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Cloning and Characterization of the Homolog of Mammalian Lipopolysaccharide-Binding Protein and Bactericidal Permeability-Increasing Protein in Rainbow Trout

Oncorhynchus mykiss

Hiroyuki Itohga,† Teruko Honda,† Chie Kohchi,† Takashi Nishizawa,† Yasutoshi Yoshiura,‡ Teruyuki Nakanishi,§ Yuichi Yokomizo,§ and Gen-Ichiro Soma*‡

We cloned two cDNAs denoted as RT-LBP/BPI-1 and RT-LBP/BPI-2, respectively, which were derived from the mRNA of head kidney from rainbow trout. They showed structural homology with LPS-binding protein (LBP) and bactericidal/permeability-increasing protein (BPI) in mammals. The full-length cDNA of RT-LBP/BPI-1 and RT-LBP/BPI-2 is 1666 and 1741 bp, respectively. Both cDNAs encoded 473 aa residues, including the amino acids conserved in mammalian LBP and BPI proteins that were assumed to be involved in LPS binding. The overall coding sequence of RT-LBP/BPI-1 has 33% amino acid homology to human LBP and 34% to human BPI, and RT-LBP/BPI-2 has 32% amino acid homology to human LBP and 33% to human BPI.

Three-dimensional structure analysis by three-dimensional/one-dimensional (3D-1D) methods also demonstrated that RT-LBP/BPI-1 and RT-LBP/BPI-2 proteins showed significant similarity to human BPI, having a boomerang shape with N-terminal and C-terminal barrels. Phylogenetic analysis showed that the LBP and BPI genes seemed to be established after the divergence of mammals from teleosts. These results suggested that RT-LBP/BPI-1 and RT-LBP/BPI-2 may be a putative ortholog for mammalian LBP and/or BPI genes. This is the first study to identify the LBP family genes from nonmammalian vertebrates.

T he innate immune system is recognized as an essential prerequisite in maintaining the integrity of the whole immune system, even in higher vertebrates (1, 2). An important and intriguing question is the mechanism by which the innate immune system distinguishes those pathogens that will be eradicated. In this respect, LPS, a complex glycolipid in the outer membrane of Gram-negative bacteria, plays an important role in the activation of the innate immune system (3).

LPS-binding protein (LBP)3 (4) and/or bactericidal/permeability-increasing protein (BPI) are thought to play a significant role in transducing cellular signals from LPS (4, 5). Recent experiments indicated that LBP played a critical role in the induction of proinflammatory cytokines by LPS (4, 6–8). LBP functions primarily by acting as a LPS transporter of LPS to CD14 (4, 9, 10) and to the Toll-like receptor complex (11, 12). These have been reported as LPS receptor-transducing cellular signals in response to the complex of LPS and LBP in vivo and in vitro (4, 11, 12).

BPI is a 50- to 60-kDa glycoprotein that has been purified from granules of neutrophils; it also binds to LPS with higher affinity than LBP (5, 13, 14). Unlike LBP, which augments the cellular response by LPS (such as TNF secretion by macrophages that enhances the affinity of LPS with its receptor(s)), BPI inhibits cellular responses by LPS in several respects, i.e., complement receptor up-regulation in neutrophil (15), TNF production in monocytes (13, 16, 17), and experimental endotoxemia (18, 19). BPI, therefore, counteracts LBP by decreasing the amount of LPS available to bind to LBP (13).

In other aspects, both LBP and BPI are thought to be essential molecules for protection from bacterial invasion (5, 20–22). LBP is required for clearance of bacterial cells from the circulation in vivo, as shown in an LBP knockout experiment (20). BPI synergized with defensins and complement exhibits a very potent bactericidal activity and plays a role of clearance of invasive bacteria at the site of inflammation (22). These facts suggest that LBP and BPI play the key role in the innate immune system by recognizing bacterial LPS. Therefore, the roles of LBP and BPI are somehow cooperative rather than counteractive.

Mammalian secreted-type mature LBP and BPI form tandem-barrel shapes (4, 23–25) in keeping with their sequence homology to the lipid transfer proteins (26, 27). Human LBP shares 45% amino acid identity with human BPI. It shares 24% identity with the phospholipid transfer protein (PLTP) and 23% with the cholesterol ester transfer protein (CETP) (28, 29). Structural similarity of LBP and BPI proteins suggested that these genes might have been evolved from a common ancestor; however, functional complexity of these genes in higher vertebrate hamsters further analyses of the biological significance of these genes. To understand the...
biological significance of LBP and BPI proteins in the innate immune systems, comparative analyses of these proteins in lower vertebrates may be useful (30). Recent data have highlighted the similarities in pathogen recognition, in signaling pathways, and in effector mechanisms of the innate immune system between *Drosophila* and mammals. One can thus hypothesize that the genes and functions related to the innate immune system are highly conserved (1). In this regard, the nucleotide and protein sequences of LBP and BPI have been reported for several mammalian species (26, 28, 29, 31, 32). However, at present, no information is available on LBP and BPI genes and proteins in other vertebrates, including teleosts fish.

In this work, we report on cloning and structural-functional relationship of two cDNAs (RT-LBP/BPI-1 and RT-LBP/BPI-2) for rainbow trout, and showed structural homology with LBP and BPI in mammals. This is the first study to identify the LBP family genes from nonmammalian vertebrates.

**Materials and Methods**

**Fish**

Rainbow trout (*Oncorhynchus mykiss*) clones AA-1 (weighing 500–750 g) and AB-7 (weighing 60–150 g) were obtained from the Nagano Prefectural Fisheries Experimental Stations (Nagano, Japan). They were maintained in a tank with fresh running water at 15°C, fed commercial pelleted trout food, and were acclimated in an aquarium for at least 2 wk before use.

**RNA preparation and construction of a cDNA library from head kidney**

A total of 1.5 mg kg⁻¹ *Escherichia coli* LPS (Sigma, St. Louis, MO) was injected i.p. into rainbow trout (33–35). Head kidneys and/or livers were collected from rainbow trout before and after LPS treatment. The total RNA from head kidney of AA-1 clone was obtained both before and after LPS treatment using a Quick prep total RNA extraction kit (Amer sham Pharmacia Biotech, Tokyo, Japan). Poly(A)⁺ RNA (5 μg) of head kidney from AA-1 clone was purified with an oligo(dT) column (Amer sham Pharmacia Biotech), and double-stranded cDNA was synthesized using a SMART PCR cDNA synthesis kit (Clontech Laboratories, Palo Alto, CA). This was followed by ligation with an EcoRI adaptor and with EcoRI-digested and calf intestine alkaline phosphatase-treated arms of *Agt*11 vector (Stratagene, La Jolla, CA). After in vitro packaging with Gigapack Gold III (Stratagene), the library was amplified once before use. The total RNA was extracted from both liver and head kidney of AB-7 before and at 3, 6, and 24 h after LPS treatment. cDNA synthesis using total RNA was done with oligo(dT)₁₂₋₁₈ (Amersham Pharmacia Biotech) and murine leukemia virus reverse transcriptase (Toyobo, Osaka, Japan).

**Screening of cDNA library**

Alkaline phosphatase labeling was performed using a Gene Images Alk-Phos Direct system (Amersham Pharmacia Biotech), according to the manufacturer’s instructions. Plaques (1 × 10⁶) of cDNA library were transferred onto nitrocellulose membranes (ADVANTEC, Tokyo, Japan). After baking at 80°C for 2 h, the membranes were prehybridized for 30 min at 55°C in an AlkPhos Direct hybridization buffer, followed by hybridization with phosphorylase-labeled DNA probes at 55°C for 16 h. After washing two times with primary wash buffer (2 M urea, 0.1% SDS, 50 mM phosphate buffer, pH 7, 150 mM NaCl, 10 mM MgCl₂) at 55°C for 10 min, hybridized probe on the membrane was detected by CDP-Star chemiluminescent detection reagent (Amersham Pharmacia Biotech).

**Analysis of cDNA sequence**

To check the inserted cDNA in *Agt*11 vector, PCR was performed to amplify the inserted DNA with *Agt*11 primers (Takara Shuzo, Tokyo, Japan). Briefly, PCR was performed for 35 cycles of 1 min each at 94°C, annealing for 1 min at 55°C, and extension for 1 min at 72°C. For sequence analysis, partially purified PCR products were analyzed by the dideoxy chain termination method using a fluorescence DNA sequencer (Applied Biosystems, Foster City, CA; model 310). Homologous sequences were sought and aligned using the Basic Local Alignment Search Tool and Clustal W. Structural homology was analyzed by three-dimensional/one-dimensional (3D-1D) methods (light balance for remote analogous proteins, LIBRA) (36).

**Suppression subtraction hybridization (SSH) method**

The SSH technique has been shown to be highly effective in the identification of differently expressed genes (37). SSH was performed by using the PCR-Select cDNA Subtraction kit (Clontech Laboratories) and conducted using the manufacturer’s protocol. Two-microgram aliquots of head kidney mRNA from either normal or LPS-treatment rainbow trout were used to make driver and tester cDNA, respectively. Subtracted PCR products were ligated into the pUC18 plasmid vector. The ligation mixture was transformed into *E. coli* XL-1 blue. The inserted DNA fragments were amplified and sequenced.

**Competitive PCR**

The competitive templates were constituted with a Competitive DNA Construction Kit (Takara) using sense (5’-TAGTGTCATTGTGCTGACTGT-3’) and antisense (5’-GCTTAATGCTGATCTCATTTGG-3’) primers, which amplify RT-LBP/BPI-1, and sense (5’-ATTTGATGACATTGGAACACT-3’) and antisense (5’-AGATTTCCTTGCAGCAGTAAA-3’) primers, which amplify RT-LBP/BPI-2. The size of PCR products amplified by RT-LBP/BPI-1 or RT-LBP/BPI-2 primers with the competitor consisted of 290 bp, respectively, and the size of PCR products amplified by RT-LBP/ BPI-1 or RT-LBP/BPI-2 primers with templates of cDNA is 352 or 376 bp, respectively.

**Southern blot analyses of genomic DNA**

Genomic DNAs were isolated from AA-1 clone rainbow trout; a 5-μg aliquot of each was digested with *HindIII*, *BamHI*, *EcoRI*, *ApaI*, and *KpnI* restriction enzymes. Each was subjected to agarose gel electrophoresis and was transferred to a nylon membrane (Biodyne, Pall Biosupport Division, East Hills, NY). A digoxigenin-labeled cDNA probe was generated by PCR using sense (5’-TCATGCCAAATCACAACAT-3’) and antisense (5’-ATTATAATTTGGACAAATGTT-3’) primers, which span the 3’-nontranslated region for RTLB BP/BPI-1, and sense (5’-CCAGTGGCAGGTCTATTAATG-3’) and antisense (5’-TACACACGTAAGATGTC-3’) primers, which span the 3’-nontranslated region for RT-LBP/BPI-2. PCR was done for 40 cycles at 55°C annealing temperature with PCR Dig Probe Synthesis kit (Roche Diagnostics, Mannheim, Germany). The membranes were hybridized at 42°C with the digoxigenin-labeled cDNA probe in Dig Easy Hyb Buffer (Roche Diagnostics), and was finally washed in 0.5× SSC containing 0.1% SDS at 65°C for 15 min. The bound probes were detected with a digoxigenin chemiluminescent detection system, i.e., alkaline phosphatase-conjugated sheep anti-digoxigenin Fab fragments and disodium 3-[4-methoxyxyspiro[1,2-dioxetane-3,2’-(5’-chloro)tricyclo[3.3.1.1³⁷]decane]-4-yl]phenyl phosphate as alkaline phosphatase substrate (Roche Diagnostics).

**Results**

**Isolation and analysis of RT-LBP/BPI cDNA and gene**

For rapid identification of differentially expressed genes, we used the recently described Clontech PCR-Select cDNA Subtraction kit method (37). We used RNA from the rainbow trout head kidney, as it is an important lymphoid organ in teleosts; this implies hemopoietic capacity without renal function (38). Using suppression and subtraction hybridization with normal head kidney and LPS-treatment head kidney mRNA, we found two cDNA fragments (317 and 588 bp) whose amino acid sequences had significant identity to those of human LBP and BPI by basic local alignment search tool analysis (data not shown). These cDNA fragments were used as probes for cloning the full-length cDNAs coding LBP-like molecules from a cDNA library of mRNA from LPS-treatment head kidney. Two cDNA (RT-LBP/BPI-1 and RT-LBP/BPI-2) consisting of 1666 and 1741 bp were cloned. Both clones had an open reading frame (1–1419) that could encode 473 aa with a predicted size of 51 kDa (Fig. 1). These sequences had a putative 18-aa signal peptide, followed by 455 aa residues namely identified as putative mature proteins. Mammalian LBP family proteins have signal peptide. This suggests that these proteins could be secreted in the mature form after cleavage.

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Multiple alignments of LBP and BPI sequences from various species

The deduced amino acid sequences of RT-LBP/BPI-1 and RT-LBP/BPI-2 were aligned with human LBP, BPI, PLTP, and CETP; mouse LBP and PLTP; mouse LBP and PLTP; rabbit LBP; and CETP and bovine BPI, primarily using the Clustal W software, as shown in Fig. 2. The identity between rainbow trout LBPs and previously reported mammalian LBP, PLTP, and CETP ranged from 20 to 34%. The rainbow trout LBPs showed 32–33% and 33–34% amino acid identity to human LBP and BPI, respectively. Each amino acid sequence identity was shown in Table I. RT-LBP/BPI-1 has high homology of 87% with LBP/BPI-2. By contrast, human LBP has only 43% identity with its BPI. Judging from the conservation of amino acid sequences among those of mammals, LBP/BPI genes in rainbow trout may have hybrid characteristics between LBP and BPI in mammals.

Structural analysis of N-terminal RT-LBP/BPIs

Rainbow trout LBP/BPIs contain a relatively high number of basic amino acid residues (Fig. 2). Structural homology analysis of LBP/BPI amino acid sequences by LIBRA (3D-1D analysis) suggests significant similarity with the 3D structure of BPI (data not shown) that was shown by x-ray crystallography (23, 39). It has been demonstrated that BPI has amino acid residues for LPS binding in the

FIGURE 1. Nucleotide and deduced amino acid sequences of RT-LBP/BPI-1 (A) and RT-LBP/BPI-2 (B). Nucleotide numbers, starting from the putative methionine initiation codon, are presented on the right side. The first 18 aa represent a putative signal sequence. The first residue of mature LBP/BPIs is numbered.

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N-terminal barrel (24, 25). Thus, we compared the LPS-binding motifs of human BPI with that of RT-LBP/BPI-1 and RT-LBP/BPI-2 (Fig. 3). The location of basic amino acid residues of RT-LBP/BPI-1 (lysine or arginine 42, 48, 92, 94, and 100) and RT-LBP/BPI-2 (lysine or arginine 42, 48, 92, 94, and 102) showed similarity to those of BPI and LBP (lysine 42, 48, 92, 95, and 99 in human BPI). An analysis of BPI by x-ray crystallography (24, 25) had previously demonstrated the position on the tip of N-terminal domain.

Structural analysis of C-terminal RT-LBP/BPIs

The function that has been reported for the C-terminal barrel portion of human LBP and BPI is that the former binds to CD14 on macrophages (4) and the latter binds to unknown receptors on neutrophils and monocytes (15). Since the functional differences between LBP and BPI have been attributed to structural differences in the C-terminal barrel, we intend to compare the amino acid sequence of the C-terminal barrel for RT-LBP/BPI-1 and RT-LBP/BPI-2. The same structural differences, which affect the functional properties of human LBP and BPI, may also account for the differences between RT-LBP/BPI-1 and RT-LBP/BPI-2. To determine whether this is the case, we compared the homology of the C-terminal and the N-terminal of human LBP and BPI with the same structures of RT-LBP/BPI-1 and RT-LBP/BPI-2. As shown in Table II, the N-terminal barrel of human LBP and BPI has a homology of 46.5%, while the homology of the C-terminal barrel is only 38.9%. Besides the homology between RT-LBP/BPI-1 and RT-LBP/BPI-2, there is also a 83.9% homology in the N-terminal barrel and 89.8% in the C-terminal barrel.

Basicity analysis of RT-LBP/BPIs

Based on the structural analyses, we examined basicity to determine whether it was one of the factors defining the binding affinity of these molecules to LPS (25). Human BPI is known as a cationic, antibacterial protein and has a somewhat higher affinity to LPS than human LBP. Basicity of the N-terminal barrel, central sheet, and C-terminal barrel in rainbow trout LBP/BPIs, mammalian BPI, and LBP is shown in Table III. Mammalian BPI proteins have high basicity in the N-terminal portion (basicity, 13–21), while LBP proteins have lower basicity (basicity, 1 to 4). Also, the basicity in the C-terminal barrel of mammalian BPI proteins is 1 to 7, and those of LBP proteins is 2 to 1. By contrast, the basicity in the N-terminal barrel of RT-LBP/BPI-1 and RT-LBP/BPI-2, there is also a 83.9% homology in the N-terminal barrel and 89.8% in the C-terminal barrel.

Phylogenetic tree of RT-LBP/BPI proteins

A phylogenetic tree of the LBP family was drawn using the neighbor-joining method (40). As shown in Fig. 4, RT-LBP/BPI-1 and RT-LBP/BPI-2 formed a cluster with mammal LBP and BPI, which was supported by a high bootstrap value. The phylogenetic analysis suggests that the LBP and BPI diverged after the divergence of mammals from teleosts.
The increase in the number of copies of RT-LBP/BPI-1 and RT-LBP/BPI-2 mRNA in head kidney and liver before and after LPS treatment

The number of copies of RT-LBP/BPI-1 and RT-LBP/BPI-2 mRNA was measured over time for a period of 24 h after LPS treatment using a competitive PCR method. As shown in Fig. 5, there was a 5-fold relative increase in the number of copies of RT-LBP/BPI-1 mRNA in head kidney. By contrast, an increase did not occur in liver after LPS treatment. RT-LBP/BPI-2 mRNA increased 2.4 times in head kidney, but liver showed no significant increase in the number of copies of RT-LBP/BPI-2 mRNA after LPS treatment. The number of copies of RT-LBP/BPI-1 mRNA in normal liver and head kidney was 8.4 × 10^7/100 ng total RNA and 2.1 × 10^3/100 ng total RNA, respectively. The number of copies of RT-LBP/BPI-2 mRNA in normal liver and head kidney was 1.4 × 10^7/100 ng total RNA and 6.9 × 10^3/100 ng total RNA, respectively. From these results, the number of copies of RT-LBP/BPI-1 mRNA was 30–60 times higher than the RT-LBP/BPI-2 level in normal liver and head kidney.

Southern blot analyses of the RT-LBP/BPI-1 and RT-LBP/BPI-2

The presence or absence of a LBP/BPI homolog in rainbow trout was confirmed by genomic Southern blot analyses. As shown in Fig. 6, Southern blot analyses revealed one major band of RT-LBP/BPI-1 corresponding to the probe for RT-LBP/BPI-1 in HindIII, EcoRI, and ApaI restriction enzyme digests. There was also one major band of RT-LBP/BPI-2 to the probe for RT-LBP/BPI-2 in HindIII, BamHI, EcoRI, ApaI, and KpmI restriction enzyme digests of rainbow trout genomic DNA. These findings strongly suggest that RT-LBP/BPI-1 or RT-LBP/BPI-2 are unique genes in the genome.

Table II. Percentage of sequence identity of RT-LBP/BPI-1 and RT-LBP/BPI-2 to human LBP and BPI corresponding to the N- and C-terminal barrel and central β sheet

<table>
<thead>
<tr>
<th></th>
<th>RT-LBP/BPI-2</th>
<th>Human LBP</th>
<th>Human BPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-terminal barrel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT-LBP/BPI-1</td>
<td>83.9</td>
<td>32.2</td>
<td>31.2</td>
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<tr>
<td>RT-LBP/BPI-2</td>
<td>31.2</td>
<td>30.6</td>
<td></td>
</tr>
<tr>
<td>Human LBP</td>
<td>46.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central β sheet</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RT-LBP/BPI-1</td>
<td>90.5</td>
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<td>RT-LBP/BPI-2</td>
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<td>36.2</td>
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</tr>
<tr>
<td>Human LBP</td>
<td>46.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-terminal barrel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT-LBP/BPI-1</td>
<td>89.8</td>
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<tr>
<td>Whole</td>
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<td>34.3</td>
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<tr>
<td>RT-LBP/BPI-2</td>
<td>43.8</td>
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</tbody>
</table>

Discussion

A comparison of amino acid sequences of the LBP family in mammals, both of RT-LBP/BPI-1 and RT-LBP/BPI-2 protein, showed similar homologies ranging from 32 to 34% with human LBP and BPI (Table I). However, the biological function of RT-LBP/BPI proteins remains unclear. To estimate the biological function of these proteins, a 3D analysis should be informative. A LIBRA analysis of RT-LBP/BPI proteins showed a high degree of structural similarity with mammal BPI (data not shown). BPI has been shown by x-ray crystallography to have a boomerang shape structure with the N-terminal barrel and the C-terminal barrel connected by central β sheet (23–25, 39). Detailed analyses of the structure of human LBP, PLTP, and CETP have shown their boomerang-shaped, two-domain structure and polar lipid-binding pockets (41, 42). Thus, RT-LBP/BPI-1 and RT-LBP/BPI-2 probably have boomerang shape structures with N-terminal and C-terminal barrels connected by a central β sheet.

LBP and BPI are distinguished from PLTP and CETP because they have the function of binding LPS with high affinity. Recent analyses suggest that conserved positive charge amino acids in the N-terminal barrel of mammal LBP and BPI (lysine 42, 48, 92, 95, and 99 in human BPI) were involved in binding the anionic portion of lipid A (25). As these residues are clustered at the tip of the NH₂-terminal domain, they may cause electrostatic interactions with negatively charged groups of LPS (25, 43). As shown in Figs. 2 and 3, basic amino acid residues in RT-LBP/BPIs (lysine or arginine 42, 48, 92, 94, and 100 in RT-LBP/BPI-1) were well conserved at a similar position. These data suggest that RT-LBP/BPI-1 and RT-LBP/BPI-2 proteins might bind LPS, and thus may have a pivotal role in the innate immune system in fish. However, because profoundly different amino acid sequences may assume the same 3D structure (39), we cannot make any conclusions about the functional properties of these genes until we have functional data using the gene products.

The function of the C-terminal barrel of mammalian LBP and BPI has been reported as binding the receptor on macrophages or neutrophils. The C-terminal barrel of human LBP is essential in binding CD14 on macrophages (4, 25). The C-terminal barrel of human BPI is associated with contacting an unknown receptor on monocytes.

Table III. Overall charge of RT-LBP/BPI-1, RT-LBP/BPI-2, and mammalian LBP/BPIs corresponding to the N- and C-terminal barrel and central β sheet

<table>
<thead>
<tr>
<th></th>
<th>N-Terminal Barrel</th>
<th>Central β Sheet</th>
<th>C-Terminal Barrel</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT-LBP/BPI-1</td>
<td>3</td>
<td>0</td>
<td>5</td>
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<tr>
<td>RT-LBP/BPI-2</td>
<td>10</td>
<td>−1</td>
<td>6</td>
</tr>
<tr>
<td>Human-LBP</td>
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<td>−2</td>
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<tr>
<td>Rabbit-LBP</td>
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<td>−1</td>
</tr>
<tr>
<td>Mouse-LBP</td>
<td>4</td>
<td>2</td>
<td>−2</td>
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<tr>
<td>Human-BPI</td>
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<td>−2</td>
<td>1</td>
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<td>Rabbit-BPI</td>
<td>21</td>
<td>ND</td>
<td>7</td>
</tr>
<tr>
<td>Bovine-BPI</td>
<td>13</td>
<td>−2</td>
<td>2</td>
</tr>
</tbody>
</table>

*Overall charge is estimated as (no. of Lys + Arg) − (no. of Glu + Asp). ND, not down for incomplete sequence data.
macrophages and neutrophils (15, 44). Thus, structural differences of C-terminal barrels in LBP and BPI should be affecting the biological differences of LBP and BPI. In fact, the homology of the N-terminal barrels between human LBP and BPI, functionally LPS-binding part, was 46.5% homology, compared with the C-terminal barrel, which only had a 38.9% homology (Table II). This suggests that the C-terminal barrel has evolved much faster than N-terminal barrel. The homology between the C-terminal barrel of RT-LBP/BPI-1 and RT-LBP/BPI-2 is higher than 80%, suggesting that the C-terminal of RT-LBP/BPI-1 and RT-LBP/BPI-2 has not yet diverged.

Also, the phylogenetic analysis suggests that mammalian LBP and BPI genes were duplicated and evolved after mammals diverged from teleosts (Fig. 4). This analysis supports the hypothesis that RT-LBP/BPI-1 and RT-LBP/BPI-2 might be the ortholog for the LBP family found in mammals.

The number of copies of RT-LBP/BPI-1 and RT-LBP/BPI-2 mRNA in liver and head kidney was measured by the competitive PCR method. Different expression patterns were observed for RT-LBP/BPI-1 and RT-LBP/BPI-2, which corresponded to the mRNA after LPS treatment of liver and head kidney. The number of copies of RT-LBP/BPI-1 was 30–60 times higher than that of RT-LBP/BPI-2 mRNA in normal liver and head kidney. The different expression of RT-LBP/BPI-1 and RT-LBP/BPI-2 suggests that the functional divergence of these molecules is developing as different innate immune responses.

Wan et al. (45) reported that LBP mRNA in rat hepatocytes isolated from livers increased 20 times during 6–12 h after i.p. administration of LPS. This is clearly different from the result shown in Fig. 5, which showed slight change of RT-LBP/BPIs mRNA in liver after LPS administration. This difference may be partly due to the lower responsiveness of LPS in rainbow trout compared with those in mammalian and/or avian reported by Kodata et al. (46). An analysis that clarifies the biological functions of the molecules will be required before we can adequately address the functional properties of RT-LBP/BPIs.

Both LBP and BPI are clearly involved in the innate immune system, but the intrinsic role of each remains elusive. Identifying the principal roles of LBP and BPI would provide clues for understanding the innate immune system. Further investigation of the protein function of the LBP/BPI gene products of rainbow trout...
may determine the functional roles of RT-LBP/BPI-1 and RT-LBP/BPI-2. This information could help explain the immune system in fish, and might also illuminate the role of the innate immune system in other species.

References