Parotid secretory protein is an HDL-associated protein with antican didal activity

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High-density lipoproteins (HDL) are heterogeneous lipoprotein particles in the density range of 1.063–1.21 g/ml that contain lipids, mainly cholesterol and phospholipids, and a variety of proteins, including apolipoprotein (apo) A-I, apo A-II, apo C, lecithin:cholesterol acyl transferase, and cholesterol ester transfer protein (CETP). HDL plays a role in a wide range of biological functions. It is best known as a key factor in protection against atherosclerosis by facilitating the removal of excess cholesterol from macrophages and the movement of cholesterol to the liver for excretion (46). In addition, HDL can inhibit cytokine-induced adhesion molecule expression on endothelial cells, thus preventing the entry of macrophages into the arterial wall. Other anti-inflammatory and antithrombotic effects of HDL have been observed (33). HDL also plays a role in innate immunity by scavenging endotoxin and ameliorating its deleterious effects (1, 9, 31, 45).

The biochemically diverse effects of HDL are carried out by different proteins that have been shown to associate with HDL, and each of these proteins plays a distinct role in the function and metabolism of HDL (22, 32). Identification of new proteins associated with HDL will eventually unravel the biological roles of HDL in a variety of physiological and pathological states.

Using two-dimensional gel electrophoresis of HDL and mass spectrometry (MS), we found a salivary protein not previously known to be in the circulation or associated with HDL. In the present study, we describe the identification of parotid secretory protein (PSP) as an HDL-associated protein. We cloned a hamster homolog of rat and mouse PSP and studied the tissue distribution. We expressed recombinant PSP in Escherichia coli and used the purified protein in the subsequent experiment and found that PSP exhibited antican didal activity. The identification of PSP on HDL thus supports the close relation between lipoproteins and innate immunity.

MATERIALS AND METHODS

Materials. ImmunoPure Plus immobilized protein G and B-Per 6xHis fusion protein purification kit were obtained from Pierce (Rockford, IL); cyanogen bromide-activated Sepharose 4B, thio propyl Sepharose 4B, Immobiline DryStrip, pH 3–10 NL (nonlinear), and immobilized pH gradient buffer from Amersham Biosciences (Piscataway, NJ); rabbit anti-hamster albumin antiserum, used to construct anti-hamster albumin columns, from Accurate Chemicals (Westbury, NY); Ultrafree centrifugal devices and C18 ZipTip from Millipore (Bedford, MA); sequencing-grade modified trypsin from Promega (Madison, WI); chemical supplies from Sigma (St. Louis, MO) or Amersham Biosciences; endotoxin (LPS) from E. coli serotype 055:B5 from Difco (Detroit, MI); RT-PCR and rapid amplification of cDNA ends (RACE) kits from Clontech (Palo Alto, CA); gel extraction kit from Qiagen (Valencia, CA); recombinant protein expression kit from Invitrogen (Carlsbad, CA); and Candida albicans 10231 from American Type Culture Collection (Manassas, VA).

Animal experiments. Syrian hamsters (8–12 wk old) were obtained from Charles River (Wilmington, MA); C57BL6 mice (6–8 wk old) from Jackson Laboratory (Bar Harbor, ME); and sialometomized mice (from which all major salivary glands were removed), castrated mice, and sham-operated mice from Charles River. Mice lacking acyl CoA:diacylglycerol acyltransferase 1 (DGAT1) were obtained as previously described (5). Rodent chow and water were provided ad libitum. In some experiments, animals were injected intraperitoneally with endotoxin; control animals were injected with normal saline. The dose of endotoxin was 100 μg/100 g body wt for hamsters and 100 μg/animal for mice. At these doses of endotoxin, the acute-phase response was induced and animals became toxic; however, these

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Protein spots were visualized using the Bio-Rad imaging densitometer. We also created a database of known HDL-associated proteins and used published two-dimensional gel patterns of HDL in several animal species for references.

**Identification of proteins by MS.** Silver-stained proteins were reduced with 1 mM dithiothreitol, alkylated with 2 mM iodoacetamide, and in-gel digested with trypsin (see http://donatello.ucsf.edu/ing.html). The peptides were extracted and purified using C18 ZipTip. An aliquot of each digest was subjected to matrix-assisted laser desorption ionization (MALDI-MS) as well as to liquid chromatography (LC)-MS-MS analysis. The MALDI-MS and MS-MS, i.e., high-energy collision-induced dissociation (CID) analyses, were performed on a Proteomics Analyzer (model 4700, Applied Biosystems, Framingham, MA) using α-cyano-4-hydroxycinnamic acid as matrix (16). LC-MS-MS was performed on a QSTAR Pulsar quadrupole-orthogonal-acceleration-time-of-flight tandem mass spectrometer equipped with an electrospray source (MDS Sciex, Toronto, ON, Canada). The peptides were separated by nano-HPLC (Ultimate HPLC system with Famous autosampler, LC Packings, San Francisco, CA) developing a 5–50% B linear gradient over 30 min on a 75-μm ID Pepmap column (LC Packings) at a flow rate of ~300 nl/min. Solvent A was 0.1% formic acid in water, and solvent B was 0.1% formic acid in acetonitrile. The eluent was directly introduced into the mass spectrometer and monitored in an information-dependent acquisition mode; 1-s mass measurements were followed by 5-s CID experiments throughout the analysis. Multiply charged precursor ions were selected by computer, and the collision energy was adjusted accordingly to the mass (m) and charge (z) of the ion fragmented. The identity of the proteins is usually determined by database searches with the uninterpreted CID data against all the proteins in the National Center for Biotechnology Information (NCBI) database using the appropriate Bioanalyst Script (MDS Sciex) and the Mascot software (www.matrixscience.com) or Protein Prospector MS-Tag (www.prospector.ucsf.edu). De novo sequencing was performed without the aid of computer programs. BLAST and MS-Profile searches were performed with the sequences database.

**Cloning of hamster PSP cDNA.** A hamster homolog of rat and mouse PSP cDNA was cloned from the hamster parotid gland RNA by RT-PCR using the rat PSP primers: 5′ ATG TTC CAA CTT GGG AGC C3′ and 5′ CCC AAC AAG GAA ATG GAG AT3′. The band was gel purified and used for sequencing. 5′ RACE and 3′ RACE were performed following the manufacturer’s instructions using hamster PSP-specific primers: 5′ CCC AAC AAG GAA ATG GAG ATT TTG TCT G3′ and 5′ CTT ATC ACC TAA GAA TGG TTT AGG GTT GC3′. All the nucleotide sequencing was performed at Sequetech (Mountain View, CA).

**Isolation of RNA and RT-PCR.** Isolation of RNA and RT-PCR were performed as previously described (20). Expression of mouse PSP mRNA was detected in various tissues by RT-PCR using the following mouse primers: 5′ CTT CTG CAG CTG TTC TCC A3′ and 5′ CCC AAC AAG GAA ATG GAG AT3′. The amplified DNA was purified using the appropriate Bioanalyst Script (MDS Sciex) and the Mascot software (www.matrixscience.com) or Protein Prospector MS-Tag (www.prospector.ucsf.edu). De novo sequencing was performed without the aid of computer programs. BLAST and MS-Profile searches were performed with the sequences database.
PSP is an HDL-associated protein with antifungal activity

A secondary antibody (Alexa Fluor chicken anti-rabbit IgG; Molecular Probes, Eugene, OR). Counterstaining for nuclei was performed with 1 mg/ml propidium iodide. After they were washed, the slides were mounted and viewed using a confocal microscope (Zeiss, Heidelberg, Germany).

In some experiments, immunohistochemistry was also performed. Endogenous peroxidase activity was blocked with 1% H2O2 in methanol for 15 min before the blocking buffer. After overnight incubation with a primary antibody, sections were incubated with a secondary antibody (biotinylated goat anti-rabbit IgG; Vector Laboratories, Burlingame, CA) followed by Vectastain and diaminobenzidine peroxidase substrate kit (Vector Laboratories). Slides were viewed with a light microscope.

Sections incubated with normal rabbit antibody (Santa Cruz Biotechnology, Santa Cruz, CA) during primary antibody incubation served as a negative control. The specificity of the anti-mouse PSP antibody was also demonstrated using peptide competition, which markedly reduced the immunoreactivity (see Fig. 5C).

Plasmid construction and bacterial expression of recombinant PSP. The sequence of hamster PSP (bp 61–708, excluding the signal sequence) was PCR amplified using a 5′ primer that introduced an XhoI site immediately upstream of bp 61 and a 3′ primer that introduced a PstI site 3′ downstream of the natural stop codon. The resulting fragment was appropriately digested and subcloned into 6xHis fusion vector pRSET A. PSP/6xHis expression in the BL21(DE3)pLysS strain of resulting fragment was appropriately digested and subcloned into the pET-22b expression vector. The recombinant protein was purified using B-Per 6xHis fusion protein purification kit. The protein was analyzed for purity and integrity by SDS-PAGE and Western blotting using anti-PSP and anti-6xHis antibodies.

Antifungal activity assay. Various concentrations of purified PSP proteins were incubated with C. albicans (1 × 10⁵/ml) in culture for 120 min, and aliquots of culture were plated to assess the number of colony-forming units.

Bioinformatic analyses. BLAST and PSI-BLAST were performed using the nonredundant database through the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST). Searches for conserved domains and predicted secondary and tertiary structures were performed using 3DPSM (http://www.sbg.bio.ic.ac.uk) as previously described (19). Multiple sequence alignments were performed using ClustalW and Boxshade (http://www.ch.embnet.org). Hydrophobicity plot was assessed by Kyte-Doolittle analysis.

RESULTS

Identification of PSP as an HDL-associated protein in hamsters. Column-purified HDL from hamster plasma was subjected to two-dimensional gel electrophoresis, and HDL-associated proteins were visualized using silver staining. A representative two-dimensional gel of proteins is shown in Fig. 1A. Using a database of HDL-associated proteins that we created and the two-dimensional gel patterns of HDL published in the literature, we selected certain protein spots for identification. For confirmation, we selected spots that represent known HDL-associated proteins. As a result, apo A-I and apo A-II were identified by MS (Fig. 1A). We then selected some “unknown protein” spots, the location of which did not match the molecular weight and isoelectric point of the known HDL-associated proteins. We, however, did not attempt to identify all protein spots in the two-dimensional gels. Some of the unknown protein spots were identified as known HDL-associated proteins, such as apo A-IV, apo E, and apo serum amyloid A (Fig. 1A). Spot 1 was among the unknown protein spots selected and submitted for identification by MS. MALDI-MS analysis of the unfraccionated digest showed a single peptide at m/z 1,651.78. Manual interpretation of the high-energy CID spectrum of this peptide yielded a partial sequence of Q/KISISLLGLR. High-energy CID fragmentation permitted the unambiguous assignments of the isomeric Leu/Ile residues (17, nomenclature as in Ref. 3). LC-MS-MS analysis with low-energy CID yielded only one additional sequence of TGI/LPTI/LTI/LGK (m/z 500.82²⁺). In low-energy CID, Leu and Ile residues are indistinguishable.

BLAST and MS-Pattern searches were performed with the two sequences determined. Both sequences showed homology to a salivary protein from rats and mice known as PSP (Fig. 2A).

To ensure that PSP is truly an HDL-associated protein and that its protein spot on the two-dimensional gel was not due to isolation artifacts from the columns, we prepared hamster HDL by ultracentrifugation and subjected it to two-dimensional gel electrophoresis. A spot corresponding to hamster PSP was also found in ultracentrifuged HDL (data not shown).

Cloning of the putative hamster PSP cDNA. After we putatively identified the hamster homolog of rat and mouse PSP in HDL, we cloned the hamster PSP cDNA. On the basis of close homology to rat PSP, we amplified the coding sequence of hamster PSP by RT-PCR using primers derived from the rat nucleotide sequence. The complete sequence of the putative hamster PSP (accession no. AY162470) contained an open reading frame of 705 bp predicted to encode a protein of 235 amino acids (Fig. 2A). The first 60 bp were predicted to encode a highly hydrophobic region, which is likely a signal peptide of 20 amino acids. Similar to rat and mouse PSP, hamster PSP is leucine rich (24%). A predicted molecular mass of the mature peptide after the signal sequence is cleaved is ~22,600 Da, and the isoelectric point is expected to be ~4.5. The predicted molecular mass is lower than that expected from the location of the spot found on the gel, suggesting that hamster PSP may be posttranslationally modified. Several sites for protein kinase C phosphorylation, casein kinase II phosphorylation, N-myristoylation, and amidation were predicted, but no glycosylation site was found. We also performed 5′ RACE and 3′ RACE, which identified ≥68 bp of the 5′-untranslated sequence and 232 bp of the 3′- untranslated sequence. Hydrophobicity plot revealed another hydrophobic domain, which likely associates with lipids in addition to a hydrophobic signal peptide (data not shown).

Analysis of the NCBI protein database revealed that hamster PSP shared 59% amino acid identity with rat PSP (accession no. NP_434695) and 57% identity with mouse PSP (accession no. NP_032979). The three cysteines are also conserved among hamster, rat, and mouse PSP (Fig. 2A). In addition, hamster PSP also showed homology to murine neonatal submandibular gland protein B (accession no. XP_141556, 42% identity) (30), BSP30, a putative bovine PSP homolog (accession no. AAB38282, 30% identity) (37), von Ebner’s salivary gland protein (accession no. NP_700467, 25% identity) (38), and PLUNC (palate, lung, and nasal epithelium clone; accession no. NP_080266, 25% identity) (47).

To obtain further insight into the potential function of hamster PSP, searches for conserved functional domains, as well as secondary and tertiary structure, were performed (19). The searches revealed that the COOH terminus of hamster PSP is homologous to a region in the NH2 terminus of a family of...
Fig. 1. Representative 2-dimensional gel electrophoresis results of HDL-associated proteins from hamster column-purified HDL (A and C) and mouse ultracentrifuged HDL (B). Hamster HDL was isolated by hamster apolipoprotein (apo) A-I column chromatography; mouse HDL was isolated by ultracentrifugation. Hamster and mouse HDL (200 μg of protein) were subjected to 2-dimensional gel electrophoresis, and protein spots were visualized using silver staining. Proteins were identified using mass spectrometry. Nonlinear pH gradient was from pH 3 to pH 10. Molecular mass markers (kDa) are shown at left. Spot 1 in A was identified as a hamster homolog of rat and mouse parotid secretory protein (PSP). Spot 1 in B was identified as mouse PSP. C: representative 2-dimensional gel electrophoresis results of hamster acute-phase HDL. Arrows, PSP. Three different sets of control and acute-phase HDL gave similar results.
proteins including LPS-binding protein (LBP), bactericidal/permeability-increasing protein (BPI), ribbit LBP, human cholesterol ester transfer protein (CETP), and mouse phospholipid transfer protein (PLTP). Amino acid residues identical in the alignment are shown in black boxes; similar residues are shown in gray. cDNA sequence of hamster PSP is available from GenBank (accession no. AY162470).

Hamster PSP was also predicted to have a tertiary structure similar to that of the NH2 terminus of human BPI. The expectation (E) value for the prediction was 0.0005, which suggested >95% certainty. A similar result was also found for mouse PSP (E = 0.0036). Because BPI consists of two domains (NH3 and COOH termini) of similar size, secondary structure, and topology, the predicted structure of hamster PSP represents only half of the BPI molecule. It is of note that the efficacy of the recombinant NH2-terminal fragment of BPI in binding LPS and neutralizing its effects is similar to that of the full-length protein (15, 34). Homologies to the NH2-terminal domain of BPI have previously been found in von Ebner’s salivary gland protein and PLUNC, two proteins closely related to PSP (4, 42).

PSP is also an HDL-associated protein in mice. To determine whether PSP is an HDL-associated protein in other species, we isolated ultracentrifuged HDL from mouse sera and subjected mouse HDL to two-dimensional gel electrophoresis and MS. Because the sequence of mouse PSP has been known and present in the NCBI database, the spot corresponding to mouse PSP should be easily identified by MS if it is associated with HDL. Indeed, mouse PSP (spot 1 in Fig. 1B) was independently identified from the two-dimensional gel without prior knowledge of the sequence from the hamster. Eight peptides selected randomly from the mass spectra (m/z 1,100 to 6,300) showed that they matched a total of 59% of the amino acid sequence of the mouse PSP (data not shown). To examine whether PSP is associated with other lipoprotein fractions, we isolated mouse lipoproteins by ultracentrifugation and performed immunoblot analysis using an antipeptide antibody against mouse PSP. PSP was detected only in HDL, and...
PSP is expressed in mouse lung, skin, and gonads. PSP is one of the major proteins found in the saliva of rats and mice (18, 35). Previous studies reported that PSP mRNA is expressed only in the salivary and lacrimal glands (29, 40). Because our study showed that PSP was found in HDL in the circulation, we hypothesized that PSP might have a wider tissue distribution than previously thought. Using RT-PCR, we found that, in addition to the salivary glands, PSP mRNA was expressed in the mouse lung, ovary, and testis (Fig. 4). Northern analysis, as well as finding numerous mRNA fragments of PSP in the EST database, further revealed that PSP was expressed in the skin (data not shown). Using a combination of RT-PCR and Northern analysis, we did not detect signals from the liver, spleen, kidney, heart, stomach, small intestine, pancreas, urinary bladder, and brain (data not shown).

Because of the relatively low levels of PSP detected in certain tissues, we hypothesized that PSP might be restrictively expressed only in a subset of cells. To localize the cellular origin of the PSP in tissues, we examined mouse organ sections using immunofluorescence and immunohistochemistry. As a positive control, we detected abundant immunoreactivity in the glandular epithelium of the parotid and submandibular glands and showed that it was blocked by the peptide (data not shown). In agreement with the mRNA data, we detected PSP immunoreactivity in the lung, skin, testis, and ovary (Fig. 5). We also screened several other tissues and did not detect specific immunoreactivity in the liver, spleen, kidney, heart, stomach, small intestine, colon, pancreas, prostate, or uterus (data not shown).

In the lung, PSP was exclusively localized to the respiratory epithelium in the bronchioles (Fig. 5). The immunoreactivity in the respiratory epithelium was discontinuous, and only a subset of cells lining the respiratory epithelium of the bronchioles expressed PSP. We did not detect specific immunoreactivity in the alveoli.

In the skin, PSP immunoreactivity was found in the keratinocytes, around the hair follicles, and in the sebaceous gland (Fig. 5).

In the testis, PSP immunoreactivity was found only in Leydig cells, and not in the seminiferous tubules (Fig. 5). In the ovary, PSP immunoreactivity was localized mainly to the stromal cells and was not found in the ovarian follicles (Fig. 5). However, we could not rule out that PSP might be expressed in the thecal cells.

Collectively, our study shows that PSP is expressed in several organs and is not restricted only to the salivary and lacrimal glands, as previously reported (29, 40). Because PSP is a secreted protein, each of the organs that expresses PSP could potentially secrete PSP into the circulation and contribute to the presence of the protein found in HDL. To examine the contribution by various organs, we selectively removed all the major salivary glands (or in sialectomized mice) or the testes (in castrated mice). At ~2 wk after surgery, HDL was isolated from pooled mouse sera of sialectomized or castrated mice, and immunoblot analyses for PSP were performed, with HDL from sham-operated mice used as a control. We found that the level of PSP in HDL was not decreased, despite removal of the salivary glands or testes (data not shown). The result suggests that other tissues or multiple tissues may contribute to the level of PSP in HDL. Less likely, the half-life of PSP in the circulation may be prolonged, and 2 wk after removal of the tissues producing PSP might not have been long enough to detect the decrease of PSP levels in HDL.

Changes in PSP levels in HDL in response to endotoxin. Because of the LBP-BPI-CETP NH2-terminal domain found in the PSP sequence, we hypothesized that PSP might be involved in the innate immune response and that PSP levels might be regulated by endotoxin. LBP and BPI, which play a role in innate immunity, are upregulated by endotoxin (7, 8). In contrast, CETP and PLTP levels are decreased after endotoxin administration (13, 26).

To determine whether the levels of PSP in HDL were affected by endotoxin, hamsters were injected with endotoxin, whereas control animals were injected with normal saline. Acute-phase HDL from plasma of endotoxin-injected hamsters and control HDL from plasma of saline-injected hamsters were separately purified using hamster apo A-I immunoadfinity columns followed by anti-hamster albumin columns. Both preparations of HDL were subjected to two-dimensional gel electrophoresis, and the amount of PSP in HDL was compared using silver staining. As shown in Fig. 1C, we found more hamster PSP in acute-phase than in control HDL (2.6-fold increase, 3 different sets of experiments). However, when we performed similar experiments in mice using ultracentrifuged HDL, we did not find a consistent increase in the amount of PSP in mouse acute-phase HDL (data not shown). These data suggest that there may be species-specific changes in the level of PSP in response to endotoxin, as seen with many other serum proteins (28).
Fig. 5. Immunoreactivity of mouse PSP in different tissues. Mouse tissues were prepared for immunofluorescence microscopy. A: confocal microscopy allowed us to visualize a strong green signal for PSP localized to different cell types (using anti-mouse PSP antibody). B: negative controls; almost no green signal was observed (using normal rabbit antibody). Strong red signals showed nuclei staining by propidium iodide. Magnification: ×400, except in the lung in B (×100) and in testis in A and B (×1,000). C: specificity of the antibody using peptide competition.
Recombinant PSP inhibits growth of C. albicans. Because PSP is an abundant protein in saliva and its structure is closely similar to BPI, which exhibits antibacterial activity, we hypothesized that PSP may be involved in antimicrobial function. When recombinant hamster PSP was incubated with Staphylococcus epidermidis or E. coli, growth of these bacteria was not inhibited (data not shown). However, when PSP was incubated with cultures of C. albicans, recombinant PSP inhibited the growth of C. albicans in a dose-dependent manner (Fig. 6).

DISCUSSION

A number of proteins are associated with HDL particles, and these proteins play different roles in the function and metabolism of HDL (22, 32). In this study, we identified PSP as an HDL-associated protein. PSP is one of the major proteins in the saliva of mice and rats. However, it has not previously been shown to be present in the circulation or associated with HDL. Although we initially hypothesized that some of the HDL-associated proteins might be lost during ultracentrifugation, using apo A-I immunoaffinity column chromatography or ultracentrifugation, we were able to identify a hamster homolog of mouse and rat PSP as an HDL-associated protein. Data from mice confirmed that PSP is associated with mouse HDL. PSP was not associated with other lipoproteins or other plasma fractions.

PSP was initially discovered in rat and mouse parotid saliva (18, 35). Rat and mouse PSP have a high content (~20%) of leucine, which suggests that PSP may be involved in protein-protein interaction. Our searches for conserved domains revealed that part of the COOH terminus of hamster and mouse PSP is homologous to the NH2-terminal domain of a family of proteins including LBP, BPI, CETP, and PLTP. The fact that PSP has this conserved domain is of special interest for a number of reasons. 1) This protein family includes LBP, BPI, CETP, and PLTP. LBP, CETP, and PLTP are known to be HDL-associated proteins (36, 43, 48), but BPI, which is found in neutrophil granules, is not. Thus it is not surprising that PSP is associated with HDL, similar to other proteins in the family. 2) LBP, CETP, and PLTP are lipid transfer proteins (43). LBP binds LPS and transfers it to soluble CD14 (49) and HDL (48). CETP mediates cholesterol ester exchange with triglyceride between HDL and triglyceride-rich lipoproteins, whereas PLTP facilitates phospholipid transfer between lipoproteins. Whether PSP could function as a lipid transfer protein in HDL requires further investigations. 3) HDL has been recognized to play a role in innate immunity. HDL is known to bind endotoxin and ameliorate its deleterious effects (1, 9, 31, 45). LBP and PLTP are two HDL-associated proteins that can bind endotoxin of bacteria and neutralize its effects (12, 48). With regard to PSP, one study has shown that PSP binds bacterial membranes (40). Data in cows also suggested that high levels of BSP30, a bovine PSP homolog in saliva, conferred protection against the bacterial disease of bloat (37). Our present study shows that PSP inhibits the growth of C. albicans. After our work was completed, human PSP was demonstrated to possess antibacterial activity (11). Collectively, these studies suggest that PSP may be part of a host defense system against microbial infection in the oral cavity and the circulation.

Previous studies reported that PSP expression was limited only to the salivary and lacrimal glands (29, 40). In this study, we provide evidence that PSP is also expressed in several additional tissues, including lung, skin, testis, and ovary. Immunofluorescence studies showed that PSP is produced by a certain subset of cells in the pseudostratified columnar epithelium of the bronchioles. The pattern of PSP expression suggests that it may be secreted by goblet cells or submucosal glands of the respiratory epithelium. Keratinocytes and sebaceous glands of the skin also express PSP. Keratinocytes and sebocytes may secrete PSP into the stratum corneum and sebum, respectively. In the sebaceous gland, alternatively, PSP may bind lipid substances in the sebum and may be involved in the organization of lipids. In the testis and ovary, however, a functional role for PSP cannot be postulated at this time. Together, the sites of tissue expression of PSP support the hypothesis that PSP is a protein produced by a variety of epithelial cells and that it might be involved in the host defense against microbes encountered at those sites. In certain tissues, PSP may also play a role in the organization of lipophilic substances.

The sequence of PSP is homologous to that of two other proteins: von Ebner’s salivary gland protein and PLUNC. Also known as tear lipocalin, von Ebner’s salivary gland protein is a protein found in human saliva, tears, nasal mucus, and sweat (39). The tissue expression of von Ebner’s gland protein in humans overlaps that of PSP (38). Also, von Ebner’s salivary gland protein can bind a number of lipid substances, including fatty acids, cholesterol, and lipid peroxidation products (24).

PLUNC is a group of proteins that also has an overlapping tissue distribution with PSP, including the lungs (4). PLUNC is found in nasal secretion and mucus, but not in saliva or tears (42). PLUNC expression is induced by a variety of stimuli, suggesting that PLUNC may also be an acute-phase protein. For example, the levels of PLUNC in human nasal lavage fluid were increased in workers exposed to an airway irritant (25). PLUNC proteins also share a tertiary structure similar to an
Our experiment in hamsters showed increased PSP levels in acute-phase HDL after injection with endotoxin. This finding is in agreement with data from PLUNC showing that PLUNC is a positive acute-phase protein (25, 42). However, we did not find an increase in PSP in mice after endotoxin injection. We think that the discordant results between hamsters and mice could represent species-specific differences. It is well known that the acute-phase response in different animal species can be different in the direction and magnitude of changes (10). For example, C-reactive protein is a positive acute-phase protein in humans, but its levels change only slightly in rats (28); in contrast, α2-macroglobulin increases in rats, but not in humans (28).

In summary, we identified PSP as an HDL-associated protein in hamsters and mice. PSP is expressed in lung, skin, and gonads, in addition to the salivary glands. The identification of the LBP/BPI/CETP domain in the PSP sequence, the homology to PLUNC, and functional data showing that PSP exhibits anticandidal activity suggest that this protein may be involved in host defense in various parts of the body. Our work, in conjunction with studies by other investigators, suggests that HDL may serve as a reservoir for antimicrobial proteins in the serum and may play an important role in innate immunity.

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PSP IS AN HDL-ASSOCIATED PROTEIN WITH ANTICANDIDAL ACTIVITY