions of synthetic gomesin from concentrations of both forms of gomesin of 0.1 to 100 μM were incubated in Eppendorf tubes with a suspension of erythrocytes (0.4%) in phosphate-buffered saline. As a positive control (100% lysis), the erythrocytes were incubated with 0.1% SDS. After 1 h at 37 °C, the samples were centrifuged (320 × g, 5 min), and the absorbance of the supernatant (50 μl) was measured at 405 nm using a microtiter plate reader.

For the antiparasitic assay, cell viability of L. (Leishmania) amazonensis (MPRO/BR/72/M 1841-LV-79) was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay as described (24) and adapted for Leishmania spp. (25). Briefly, 5 × 10^5 stationary phase promastigotes suspended in buffered saline (118 mM NaCl, 5.4 mM KCl, 0.8 mM MgSO_4, 5.5 mM d-glucose, 10 mM MOPS, pH 7.2) were incubated with two times serial dilutions of synthetic gomesin and hemoglobin fragment (33–61 of bovine α-hemoglobin) (26) from 0.3 to 80 μM. After a 1-h incubation at 22 °C, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide was added to a final concentration of 1 mg/ml, and the reaction was measured after 40 min in a microtiter plate reader.

RESULTS

Purification and Primary Structure Determination of Gomesin—We first performed a pilot experiment to compare the antibacterial activity against M. luteus of crude extracts of hemocytes from unchallenged and bacteria-challenged A. gomesiana spiders. Because no increase in antibacterial activity was found in the immunized spiders, we decided to purify the antimicrobial compounds from native spider hemocytes.

The antimicrobial compounds from the hemocytes were extracted under acidic conditions after sonication. The acidic extract was purified by solid phase extraction. The three eluted fractions obtained (5, 40, and 80%) were submitted to reversed phase HPLC and to the antibacterial assay. Among the different fractions obtained from the 40% Sep-Pak fraction, three were found to have activity against M. luteus and E. coli, and the two bacterial strains used for the bioassay (Fig. 1A). In the present study, we focused our attention on fraction AGH2, which has activity against both strains of bacteria. MALDI-MS analysis of fraction AGH2 revealed the presence of three different molecules with molecular masses of 413.4, 2270.4, and 7179.8 Da. To separate these molecules, the second step of purification was performed using size exclusion chromatography (Fig. 1B). Among the different fractions obtained, only one peak was found to be active against M. luteus and E. coli. Because a single molecular mass of 2270.4 Da was obtained by mass spectrometry, the molecule was considered to be pure enough for sequencing by Edman degradation.

Because no PTH-derivative signal was observed in the 25 cycles of Edman degradation, this molecule was supposed to be a N-terminally blocked peptide. To verify this hypothesis, the reduced and S-pyridylethylated molecule was subjected to pyrogglutamate aminopeptidase digestion to remove a potential pyrogglutamic acid. After purification by reversed phase HPLC of the digestion products, two fractions were obtained (data not shown). Mass spectrometry analysis revealed a difference of 111 Da between the two molecules that corresponded to the extramolecular mass of a pyrogglutamic acid. For this reason, only the compound with the smallest molecular mass (2583.8 Da) was subjected to Edman degradation. The following 17-residue sequence was obtained: C*RRLC*YKQRC*VTYC*RGR, where C* stands for a pyridylethylated cysteine residue. The identification of the four pyridylethylated cysteine residues after Edman degradation is in agreement with the increased mass (424.3 Da) of the native peptide following reduction/alkylation with vinyl pyridine (2694.7 Da). From the primary se-
Antimicrobial Activity Spectrum of the Amidated Gomesin—

The activity of amidated gomesin was compared to a non-amidated form (gomesin-fa) and to androconin a four cysteine-rich antimicrobial peptide from the scorpion A. australis (9).

### Table I

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Minimal inhibitory concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gomesin-fa</td>
</tr>
<tr>
<td><strong>Gram-positive bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Aerococcus viridans</td>
<td>0.8–1.6</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>6.25–12.5</td>
</tr>
<tr>
<td>Bacillus megaterium</td>
<td>0.2–0.4</td>
</tr>
<tr>
<td>Bacillus thuringiensis</td>
<td>1.6–3.15</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>6.2–12.5</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>0.8–1.6</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>0.4–0.8</td>
</tr>
<tr>
<td><strong>Gram-negative bacteria</strong></td>
<td></td>
</tr>
<tr>
<td>Agrobacterium tumefaciens</td>
<td>ND</td>
</tr>
<tr>
<td>Alcaligenes faexsis</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>0.8–1.6</td>
</tr>
<tr>
<td>Escherichia coli D22</td>
<td>0.4–0.8</td>
</tr>
<tr>
<td>Escherichia coli D31</td>
<td>0.8–1.6</td>
</tr>
<tr>
<td>Escherichia coli SBS363</td>
<td>0.4–0.8</td>
</tr>
<tr>
<td>Erwinia carolovora carolovora</td>
<td>3.15–6.25</td>
</tr>
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<td>Enterobacter cloacae pL2</td>
<td>3.15–6.25</td>
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<tr>
<td>Kibblesia pneumonia</td>
<td>3.15–6.25</td>
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<tr>
<td>Pseudomonas aeruginosa</td>
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<tr>
<td>Salmonella thphinyurium</td>
<td>0.8–1.6</td>
</tr>
<tr>
<td>Serralia marcescens Db11</td>
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<tr>
<td>Xanthomonas caminostr pv. orizae</td>
<td>3.15–6.25</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
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<tr>
<td>Alternaria brassica</td>
<td>0.4–0.8</td>
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<tr>
<td>Aspergillus fumigatus</td>
<td>1.6–3.15</td>
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<tr>
<td>Beauveria bassiana</td>
<td>12.5–25</td>
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<tr>
<td>Fusarium culmorum</td>
<td>0.4–0.8</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>0.4–0.8</td>
</tr>
<tr>
<td>Neurospora crassa</td>
<td>0.4–0.8</td>
</tr>
<tr>
<td>Nectria haematococca</td>
<td>0.2–0.4</td>
</tr>
<tr>
<td>Tricoderma viride</td>
<td>0.4–0.8</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>0.8–1.6</td>
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<tr>
<td><strong>Yeasts</strong></td>
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<td>Candida albicans</td>
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</tr>
<tr>
<td>Candida glabrata</td>
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<tr>
<td>Candida tropicalis</td>
<td>3.15–6.25</td>
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<tr>
<td>Cryptococcus neoformans</td>
<td>0.8–1.6</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>1.6–3.15</td>
</tr>
</tbody>
</table>

* ND means not detected in the range of the concentrations tested up to 100 μM (gomesin-fa) and 50 μM (androconin).

** NM means not measured.
Activity of Gomesin against Eukaryotic Cells—To determine the effect of gomesin against eukaryotic cells, various concentrations of synthetic peptide (0.1–100 μM) were tested against the parasite L. (L.) amazonensis and human erythrocytes using viability and hemolytic assays, respectively. After 1 h of incubation, the viability of the parasite was found to be dependent on the gomesin concentration used (Fig. 3). Using as control a hemoglobin fragment (33–61 of bovine α-hemoglobin) (26), which exhibits some effect on the viability of the parasite at high concentrations (>40 μM), gomesin was found to strongly reduce viability even at concentration as low as 2.5 μM. In addition, in an hemolytic assay against human erythrocytes, the percentage of cell lysis was found to be dependent of the gomesin (amidated and non-amidated) concentrations tested. From 0.1 μM (the lowest concentration tested) up to 0.2 μM, the percentage of hemolysis remains rather low (<5%), whereas increasing the peptide concentration up to 1 μM results in a rapid and significant elevation of the percentage of cell lysis (up to approximately 16%). However, an increase in the concentration of gomesin of 2 orders of magnitude (final concentration of 20 μM) results in only little effect on human erythrocyte lysis (Fig. 4). This contrast to the gomesin activity against the parasite L. (L.) amazonensis where cell viability was found to be almost linearly dependent of the peptide concentration (see Fig. 3).

DISCUSSION

To gain information on the involvement of antimicrobial peptides in arachnid immunity, we started to investigate the antimicrobial peptides of the tarantula spider A. gomesiana, which can live over 23 years (Butantan Institute, São Paulo, registration book). We have purified and characterized from A. gomesiana hemocytes an antimicrobial peptide that we named gomesin. The level of antibacterial activity found in the hemocytes has not changed in bacteria-challenged spiders, such as scorpions (9), ticks (26), shrimps (27), and bivalve mollusks (28), suggesting that the antimicrobial peptides are in fact stored in the spider hemocytes and may be released into the plasma upon infection, as established in mollusks (28). This remains to be confirmed by gomesin mRNA quantification.

Gomesin is an 18-residue peptide with four cysteine residues engaged in disulfide bridges, a pyroglutamic acid as the N terminus, and an Arg α-amide as C-terminal residue. The presence of two internal disulfide bridges plus a C-terminal amimation certainly contributes to the stability of the peptide to proteases within the hemocytes or if released into the hemolymph. Gomesin has six positively charged residues (five Arg and one Lys for a calculated pI of 12.7) and showed similarities to (i) antimicrobial peptides from horseshoe crabs: tachyplesins and polyphemusins (50%, determined by using Clustal V method with PAM250 residue weight table) (12, 13), (ii) androctonin (23%), from scorpions (9), and finally (iii) protegrins (17%), porcine leukocyte antimicrobial peptides (14) (Fig. 5). In gomesin, the four cysteine residues form two disulfide bridges linking Cys5 to Cys15 and Cys8 to Cys11. Such a disulfide array (Cys1-Cys4 and Cys2-Cys3) is identical for all antimicrobial peptides (Fig. 5). Interestingly, in gomesin an equal number of three residues is observed on each segment upstream and downstream the disulfide bridge Cys2-Cys3 (Arg11-Arg1-Leu5 and Val12-Thr14-Tyr14, gomesin numbering) as in tachyplesin I and polyphemusin II. A similar balance in the number of amino acids between the two disulfide bridges is also found in protegrin PG-1 (one residue in each segment). Unlike gomesin, the horseshoe crab peptides and protegrin PG-1, androctonin presents an unequal number of residues: five residues (Arg-Gln-Lys-Ile) upstream Cys2 and three amino acids (Tyr-Tyr-Lys) downstream Cys3. This suggests that gomesin will adopt a β-hairpin structure close to the one found in tachyplesin (29,
Gomesin is a Spider Antimicrobial Peptide

Gomesin, a Spider Antimicrobial Peptide

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Gomesin was compared with (i) tachyplesin I and (ii) polyphemusin II from the horseshoe crabs Tachypleus tridentatus and Limulus polyphemus, respectively (12, 13); (iii) androctonin from the scorpion A. australis (9); and (iv) protegrin PG-I from porcine leukocytes (14). Z stands for pyroglutamic acid, and asterisks indicate a C-terminal α-amide. Identical and conserved residues are shaded.

Fig. 5. Amino acid sequence comparison of gomesin to vertebrate and invertebrate antimicrobial peptides. Gomesin was compared with (i) tachyplesin I and (ii) polyphemusin II from the horseshoe crabs Tachypleus tridentatus and Limulus polyphemus, respectively (12, 13); (iii) androctonin from the scorpion A. australis (9); and (iv) protegrin PG-I from porcine leukocytes (14). Z stands for pyroglutamic acid, and asterisks indicate a C-terminal α-amide. Identical and conserved residues are shaded.

In addition to being antibacterial, gomesin has marked activity against human erythrocytes at a rather low concentration (16% at 1 μM), the effect on these human cells does not increase, whereas the peptide concentration reaches 100 μM. This is in contrast to the hemolytic effect of tachyplesin, a response which was found to be linearly dependent on the peptide concentration tested, 5% up to 20 μM up to 100% hemolysis at 100 μM (34). Surprisingly, Rao (36) observed only 30% hemolysis with 100 μM of tachyplesin. On the other hand, the scorpion androctonin is not hemolytic even at high 150 μM (9). The differences in hemolytic activity observed between androctonin and gomesin and tachyplesin, could be attributed to (i) a longest C terminus and (ii) charge differences in this section of the molecule. Gomesin was found to be more toxic to Leishmania (L) amazonensis promastigote than to human erythrocytes. In fact, in the presence of 2.5 μg gomesin only 50% of promastigotes were viable. Such effect was also observed with the cecropin A-melittin hybrid peptide when tested against Leishmania donovani promastigotes (39). This activity on Leishmania promastigotes could be attributed to a higher percentage of anionic phospholipids in their plasma membrane compared with the plasma membrane of mammalian cells and of ergosterol instead of cholesterol (40). The presence of a large number of positively charged amino acids (five Arg and one Lys) in gomesin contributes to a higher specificity of the peptide for prokaryotic cells because of a higher electronegativity of prokaryotic cells when compared with the eukaryotic cell membrane. The participation of hydrophobic residues in the interaction may occur during later events, resulting in bacterial lysis. To obtain a better understanding of the mode of action of gomesin, its three-dimensional structure should be elucidated using NMR spectroscopy.

In conclusion, gomesin appears to have an interesting potential for therapeutic application because of its strong and broad activity spectrum (affecting bacteria, fungi, yeasts, and eukaryotic parasites) and structural properties conferring high stability to the molecule. In addition, the conservation of the activity in a physiological condition supports the possibility to develop this peptide as a new drug for human therapy. Despite hemolytic activity of the gomesin against human erythrocytes, this activity could be decreased or abolished through the modifica-
tion by molecular biology and/or chemical approach as obtained in tachyplesin analogs (41).

Acknowledgments—We are grateful to Dr. Sandrine Uttenweiler-Joseph for mass spectromotography analysis of native gomesin and Jose’ Mario de Freitas Balanco for antiparasitic assay.

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Gomesin, a Spider Antimicrobial Peptide
Isolation and Characterization of Gomesin, an 18-Residue Cysteine-rich Defense Peptide from the Spider *Acanthoscurria gomesiana* Hemocytes with Sequence Similarities to Horseshoe Crab Antimicrobial Peptides of the Tachyplesin Family

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