Negative Regulation of Platelet Clearance and of the Macrophage Phagocytic Response by the Transmembrane Glycoprotein SHPS-1*

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SHPS-1 is a receptor-type glycoprotein that binds and activates the protein-tyrosine phosphatases SHP-1 and SHP-2, and thereby negatively modulates intracellular signaling initiated by various cell surface receptors coupled to tyrosine kinases. SHPS-1 also regulates intracellular communication in the neural and immune systems through its association with CD47 (integrin-associated protein) on adjacent cells. Furthermore, recent studies with fibroblasts derived from mice expressing an SHPS-1 mutant that lacks most of the cytoplasmic region suggested that the intact protein contributes to cytoskeletal function. Mice homozygous for this SHPS-1 mutation have now been shown to manifest thrombocytopenia. These animals did not exhibit a defect in megakaryocytopenia or in platelet production. However, platelets were cleared from the bloodstream more rapidly in the mutant mice than in wild-type animals. Furthermore, peritoneal macrophages from the mutant mice phagocytosed red blood cells more effectively than did those from wild-type mice; in addition, they exhibited an increase both in the rate of cell spreading and in the formation of filopodia-like structures at the cell periphery. These results indicate that SHPS-1 both contributes to the survival of circulating platelets and down-regulates the macrophage phagocytic response.

SHPS-1 is a transmembrane glycoprotein that is abundant in neural and myeloid tissues (1–6). This molecule is also known as SIRPα1 (7), BIT (8), MFR (9), and p84 neural adhesion molecule (10). The cytoplasmic region of SHPS-1 contains two immunoreceptor tyrosine-based inhibitory motifs, which recruit and activate the Src homology 2 domain-containing protein-tyrosine phosphatases SHP-1 and SHP-2 in a phosphorylation-dependent manner (1, 7, 11). The putative extracellular region of this protein comprises three immunoglobulin (Ig)-like domains, of which the most amino-terminal, IgV-like domain associates with the ligand CD47, also known as integrin-associated protein (6, 12, 13).

Tyrosine phosphorylation of SHPS-1 is induced by soluble growth factors (1, 7, 14, 15), integrin-mediated cell adhesion (16–18), or cross-linking of Fcγ receptors (19). Overexpression of SHPS-1 inhibits the activation of extracellular signal-regulated kinases induced by growth factors such as insulin, epidermal growth factor, and platelet-derived growth factor (7); it also inhibits promotion of the motility and survival of glioblastoma cells by epidermal growth factor (20). Furthermore, SHPS-1 inhibits IgE-induced mediator secretion and cytokine synthesis by mast cells (21). These observations suggest that SHPS-1, presumably by recruiting SHP-1 or SHP-2, negatively modulates a wide range of cellular activation signals initiated by tyrosine kinase-coupled receptors. However, the physiological significance of these observations remains unclear.

Recent studies have suggested that SHPS-1, through its association with CD47, contributes to cellular functions that depend on intercellular communication, including T cell activation (13), T cell arrest on inflammatory vascular endothelium (22), B cell aggregation (23), macrophage multinucleation (24), and phagocytosis of red blood cells (RBCs) by splenic macrophages (25, 26). SHPS-1-CD47 interaction also promotes the adhesion of cerebellar neurons (12) and modifies synaptic activity in the retina (27). Thus, SHPS-1 appears to interact with CD47 on adjacent cells and thereby regulates various cellular responses in the neural and immune systems. The biological consequences of this interaction in vivo, however, remain to be clarified.

We recently generated mice that lack most of the cytoplasmic region of SHPS-1. Characterization of immortalized fibroblasts from these mice revealed important roles for SHPS-1 in both integrin-mediated cytoskeletal reorganization and the down-regulation of growth-factor-induced activation of mitogen-activated protein kinase cascades (28). To elucidate further the physiological roles of SHPS-1, we have now characterized the phenotype of the SHPS-1 mutant mice. The mutant animals were found to exhibit thrombocytopenia, which results from an increased rate of clearance of circulating platelets. Further-
more, peritoneal macrophages (PEMs) from these mice exhibit an enhanced phagocytic response.

**EXPERIMENTAL PROCEDURES**

*Animals*—The generation of mutant mice that lack most of the cytoplasmic region of SHPS-1 has been described (28). The mice were housed and maintained by brother-sister mating on a mixed C57BL/6 x 129Sv genetic background. Genotyping of the mice was performed by polymerease chain reaction analysis as described (28). Mice were housed under pathogen-free conditions and handled in accordance with the animal care guidelines of Kobe University.

**Immunoprecipitation and Immunoblot Analysis**—Mouse tissues were homogenized on ice in lysis buffer (20 mM Tris-HCl (pH 7.6), 140 mM NaCl, 1 mM EDTA, 1% Nonidet P-40) containing 5 mM NaF, 1 mM phenylmethylsulfonyl fluoride, aproatin (10 μg/ml), and 1 mM sodium vanadate. The lysates were centrifuged at 10,000 × g for 15 min at 4 °C, and the resulting supernatants were subjected to immunoprecipitation and immunoblot analysis as described (28). A rat monoclonal antibody (mAb) to p84 (SHPS-1) was kindly provided by C. Lagenaur; rabbit polyclonal antibodies to SHP-2 or to SHP-1 were described previously and immunoblot analysis as described (28). A rat monoclonal antibody and the resulting supernatants were subjected to immunoprecipitation for 30 min with modified Tyrode buffer containing 5 mM NaF, 1 mM sodium vanadate. The lysates were centrifuged at 10,000 × g for 15 min at 4 °C, and the resulting supernatants were subjected to immunoprecipitation and immunoblot analysis as described (28). A rat monoclonal antibody (mAb) to p84 (SHPS-1) was kindly provided by C. Lagenaur; rabbit polyclonal antibodies to SHP-2 or to SHP-1 were described previously (1); and normal rat IgG were obtained from Santa Cruz Biotechnology.

**Tissue Histology and Peripheral Blood Counts**—Tissues were fixed in 3.7% formaldehyde in phosphate-buffered saline, embedded in paraffin, sectioned, and stained with Mayer's hematoxylin-eosin. Peripheral blood samples were obtained from the retro-orbital plexus with 75-mm heparinized capillary tubes (Funakoshi, Tokyo, Japan). Complete blood cell counts were performed with the Sysmex automatic microcell counter F-800 (Toa Medical Electronics, Kobe, Japan). To determine the number of circulating leukocyte subsets, we stained blood smears for the characteristic light scatter of these cells.

**EXPERIMENTAL PROCEDURES**

*Animals*—The generation of mutant mice that lack most of the cytoplasmic region of SHPS-1 has been described (28). Crossing of mice heterozygous for the SHPS-1 mutation revealed that, among the resulting 44 offspring, the ratio of genotypes was consistent with that predicted by Mendelian inheritance (wild-type, 1.73 ± 0.22; heterozygous, 3.34 ± 0.28; homozygous mutant, 1.91 ± 0.23; these values are the number of mice per litter). Mice homozygous for the SHPS-1 mutation appeared healthy and remained viable for >18 months, although their mean body mass was reduced by ~10% compared with that of wild-type littermate controls. Expression of SHPS-1 was examined in lysates of the brain and PEMs by immunoprecipitation and immunoblot analysis with a mAb to SHPS-1 that recognizes the extracellular region of the protein and thus reacts with both the wild-type and mutant forms. Only the truncated form of SHPS-1 was detected in both the brain (Fig. 1A) and PEMs (Fig. 1B) of the homozygous mutant mice, although the abundance of this protein was markedly reduced compared with that of the full-length protein in wild-type animals. Unlike wild-type SHPS-1, the mutant neither underwent tyrosine phosphorylation nor associated with SHP-1 in response to exposure of PEMs to pervanadate, which greatly increases the extent of tyrosine phosphorylation of cellular proteins (Fig. 1B; data not shown). These results indicate that the truncated SHPS-1 is unable to recruit and activate SHP-1, and are consistent with our previous observations with embryonic fibroblasts from these mice (28).

The tissue histology of the homozygous mutant mice and wild-type littermate controls was examined at 3 weeks, 7 weeks, 11 weeks, and 1 year of age. No marked differences were apparent in most tissues, including the brain, heart, lung, kidney, thymus, and bone marrow, between the two types of mice, although the liver of the mutant animals exhibited a markedly increased fat content (data not shown). However, at 11 weeks of age, a large number of megakaryocytes were observed in the spleen of the homozygous mutant mice (Fig. 2,
Fig. 1. Expression of a truncated form of SHPS-1 in the brain and macrophages of mutant mice. A, brain lysates derived from wild-type (+/+) and homozygous SHPS-1 mutant (−/−) mice were subjected to immunoblot analysis either with a mAb to SHPS-1 (upper panel) or with polyclonal antibodies to SHP-2 (lower panel). The positions of wild-type and mutant (ΔSHPS-1) SHPS-1, SHP-2, and molecular size standards (in kilodaltons) are indicated. B, thiglycolate-elicited PEMs were incubated for 5 min at room temperature in the absence (−) or presence (+) of 100 μM pervanadate (NaVO₄), after which cell lysates were subjected to immunoprecipitation (IP) with a mAb to SHPS-1 (αSHPS-1) or with normal rat IgG (NRG). The resulting precipitates were subjected to immunoblot analysis either with the mAb to SHPS-1 (upper panel) or with polyclonal antibodies to SHP-1 (lower panel). Data in both panels are representative of three independent experiments.

Fig. 2. Histological changes in the spleen of SHPS-1 mutant mice. The spleen was removed from 11-week-old wild-type (left panels) or homozygous SHPS-1 mutant (right panels) mice, fixed, and stained with Mayer’s hematoxylin–eosin. Sections were examined with a light microscope equipped with phase-contrast optics, and megakaryocytes are indicated by arrowheads. Data are representative of three independent experiments. Original magnification: ×100 (upper panels) or ×400 (lower panels).

Absence (elicited PEMs were incubated for 5 min at room temperature in the B/H9004 panel subjected to immunoblot analysis either with a mAb to SHPS-1 (wild-type/H11001 or homozygous SHPS-1 mutant/H11002) or presence (H9251 of SHPS-1). The spleen was removed from 11-week-old wild-type/H11003 mice. Apendent experiments. Original magnification: microscope equipped with phase-contrast optics, and megakaryocytes with Mayer’s or homozygous SHPS-1 mutant/H11003 mice, fixed, and stained with Mayer’s hematoxylin–eosin. Sections were examined with a light microscope equipped with phase-contrast optics, and megakaryocytes were indicated by arrowheads. Data are representative of three independent experiments. Original magnification: ×100 (upper panels) or ×400 (lower panels).

Megakaryocytopenia in SHPS-1 Mutant Mice—We next compared peripheral blood cell counts among the homozygous mutant mice, heterozygous mutant mice, and their wild-type littermates at various ages. No significant differences in the numbers of circulating RBCs or reticulocytes or in the percent-ages of leukocyte subsets were apparent among the three types of mice (Table I; data not shown). In addition, mean corpuscular volume, mean corpuscular hemoglobin concentration, and mean platelet cell volume were similar in these animals. In contrast, the circulating platelet count of the homozygous SHPS-1 mutant mice was significantly reduced (by ~25%) compared with that of gender- and age-matched wild-type littermates (Table I); the number of platelets in female mice was 15–20% smaller than that in males regardless of genotype. The difference in platelet number between the homozygous mutant and wild-type mice was detected as early as 4 weeks after birth and remained apparent at 13 weeks of age (Fig. 3). Thus, targeted deletion of the cytoplasmic region of SHPS-1 in mice resulted in moderate thrombocytopenia. The platelet count of the heterozygous mutant mice was variably reduced (by ~20%) compared with wild-type littermates; however, this reduction was not statistically significant (data not shown). We therefore utilized the homozygous mutant mice for the subsequent analyses with wild-type mice as controls.

Megakaryocytopenia apparent in SHPS-1 mutant mice might have resulted either from reduced proliferation or maturation of the megakaryocyte-platelet lineage or from an increased rate of clearance of circulating platelets. To test the former possibility, we counted the number of megakaryocytes in femoral bone marrow and examined their ploidy distribution by flow cytometry. Neither of these parameters differed significantly between wild-type and homozygous mutant mice (Table II). Given that platelets contain SHPS-1 mRNA (data not shown), megakaryocytes might also express the SHPS-1; we therefore examined the ability of bone marrow or spleen cells from the mutant mice to undergo megakaryocytogenesis with a CFU-MK assay in vitro. The CFU-MK from either the bone marrow or spleen of SHPS-1 mutant mice yielded statistically similar numbers of colonies in response to incubation with PEG-rHuMGDF as did those of wild-type mice (Table II). The serum concentrations of thrombopoietin were also similar in wild-type and mutant mice (320 ± 17.4 versus 337 ± 19.5 pg/ml, respectively), indicating that the generation of endogenous thrombo-poietin was not affected by the SHPS-1 mutation. Together, these results demonstrate that neither the generation of megakaryocyte progenitors nor the maturation of these cells is affected by the absence of the cytoplasmic region of SHPS-1, and that suppression of megakaryocytogenesis therefore is not responsible for the thrombocytopenia of the mutant animals.

Shortened Life Span of Circulating Platelets in SHPS-1 Mutant Mice—We next examined the turnover rate of circulating platelets in the homozygous mutant and wild-type mice after myelosuppression with 5-FU. Administration of a single high
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TABLE I
Peripheral blood cell counts in SHPS-1 mutant (−/−) and wild-type (+/+ ) mice at 4 weeks of age

| Parameter | Male (+/+) | Female (+/+ ) | Male (−/−) | Female (−/−) |
|-----------|------------|---------------|------------|--------------|
| RBCs (10^6/μl) | 925 ± 25 | 924 ± 20 | 973 ± 27 | 932 ± 8 |
| WBCs (10^9/μl) | 14.6 ± 1.4 | 25.0 ± 1.8 | 23.2 ± 1.8 | 17.2 ± 0.7 |
| Hb (g/dl) | 15.7 ± 0.33 | 14.8 ± 0.44 | 16.6 ± 0.54 | 15.7 ± 0.26 |
| MCV (fl) | 57.6 ± 0.67 | 54.6 ± 0.68 | 57.2 ± 0.53 | 55.6 ± 0.57 |
| PLTs (10^4/μl) | 98.2 ± 4.1 | 75.6 ± 3.5 | 84.3 ± 4.3 | 64.9 ± 2.3 |
| MPV (fl) | 5.56 ± 0.07 | 5.76 ± 0.05 | 5.70 ± 0.12 | 5.52 ± 0.13 |

* *p < 0.005 versus corresponding wild-type animals (Student’s *t* test).

FIG. 3. Circulating platelet counts in SHPS-1 mutant and wild-type mice at 4–13 weeks of age. The number of platelets in peripheral blood was determined for male and female wild-type and homozygous mutant mice at the indicated ages. Data are means of triplicate determinations for five animals per group. The platelet count differed significantly (p < 0.05) between wild-type and mutant mice of each gender at all ages.

A dose (150 mg/kg) of 5-FU resulted in a decrease in the platelet count of peripheral blood at 5 days and subsequent platelet recovery by 10 days in both types of mice (Fig. 4). Although the initial platelet number was smaller in the mutant mice than in the wild-type animals, the increase in the number of circulating platelets apparent from 5 to 10 days after 5-FU administration did not differ significantly between the two genotypes. At 15 days after 5-FU injection, while the platelet count of wild-type mice remained unchanged compared with the value at 10 days, that of the mutant mice had decreased by ~23%. The number of circulating platelets in the mutant mice remained smaller than that in wild-type mice at all times after 5-FU treatment, with the most marked difference being observed at 15 days (117 ± 41.2) × 10^4 versus 182 (± 13.7) × 10^4 platelets/μl, respectively. These results indicate that platelet production is not affected in SHPS-1 mutant mice, but that the life span of circulating platelets is shortened by the lack of the SHPS-1 cytoplasmic region.

To characterize further the life span of circulating platelets, we biotinylated platelets in vivo and then counted the number of labeled cells remaining in peripheral blood at various times.

TABLE II
Megakaryocytopoiesis in SHPS-1 mutant (−/−) and wild-type (+/+ ) mice at 10 to 12 weeks of age

| Parameter | Male (+/+ ) | Female (+/+ ) | Male (−/−) | Female (−/−) |
|-----------|------------|---------------|------------|--------------|
| AChE-positive cells per femur* | 11,994 | 11,647 |
| Mean ploidy (n) | 14.8 | 15.3 |
| CFU-MK per femura | 729 | 508 |
| CFU-MK per spleena | 112 | 149 |

* The number of megakaryocytes per well was multiplied by the total number of nucleated cells obtained from one femur and divided by the number of colonies plated per well.

a The number of colonies per dish was multiplied by the total number of nucleated cells obtained from either one femur or one spleen and divided by the number of cells plated per dish.

FIG. 4. Platelet turnover in SHPS-1 mutant and wild-type mice after 5-FU administration. Female mice (10–12 weeks of age) were injected intravenously with 5-FU (150 mg/kg), and the number of platelets in peripheral blood was counted at the indicated times thereafter. Data are mean ± S.E. of triplicate determinations for five mice of each genotype. The platelet count differed significantly (p < 0.05) between wild-type and mutant mice at 15 days.

The time course of the reduction in the percentage of biotinylated platelets in peripheral blood revealed that the life span of platelets in the mutant mice was significantly shorter than the corresponding value for wild-type mice (Fig. 5).
Enhanced Phagocytic Activity of PEMs from SHPS-1 Mutant Mice — SHPS-1 expressed in splenic macrophages contributes to the survival of RBCs by negatively regulating phagocytosis (25, 26). This effect requires the interaction of SHPS-1 with CD47 expressed on the surface of RBCs and appears to be mediated by SHP-1 (19, 25, 26). Given that the truncated form of SHPS-1 lacks the immunoreceptor tyrosine-based inhibitory motifs that bind SHP-1 (Fig. 1B; Ref. 28), it might be expected to have lost the ability to suppress phagocytosis by macrophages. We tested this hypothesis with an ex vivo phagocytosis assay in which thioglycolate-elicited PEMs were incubated with PKH26-labeled RBCs from wild-type donor mice; RBCs were used instead of platelets because of technical limitations. Whereas PEMs derived from wild-type mice phagocytosed few RBCs, a substantial proportion of PEMs from the homozygous mutant mice exhibited phagocytosis of RBCs (Fig. 6). The phagocytic activity of PEMs was thus markedly enhanced as a result of the absence of the cytoplasmic region of SHPS-1.

Morphological Changes in PEMs from SHPS-1 Mutant Mice — Proteins of the Rho family of GTPases, including Rac1 and Cdc42, regulate phagocytosis by macrophages (33, 34). These proteins might thus also play a role in the enhanced phagocytosis apparent in macrophages from SHPS-1 mutant mice. Because biochemical assays failed to detect a substantial amount of activated Rac1 and Cdc42 in PEMs (data not shown), we examined macrophage spreading on the extracellular matrix as a more sensitive indicator for the activities of these GTPases. PEMs from the mutant mice spread more extensively than did wild-type PEMs on fibronectin-coated coverslips for up to 1 h after attachment; they also exhibited a marked increase in the number of filopodia-like structures at the cell periphery (Fig. 7, A–D), indicative of enhanced activities of Rac1 and Cdc42. After 4 h, however, no marked difference in the extent of spreading was apparent between macrophages from the two types of mice, although PEMs from the mutant mice appeared less polarized than did those from wild-type animals (Fig. 7, E and F).

DISCUSSION

We recently generated mutant mice that express a truncated form of SHPS-1 lacking most of the cytoplasmic region of this protein. We have now characterized these mutant mice and demonstrated that the absence of the cytoplasmic region of SHPS-1 results in thrombocytopenia. This condition is attributable to an increased metabolism of circulating platelets, with platelet production being unaffected. Our results have also revealed an important role for the cytoplasmic region of SHPS-1 in down-regulation of the macrophage phagocytic response. However, we need to be cautious in interpreting these results given that the mutant mice would possess functional

Fig. 5. Platelet survival in SHPS-1 mutant and wild-type mice. Female mice at 9 weeks of age were injected intravenously with sulfosuccinimidyl succinimidyl succinate (sulfo-NHS-LC-biotin, 1.5 mg), and the number of biotinylated platelets in peripheral blood was determined as a percentage of the total number of circulating platelets at the indicated times thereafter (A); the total number of circulating platelets is shown in B. Data are mean ± S.E. of triplicate determinations for five mice per group.

Fig. 6. Phagocytosis of RBCs by PEMs from SHPS-1 mutant and wild-type mice in vitro. PKH26-labeled RBCs from wild-type donor mice were overlaid on thioglycolate-elicited PEMs that were derived from wild-type (+/+) or homozygous SHPS-1 mutant (-/-) mice and adherent to fibronectin-coated coverslips. After incubation for 2 h, nonattached RBCs were washed away and attached (but not ingested) RBCs were removed by hemolysis. The cells were examined by confocal microscopy (upper panels), and the number of RBCs phagocytosed by PEMs was counted in at least six fields. Quantitative data are expressed as the number of RBCs ingested per 100 macrophages (phagocytosis index) (lower panel) and are mean ± S.E. of values from three independent experiments. Arrowheads in the upper panel indicate phagocytosed RBCs; original magnification, ×630.
extracellular regions of SHPS-1 and thus represent a hypomorphic or neomorphic mutation as opposed to a true loss-of-function.

The mice homozygous for the SHPS-1 mutation exhibited mild thrombocytopenia. Both the generation of megakaryocyte progenitors in bone marrow and the ability of these progenitors to grow and differentiate into mature polyploid megakaryocytes in this tissue appeared unaffected in the mutant mice. Furthermore, these animals exhibited no significant defect in the recovery of platelet counts after transient myelosuppression. In contrast, platelets were cleared from the bloodstream more rapidly in the mutant mice than in wild-type mice. Thus, the thrombocytopenia of the SHPS-1 mutant mice appears to be attributable, at least in part, to an increased rate of consumption of circulating platelets, and not to a defect in the production of these cells.

At least two possible mechanisms could account for the accelerated clearance of circulating platelets in SHPS-1 mutant mice. Reverse transcription-polymerase chain reaction analysis has revealed the presence of SHPS-1 mRNA, albeit in small amounts, in platelets (data not shown). The deletion of the cytoplasmic region of SHPS-1 expressed in platelets might therefore increase the fragility of these cells and thereby render them more susceptible to destruction, although platelet functions such as aggregation in response to collagen or to ADP are not affected by the SHPS-1 mutation. The second, and more likely, possibility is that the enhanced rate of platelet clearance in the mutant mice results from expression of the mutant SHPS-1 protein in other cell types. The sequestration of aged platelets is mediated by phagocytosis within the reticuloendothelial system, especially by splenic and hepatic macrophages (35). In addition, thrombocytopenia with abnormal platelet sequestration has been observed in various diseases including sepsis syndrome (36) and chronic immune thrombocytopenic purpura (37). Administration of cytokines such as granulocyte-macrophage colony stimulating factor and macrophage colony stimulating factor also results in either the clearance of circulating platelets or the stimulation of megakaryocytopenia, presumably by activating the monocyte-macrophage system (38–40). Given the fact that SHPS-1 is abundant in monocytes-macrophages and that this protein negatively regulates cytokine synthesis (21), enhanced phagocytosis of cytokine production by monocytes-macrophages is likely responsible for the thrombocytopenia in SHPS-1 mutant mice.

In splenic macrophages, SHPS-1 generates an intracellular signal that inhibits phagocytosis of RBCs (25, 26) in a manner that depends both on the binding of its extracellular IgV-like domain to CD47 expressed by the RBCs and on the recruitment of SHP-1 to the immunoreceptor tyrosine-based inhibitory motifs of SHPS-1 (19, 25, 26). CD47 engagement of macrophage SHPS-1 has also been shown to prevent the clearance of lymphohematopoietic cells (41). Given that the truncated SHPS-1 expressed in macrophages of the homozygous mutant mice is not able to bind SHP-1, these cells would not be expected to transmit the inhibitory signal initiated by the interaction with CD47. CD47 is abundant in platelets (42, 43). If the functional consequences of the interaction of macrophage SHPS-1 with CD47 expressed on platelets are similar to those of the interaction of macrophage SHPS-1 with CD47 on RBCs or lymphocytes, then platelets would be expected to be phagocytosed by splenic macrophages more effectively in SHPS-1 mutant mice.

In agreement with the lack of a signal that prevents phagocytosis, the number of CD4- and CD8-positive T lymphocytes in spleen was reduced in SHPS-1 mutant mice. In these mice, however, we failed to detect any sign of accelerated RBC clearance such as anemia and reticulocytosis. Also, no marked reduction in the survival ratio of transfused RBCs was observed in the mutant mice. These results appear inconsistent with what might have been predicted from the previous reports (25, 26). This apparent discrepancy presumably results from the difference in the experimental approach adopted; whereas we mutated SHPS-1 to interrupt the inhibitory signal by CD47, Oldenburg et al. (25, 26) followed the fate of RBCs deficient in CD47. Redundant pathways may also explain the differences in phenotype between the SHPS-1-deficient and CD47-deficient mice. Nevertheless, our ex vivo analyses have demonstrated that PEMs from SHPS-1 mutant mice phagocytosed RBCs more effectively than did wild-type cells, supporting the notion that the cytoplasmic region of SHPS-1 negatively regulates the macrophage phagocytic response.

PEMs from SHPS-1 mutant mice are reminiscent of macrophages (44), lymphocytes (45), or neutrophils (46) from SHP-1-deficient mice, in that they manifest an increase in the actin polymerization. Although this similarity suggests that the inability of the truncated SHPS-1 to recruit SHP-1 might partly account for the phenotype of the mutant PEMs, the mechanism by which SHPS-1 truncation stimulates phagocytosis remains unclear. Rac1 and Cdc42 positively regulate Fc receptor-mediated phagocytosis by macrophages (33, 34), indicating that up-regulation of the small GTPases might play a causal role. We could not, however, provide direct evidence for this hypothesis presumably because of the low stoichiometry and transient nature of the activation of these GTPases in PEMs. This idea nevertheless appears consistent with the observation that cell spreading and filopodia formation, which correlate well with the activation of Rac1 and Cdc42, were enhanced in SHPS-1-deficient macrophages. Phagocytosis and Rac-mediated cyto-
Indexing terms: platelets, tyrosine kinase, SHPS-1, megakaryocytes 

parsimonious that this inhibition and thus facilitate megakaryocyteopoiesis in the spleen. In conclusion, our results demonstrate that SHPS-1 inhibits the clearance of circulating platelets as well as downregulates the macrophage phagocytic response, thereby possibly contributing to hemostasis and host defense.

Acknowledgments—We thank C. Lagenaur for providing the rat mAb to p84 (SHPS-1), as well as H. Miyazaki and T. Kawai for critical discussions throughout the study.

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