Deficiency of Src Homology 2-Containing Phosphatase 1 Results in Abnormalities in Murine Neutrophil Function: Studies in Motheaten Mice

Joshua Kruger, Jeffrey R. Butler, Vera Cherapanov, Qin Dong, Hedy Ginzberg, Anand Govindarajan, Sergio Grinstein, Katherine A. Siminovitch and Gregory P. Downey

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Neutrophils, an essential component of the innate immune system, are regulated in part by signaling pathways involving protein tyrosine phosphorylation. While protein tyrosine kinase functions in regulating neutrophil behavior have been extensively investigated, little is known about the role for specific protein tyrosine phosphatases (PTP) in modulating neutrophil signaling cascades. A key role for Src homology 2 domain-containing phosphatase 1 (SHP-1), a PTP, in neutrophil physiology is, however, implied by the overexpansion and inappropriate activation of granulocyte populations in SHP-1-deficient motheaten (me/me) and motheaten viable (me/+me*) mice. To directly investigate the importance of SHP-1 to phagocytic cell function, bone marrow neutrophils were isolated from both me/me and me/+me* mice and examined with respect to their responses to various stimuli. The results of these studies revealed that both quiescent and activated neutrophils from motheaten mice manifested enhanced tyrosine phosphorylation of cellular proteins in the 60- to 80-kDa range relative to that detected in wild-type congenic control neutrophils. Motheaten neutrophils also demonstrated increased oxidant production, surface expression of CD18, and adhesion to protein-coated plastic. Chemotaxis, however, was severely diminished in the SHP-deficient neutrophils relative to control neutrophils, which was possibly attributable to a combination of defective deadhesion and altered actin assembly. Taken together, these results indicate a significant role for SHP-1 in modulating the tyrosine phosphorylation-dependent signaling pathways that regulate neutrophil microbialicidal functions. The Journal of Immunology, 2000, 165: 5847–5859.

Abbreviations used in this paper: PMN, polymorphonuclear leukocytes; PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; SH2, Src homology 2; SHP-1, SH2-containing phosphatase-1; SDF-1, stromal cell-derived factor 1; PIR-B, paired Ig-like receptor B; PI, phosphatidylinositol; KRPD, Krebs-Ringer-phosphate dextrose; INLP, N-formyl-norleucyl-phenylalanine.
the transmembrane PTP expressed in PMN, CD45, has been implicated in regulation of motility (28) and Fe-mediated phagocytosis (29), but the mechanisms linking CD45 to these behaviors are not known. The Src homology 2 (SH2) domain-containing cytosolic phosphatase 1 (SHP-1) is known to be expressed in myeloid cells, including the cultured cell lines HL-60 and U937, macrophages (30–34), and peripheral blood PMN (35), and modulates proliferation, apoptosis, oxidant production, and adhesion in cultured U937 cells (36). These data suggest integral roles for SHP-1 in governing PMN biology. This contention is consistent with the profound myeloid defects observed in mice completely or partially deficient in SHP-1, which are denoted motheaten (me/me) and motheaten viable (me/me+) mice, respectively. Both the me and me+ mutations result in defective SHP-1 RNA splicing. The me mutation is a deletion of a cytidine residue that generates a novel splice donor site in the first SH2 domain generating a frameshift in SHP-1 (37). The recombinant protein was used to generate polyclonal Abs to SHP-1 that were affinity purified and have been shown to be suitable for immunoblotting and immunoprecipitation (35, 54). A rat mAb (clone RB6-8C5) recognizing the myeloid differentiation Ag Ly-6G (previously known as Gr-1) was obtained from The Jackson Laboratory (Bar Harbor, ME) and were maintained at the Samuel Lunenfeld Research Institute, Mt. Sinai Hospital (Toronto, Ontario, Canada). All mice were genotyped using PCR amplification of tail DNA as described previously (37).

Identification of SHP-1 mutation as the defect responsible for the motheaten phenotype has resulted in intensive investigation of the functions of this phosphatase. Such studies have revealed that SHP-1 participates in down-regulating a broad spectrum of growth-promoting receptor-evoked activation cascades. These include, for example, the signaling pathways triggered by receptor tyrosine kinases (41, 42) and the CSF-1 receptor (43), cytokine receptors such as the IL-3 (41) and IFN-γ (44) receptors, and immune receptors containing the immune receptor tyrosine-based inhibitory motif such as CD22 (45) and paired Ig-like receptor B (PIR-B) (32, 46). SHP-1 modulates these signaling cascades via a diversity of molecular interactions including dephosphorylation of receptor tyrosine kinases (41, 43, 47, 48), interaction with non-catalytic subunits of receptors (e.g., cytokine receptors), and dephosphorylation of associated Janus family tyrosine kinase (44, 49) or via interactions with cytosolic signaling effectors such as Vav, slp-76 (50), and lck (45, 51–57).

While SHP-1 functions in lymphocytes have been extensively studied, the role of this PTP in the regulation of microbicidal responses in myeloid cells is poorly understood. SHP-1-deficient bone marrow myeloid progenitor cells and macrophages demonstrated enhanced chemotactic responses to stromal cell-derived factor 1 (SDF-1), a CXC chemokine (58). SHP-1-deficient bone marrow myeloid cells (59) and macrophages (43, 60) were hyper-responsive to growth factor stimulation and adhered and spread on surfaces to a greater extent than normal macrophages (33). Interestingly, SHP-1-deficient macrophages were impaired in their ability to detach from the substratum apparently due to defective regulation of phosphatidylinositol (PI) 3-kinase (33).

In light of the apparent importance of SHP-1 in regulating macrophage function, we sought to delineate SHP-1 roles in modulating PMN behavior. To this end, we characterized the functional properties of bone marrow PMN isolated from me/me and me+/me+ mice, which express no and catalytically inert SHP-1, respectively. As described herein, analyses of these cells revealed that SHP-1 deficiency is associated with an increase in total cellular protein tyrosine phosphorylation and a state of hyperresponsiveness as connoted by increased surface expression of the β2 integrin, CD11b, increased adhesion to protein-coated surfaces, and increased oxidant production. Additionally, SHP-1-deficient PMN have markedly diminished chemotactic ability that may reflect impairment in deadhesion and altered cytoskeletal regulation.

Materials and Methods

**Reagents**

Percoll and Dextran T-500 were obtained from Pharmacia LKB Biotechnology (Baie D’Urfe, Quebec, Canada). Reagents for Krebs-Ringer-phosphate dextrose (KRPD) were obtained from Mallinkrodt (Paris, KY). HEPES, PMI, N-formyl-norleucyl-phenylalanine (NLP), cytochlasin B, scopoletin, HRP, and mouse IgG were from Sigma (St. Louis, MO). H2O2 was from Caledon Laboratories (Toronto, Ontario, Canada). Caffeine-ace toxymethyl ester and dihydrodiamine were from Molecular Probes (Eugene, OR). KC was from R&D Systems (Minneapolis, MN). H2O2 was from Caledon Laboratories.

**Buffers**

Na buffer contained (in mM) 140 NaCl, 4 KCl, 10 glucose, 10 HEPES, 1 MgCl2, and 1 CaCl2 (pH 7.4 at 37°C). KRPD contained (in mM) 120 NaCl, 4.8 KCl, 1.2 MgSO4, 0.93 CaCl2, 3.1 NaH2PO4, and 12.5 Na2HPO4 (pH 7.4 at 37°C).

**Antibodies**

A GST-fusion protein of wild-type murine SHP-1 encompassing its two SH2 domains (amino acids 1–296) was generated as previously described (37). The recombinant protein was used to generate polyclonal Abs to SHP-1 that were affinity purified and have been shown to be suitable for immunoblotting and immunoprecipitation (35, 54). A rat mAb (clone RB6-8C5) recognizing the myeloid differentiation Ag Ly-6G (previously known as Gr-1) was obtained from Pharmingen Canada (Mississauga, Ontario, Canada). Rabbit anti-lactoferrin Abs were obtained from Transduction Laboratories (Lexington, KY). Anti-phosphotyrosine Ab 4G10 was purchased from Upstate Biotechnology (Lake Placid, NY). Rat mAbs against mouse CD11b (clone M1/70) were obtained from Biosource International (Nivelles, Belgium). Rabbit anti-lactoferrin Abs were obtained from Sigma.

**Motheaten mice**

Mice homozygous for the motheaten (me) mutation were obtained by mating C3H HeF1 me/me+ breeding pairs. Mice homozygous for the motheaten viable (me+) mutation were obtained by mating C57BL/6 mice obtained from Transduction Laboratories with C3H mice obtained from Jackson Laboratory. Mouse anti-lactoferrin Ab (clone RB6-8C5) recognizing the myeloid differentiation Ag Ly-6G (previously known as Gr-1) was obtained from Pharmingen Canada (Mississauga, Ontario, Canada). Rabbit anti-lactoferrin Abs were obtained from Sigma.

**Isolation of bone marrow PMN**

PMN were isolated from bone marrow according to the method of Lowell and Berton (61) with minor modifications. Long bones from mice (humerus, femur, and tibia) were removed, and the ends were clipped and then flushed using a 27-gauge needle and ice-cold calcium- and magnesium-free HBSS. Clumps of marrow were broken up by repeated pipetting. Cells from the marrow were sedimented by centrifugation at 500 x g at 4°C for 5 min and resuspended in 4 ml of HBSS. The unpurified marrow was placed in a 15-ml polypropylene tube and layered over a three-step gradient (52%, 65%, and 75% Percoll diluted with HBSS). For these studies, 100% Percoll is defined as nine parts Percoll and one part 10× HBSS (Ca2+ free). The tube containing the Percoll gradient was centrifuged at 1500 x g for 30 min at 4°C in a swinging bucket rotor using a slow brake to prevent disruption of the layers during deceleration. The cells were then removed from the PMN-enriched fraction at the interface of the 65% and 75% layers, diluted with an equal volume of HBSS, and sedimented in a microcentrifuge for 10 s at 75% maximum speed. These cells were resuspended in 1 ml of RPMI 1640 and counted using a Coulter counter. An aliquot of the purified cells was sedimented onto a glass coverslip using a cytocentrifuge (Shandon, Pittsburgh, PA), fixed, and stained using a modified Wright-Giemsa stain (Diff-Quick, Dade Diagnostics, Aquanda, PR). Purification of bone marrow PMN from motheaten mice required a slight modification using 62% as the second step of the gradient to yield maximum purity.

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Ly6G (GR-1) labeling and flow cytometry

Surface expression of Ly6G (GR-1) was quantified with purified rat mAbs (clone RB6-8C5, Pharmingen) or using unlabeled supernatant from the hybridoma cell line. Cells in suspension were fixed with 1.6% paraformaldehyde in PBS for 30 min, washed with PBS twice, and incubated with the purified Ab (40 μg/ml) or unlabeled hybridoma supernatant for 1 h at room temperature. Cells were again washed three times with PBS and resuspended in a 1:1500 dilution of anti-rat FITC-conjugated secondary Ab for 1 h at room temperature and washed twice with PBS. The surface expression of Gr-1 was then quantified using a FACSscan flow cytometer (Becton Dickinson, San Jose, CA).

SDS-PAGE and immunoblot analysis

For assessment of cellular tyrosine phosphorylation, 1 × 10^6 cells were suspended in 0.5 ml of Na buffer at 37°C and exposed to agonists at 37°C as specified. The cells were fixed by exposure to 1 ml of ice-cold Na acetate buffer and the cells were sedimented and resuspended in 50 μl of boiling Laemmli sample buffer. The cell lysates were resolved on a 10% polyacrylamide gel using SDS-PAGE, transferred to nitrocellulose, and blotted with anti-SHP-1 or 4G10 anti-phosphotyrosine Abs as indicated.

SHP-1 immunoprecipitation and phosphatase assay

Bone marrow PMN were suspended in 1 ml ice-cold lysis buffer (PBS (ph 7.4), 1% Nonidet P-40, 1 mM PMSF, 0.5 mM benzamidine, 10 μg/ml aprotinin, and 10 μg/ml leupeptin). Lysates were centrifuged at 15,000 × g for 15 min, and supernatants were mixed with 10 μl polyclonal anti-SHP-1 at 4°C for 2 h and then incubated with 50 μl protein G/A plus agarose rotating overnight at 4°C. The washed beads were analyzed by SDS-PAGE and Western blotting with monoclonal-SHP-1 Abs. Tyrosine phosphatase activity was determined using the malachite green phosphatase assay with a phosphopeptide substrate, RRLIEDAEpYAARG (Upstate Biotechnologies). The activity was normalized to the amount of immunoreactive SHP-1 protein as determined by Western blotting of the immunoprecipitates with monoclonal anti-SHP-1 Abs followed by densitometric analysis as described below. The intensity of the SHP-1 band in each sample was determined using IP Lab Gel-D10 (Scanalytics, Fairfax, VA). The SHP-1 activity was calibrated by running varying amounts of recombinant GST-SHP-1 fusion protein on the same blot to ensure that samples were in the linear range of the x-ray film.

Chemotaxis

Chemotaxis was determined using a micro-Boyden chamber from Neuroprobe (Cabin John, MD). Zymosan-activated mouse serum (heterologous) was used to provide a constant flow rate of the buffer across the filters. Immediately upstream of each filter chamber, a pressure transducer (Validyne Engineering, Northridge, CA) connected to a strip chart recorder continuously measured pressure. A cell suspension (0.5 × 10^6 cells/ml) was filtered through a 3-μm pore filter (Costar) and a 3-μm pore diameter filter (both filters were 37°C). The filters were calibrated by using varying amounts of recombinant GST-SHP-1 fusion protein on the same blot to ensure that samples were in the linear range of the x-ray film.

Chemokinesis

Glass coverslips (Fisher Scientific, Pittsburgh, PA) were coated with fibrinogen (Sigma) for 2 h at 37°C. PMN (7.5 × 10^5 cells) were allowed to adhere to the coverslips for 10 min at 37°C. Following incubation, the coverslips were inserted into a stage of a microscope (Deerfield, IL) DM-IRB microscope, covered with HBSS containing 1% BSA, and maintained at 37°C. IL-8 (final concentration 10^{-8} - 10^{-9} M) was then added and dispersed using gentle pipetting. For analysis, fields containing equal leukocyte density were chosen for observation, and differential interference contrast images at × 20 magnification were acquired at 20-s intervals for a total of 20 min using a Princeton Instruments (Trenton, NJ) Pentamax cooled charge-coupled device camera controlled by MetaFluor software (Universal Imaging, Media, PA). Following acquisition, 30 adherent PMN were chosen at random from each mouse, and the movement of their centroids were tracked using Metamorph software (Universal Imaging). Only cells that remained within the field of observation for the entire experiment were chosen. Two indices of movement were derived. The total distance traveled by each cell was represented by the sum of the distances between the position of the cell centroid in each 30-s interval over the period of observation. The net distance represents the distance between the cell centroid at the start and the end of the observation period.

Measurement of cytoskeletal alterations

The quantity of polymerized F-actin in neutrophils was measured as previously described (62). Briefly, after exposure to chemoattractant or vehicle control, cells were simultaneously fixed and permeabilized with lyso-phosphatidylcholine (0.1 mg/ml final concentration) in buffered formalin. After a 5-min incubation at 37°C, nitrobenzoxadiazole-phallacidin was added to a final concentration of 1.65 × 10^{-7} M. Cells were examined on a FACSscan (Becton Dickinson) and were gated on the forward and right angle light scatter to remove debris and cell clumps. Cellular fluorescence was quantified using the FL1 detector (488-nm excitation and 530-nm emission wavelengths), and values are expressed as relative fluorescence index by dividing the linearized fluorescence of the experimental group by the value for the unstimulated control cells. This method has been shown to correlate with biochemical measurements of F-actin.

The distribution of F-actin within cells was examined using Alexa-Red phallidin (Molecular Probes) essentially as previously described (63). Briefly, after exposure to chemoattractant or vehicle control, cells were fixed with 4% paraformaldehyde, washed, and allowed to settle on coverslips that were previously coated with 1 mg/ml poly-l-lysine. After 20 min, the coverslips were rinsed in PBS and the neutrophils permeabilized by incubation in 0.1% Triton X-100 in KRPI for 15 min. The cells were stained with 1.65 × 10^{-7} M Alexa-Red phalloidin for 10 min at 37°C and then washed with several rinses in PBS. The coverslips were mounted with fluorescence mounting medium (Dako, Carpenteria, CA). The slides were viewed using a Leica DM-IRB inverted fluorescence microscope and digital images captured as TIFF files using a Princeton Instruments Micromax cooled charge-coupled device camera controlled by Metamorph software. The images were imported into Adobe Photoshop, labeled, and printed on a Hewlett Packard Ink Jet printer.

Measurement of cell deformability

PMN deformability was assessed by measuring the pressure needed to pass a suspension of PMN through a polycarbonate filter (Poretics, Livermore, CA) with a uniform pore diameter of 6.5 μm (range 6.0–7.0 μm; coefficient of variation <10%) as previously described (1–3, 64, 65). In brief, polycarbonate filters, polypropylene chambers, and siliconized plastic I.V. tubing were used to coat the chamber before incubation in 20% heat-inactivated murine plasma at 37°C for 2 h to make a pressure across the filters. Chambers were connected to a multichannel infusion pump (Harvard Apparatus, Millis, MA) and used to provide a constant pressure of the buffer across the filters. Immediately upstream of each filter chamber, a pressure transducer (Validyne Engineering, Northridge, CA) connected to a strip chart recorder continuously measured pressure. A cell suspension (0.5 × 10^6 cells/ml) was filtered through a 3-μm pore constant flow rate of 300 s. Where indicated, 10^{-8} M IL-8 was added to the cell suspension. The maximum pressure attained was recorded for each experiment.

Measurement of oxidant production

Oxidant production by murine PMN was quantified in two ways. To analyze the kinetics of oxidant production, a fluorescence assay with scopoletin (the fluorescence of which decreases in the presence of H_2O_2) was used as previously described (36, 66). Typically, 5 × 10^{-1} × 10^5 cells were incubated in a physiological buffer containing 200 μM scopoletin, 2.4 μM HRP, and 0.01% sodium azide. PMA (10^{-8} M) was added to cells suspended in scopoletin assay buffer and incubated in the fluorometer with stirring at 37°C for 3–5 min. Reduction in fluorescence of scopoletin was quantified in a Hitachi F-2000 fluorescence spectrophotometer using an excitation wavelength of 365 nm and an emission wavelength of 473 nm. A continuous readout of fluorescence vs time was obtained, and the slope of this line was calculated using graphical analysis. Standard curves were generated using known amounts of H_2O_2. To calculate the lag time of oxidase activation, the time from the addition of stimulus to the intercept of the line of maximal slope of the curve on the abscissa was measured. Oxidant production was also measured by flow cytometry using the oxidant-sensitive dye dihydrodihorhodamine 123 (67) as previously described. In brief, 5 × 10^5 cells in suspension were incubated in the presence of 2 μM dihydrodihorhodamine for 20 min at 37°C. Cells were fixed with 1.5% paraformaldehyde before analysis on a FACSscan flow cytometer (Becton Dickinson).
Phagocytosis

The phagocytic ability of PMN was assayed by incubating serum- or murine IgG-opsonized zymosan with cells in the presence of the permeal fluid-phase marker Lucifer Yellow as previously described (68). PMN (3 × 10^5) were allowed to settle on glass coverslips for 30 min at room temperature. To synchronize phagocytosis, the opsonized zymosan (6 × 10^6 particles) was added to cells and allowed to bind for 10 min at 4°C. The temperature was then rapidly raised to 37°C and phagocytosis allowed to proceed for 10 min in the presence of 2 mg/ml Lucifer Yellow. The coverslips were cooled in an ice-water bath, and phagosomes were counted using a fluorescence microscope (Nikon, Melville, NY).

Flow cytometric analysis of CD11b and lactoferrin

Purified PMN (1 × 10^6) were fixed with 1.6% paraformaldehyde for 15 min at room temperature, washed, and then incubated with 20% goat serum for 30 min to block nonspecific binding. Cells were washed and then incubated with 10 μg/ml rat anti-murine CD11b Ab (clone M1/70; Biosource International) or rabbit anti-human lactoferrin (Sigma) for 1 h at 4°C and washed and then incubated with FITC-labeled goat anti-rat or goat-anti-rabbit Ab. Cells were washed and resuspended in PBS and analyzed by flow cytometry (FACStar, Becton Dickinson). Where indicated, cells were first permeabilized with 0.5% Triton X-100 before incubation with the primary Abs.

Adhesion assay

Purified PMN (2 × 10^6) were labeled with 1.5 μM calcine-acetoxyethyl ester for 20 min at 37°C with gentle agitation followed by washing and resuspension in Na buffer. Subsequently, cells were added to 24-well tissue culture plates (5 × 10^5 cells/well) precoated with FBS and incubated for an additional 2 h at 37°C. Each assay was done in quadruplicate. After incubation, cells were fixed with 1.6% paraformaldehyde for 40 min at room temperature, and then wells were washed two times with PBS using a gravity washing device. Calcine was extracted by adding methanol to the remaining adherent cells followed by vigorous pipetting. Fluorescence was detected using a Hitachi F-2000 fluorescence spectrophotometer with an excitation wavelength of 490 nm and an emission wavelength of 520 nm. All values were normalized to the number of cells added, which was determined by measuring the mean fluorescence of three separate aliquots of 5 × 10^5 calcine-labeled cells by methanol extraction.

For experiments using blocking anti-CD11b Abs, cells were preincubated with or without blocking anti-CD11b Abs (clone M1/70, 25 μg/ml) for 20 min at 4°C and then added to 96-well plates (1 × 10^5 cells/well) precoated with FBS. The plates were incubated for an additional 2 h at 37°C in the presence or absence of stimulant as indicated and in the presence of anti-CD11b Abs or buffer control. The cells were fixed with 1.6% paraformaldehyde for 40 min at room temperature, and then the wells were washed two times with PBS using a gravity washing device. The fluorescence of each well was then determined using a plate-reading fluorescence spectrophotometer (Cytofluor, PE Biosystems, Mississauga, Ontario, Canada). All values were normalized to the number of cells added, which was determined by measuring the mean fluorescence of three separate aliquots of 1 × 10^5 calcine-labeled cells.

Data analysis

Data were analyzed by ANOVA with correction for multiple comparisons (Shelfiff) or by paired or unpaired Student’s t test, as indicated. Statistical significance was considered for p values of < 0.05.

Results

Purification of myeloid leukocytes from the bone marrow of me/me and me/me’ mice

To investigate the roles of SHP-1 in modulating PMN, we took advantage of the availability of SHP-deficient mice in which SHP-1 protein is either absent (me/me) or catalytically impaired (me/me’). Initial attempts to purify PMN from peripheral blood using discontinuous plasma-Percoll gradients (69) yielded insufficient numbers of cells for study. These cells were therefore instead purified from bone marrow using discontinuous Percoll gradient centrifugation and a modification of previously published methods (70). This strategy allowed for the isolation of ~3 × 10^6 cells per mouse that were 88–95% mature myeloid cells as determined by modified Wright-Giemsa staining (Fig. 1, c and f).

To assess the maturity of these cells, the surface expression of the myeloid differentiation Ag Ly-6G (71–73), which was previously known as Gr-1, was assessed by flow cytometric analysis. As illustrated by the data shown in Fig. 1, analysis of Ly-6G surface expression in unpurified bone marrow revealed three differentially stained subpopulations of both wild-type (Fig. 1a) and me/me (Fig. 1d) cells. Among these populations, the subpopulation characterized by an intermediate level of Ly-6G surface expression appeared relatively increased in me/me (Fig. 1d, second peak) compared with wild-type mice (Fig. 1a, second peak). Thus, this population (i.e., intermediate Ly-6G expression) may represent the expanded population of myelomonocytic cells that develops in motheaten mice (74). Purification using the plasma-Percoll gradients yielded a population with the highest levels of Ly-6G expression (Ly-6Ghigh). These Ly-6Ghigh subpopulations (Fig. 1, b and e) from bone marrow are morphologically identical (Fig. 1, c and f) to peripheral blood PMN (not shown) and therefore were designated as the bone marrow PMN population and used for all of the studies described below. Bone marrow cells from me/me’ mice exhibited a pattern of Ly-6G staining similar to me/me mice (not illustrated).

To determine the level of expression of SHP-1 in bone marrow PMN, the purified cell populations were subjected to anti-SHP-1 immunoblotting analysis. As indicated in Fig. 1g, the results of these analyses confirmed the presence of SHP-1 in PMN from wild-type mice (lanes 1 and 3) and absence of SHP-1 in PMN from me/me mice (lane 2). Two immunoreactive bands of ~68 and 71 kDa were detected in PMN from me/me’ mice (Fig. 1g, lane 4) and consistent with previous data demonstrating two SHP-1 species, probably representing splice variants, in me/me’ bone marrow cells and human peripheral blood PMN (35). Tyrosine phosphatase activity was severely diminished in anti-SHP-1 immunoprecipitates from bone marrow PMN from me/me’ mice (~<10% control; data not illustrated), consistent with previous reports in me/me’ lymphocytes (37).

Following confirmation of impaired SHP-1 PTP activity, bone marrow PMN from the various mice were compared with respect to the intensity and pattern of cellular protein tyrosine phosphorylation. As shown in Fig. 2, the results of anti-phosphotyrosine immunoblotting analysis revealed tyrosine phosphorylation of multiple polypeptides to be enhanced in me/me and me/me’ relative to wild-type PMN. These increases in tyrosine phosphorylation level were observed both in quiescent cells (Fig. 2, a and b, lanes 1 and 5) and in cells activated by agonists such as the chemoattractant formyl peptide fMLP (lanes 2 and 6), PMA (lanes 3 and 7) and the phagocytic stimulus opsonized zymosan (OP-Z; lanes 4 and 8). These data reveal that the loss of SHP-1 activity is associated with increases in cellular protein tyrosine phosphorylation that is likely to impact upon the function of multiple PMN signaling effectors.

Oxidant production is enhanced in motheaten PMN

The microbicidal function of myeloid cells is dependent in part on their ability to produce reactive oxygen intermediates such as O_2^- and H_2O_2 by a multicomponent enzyme complex termed the NADPH oxidase (75). To determine the relevance of SHP-1 to the regulation of the NADPH oxidase, PMN from the motheaten and wild-type mice were compared with respect to their H_2O_2 production following treatment with fMLP, CSa, and PMA, the latter of which is a direct activator of protein kinase C and a potent agonist of the NADPH oxidase. The results of this analysis revealed that agonist-induced oxidant production was markedly increased in me/me and me/me’ PMN when compared with cells from wild-type mice (Fig. 3a). As is evident from the figure, levels of PMN...
FIGURE 1. Purification of mature PMN from bone marrow of wild-type and motheaten mice. Cells from the marrow of long bones of wild-type and me/me mice were removed, washed, and saved for analysis (a and d) or subjected to further purification using a three-step Percoll gradient as described in Materials and Methods (b and c, e and f). Cells from the PMN-enriched fraction, the interface of the 65% (wild type) or 62% (motheaten) and 75% layers, were removed and washed. Surface expression of Ly6G (GR-1) on unpurified (i.e., before Percoll gradient purification; a and d) and purified (b and e) bone marrow cells from wild-type and me/me mice was determined by flow cytometry using an mAb (clone RB6-8C5) and secondary anti-rat FITC-conjugated Ab. Note that in the unpurified marrow from me/me mice (d), a larger proportion of cells have an intermediate level of Ly6G expression (population of cells delimited by the second gate). The expression of Ly6G on cells from me/me mice was similar to those from me/me mice (not illustrated). The Percoll gradient centrifugation yielded cells with the highest level of Ly6G expression (b and e). An aliquot of the purified Ly6G<sup>high</sup> cells was sedimented onto a glass coverslip using a cytocentrifuge and stained using a modified Wright-Giemsa stain (c and f). Note the presence of ring-shaped nuclei in many of the cells, which is characteristic of mature PMN from both the marrow and the peripheral blood (not illustrated). Cells purified from the marrow of me<sup>v</sup>/me<sup>v</sup> mice were similar to those from me/me mice (not illustrated). g, Expression of immunoreactive SHP-1 protein by purified bone marrow PMN from congenic wild-type (C3H and C57BL/6), me/me, and me<sup>v</sup>/me<sup>v</sup> mice. Proteins from whole-cell lysates of bone marrow PMN purified by Percoll gradient centrifugation from wild-type, me/me, and me<sup>v</sup>/me<sup>v</sup> mice were separated by SDS-PAGE gels and subjected to immunoblotting with affinity-purified polyclonal anti-SHP-1 Abs. For each wild-type and mutant pair, equal cell equivalents were loaded onto each lane. The two immunoreactive bands of ~68 and 71 kDa in cell extracts from me<sup>v</sup>/me<sup>v</sup> PMN (lane 4) likely represent splice variants of SHP-1. The Western blots illustrated are representative of three experiments with cells from separate animals.
oxidant production were higher in the me/me than in the me/me mice, possibly reflecting the presence of residual SHP-1 activity in these latter cells. To analyze the kinetics of oxidant production, H$_2$O$_2$ generation was also evaluated using a continuous assay based on scopoletin reduction. As shown in Fig. 3, b and c, the results of this analysis revealed that the maximal rate of H$_2$O$_2$ generation (as indicated by the slope of the line) was greater in me/me than in wild-type PMN, while me/me opposed PMN showed maximum rates of oxidant production that were intermediate between wild-type and me/me mice, possibly reflecting the presence of residual SHP-1 activity in these latter cells. To analyze the kinetics of oxidant production, H$_2$O$_2$ generation was also evaluated using a continuous assay based on scopoletin reduction. As shown in Fig. 3, b and c, the results of this analysis measured the rate rather than the total (cumulative) amount of oxidant production. These conditions were substrate limited, and the flattening of the curve in the latter part of the assay represents substrate depletion rather than termination of oxidant production. The traces are representative of four separate experiments. Responses of bone marrow PMN from me/me mice were similar to those from me/me mice (not illustrated). c, Graphical representation of the data from four experiments comparing the rates of PMA-stimulated oxidant production between PMN from wild-type and me/me mice. Data were analyzed by ANOVA with correction for multiple comparisons (Shef penalties). Results of a quantitative comparison of basal and stimulated adhesion of bone marrow PMN from wild-type and me/me mice (Fig. 4b) also revealed that me/me PMN were hyperadherent and indicated a relative inability of these latter cells to modulate their adhesion in response to C5a, fMLP, or PMA. PMN from me/me mice exhibited a similar degree of hyperadhesiveness, as did the me/me cells (not illustrated).
Surface expression of $\beta_2$ integrins is increased on motheaten PMN

As adhesion of myeloid leukocytes to endothelial cells and also extracellular matrix proteins is mediated primarily by $\beta_2$ integrins (81, 82), me/me and me/me+ wild-type PMN were evaluated for surface expression of CD11b, the major isoform of the $\alpha$-chain expressed by these cells. Flow cytometric analysis of cells stained with a mAb that recognizes CD11b revealed that surface expression of this adhesion receptor was increased in both the me/me and me/me+ cells compared with wild-type PMN in the absence of activating stimuli. Additionally, the relative increase in surface expression of CD11b in response to agonist stimulation was less (and statistically insignificant) in motheaten compared with wild-type PMN (Fig. 5a). As is consistent with this observation, pretreatment with a blocking anti-CD11b Ab (M1/70) reduced both the basal and agonist-stimulated adhesion of motheaten cells to a level similar to that of wild-type PMN (Fig. 5b). These data therefore suggest that modulation of $\beta_2$ integrins contributes to the enhanced adhesive properties of SHP-1-deficient PMN.

The increased plasma membrane levels of CD11b in motheaten PMN could be accounted for by a shift in the cellular distribution of CD11b/integrins. To determine the relative contributions of the plasma membrane (cell surface) pool of CD11b and lactoferrin, the fluorescence in intact cells reflects the plasma membrane (cell surface) pool of CD11b or lactoferrin, whereas the fluorescence in permeabilized cells reflects the total cellular stores accessible to Ab (plasma membrane and intracellular stores).
from their intracellular stores in secondary granules and vesicles to the cell surface. To investigate this possibility, we compared the subcellular distribution (cell surface vs intracellular) of CD11b and lactoferrin (a component of secondary granules) in intact and Triton X-100-permeabilized cells by flow cytometry using specific Abs. The distribution was compared in wild-type and motheaten PMN. This analysis (Fig. 5c) revealed that the majority of both CD11b and lactoferrin was in an intracellular compartment. The cell surface levels of CD11b were higher in motheaten compared with wild-type PMN, confirming early observations (Fig. 5a). The amount of intracellular CD11b was slightly less in me/me compared with wild-type PMN, but this difference did not reach statistical significance. Surface levels of lactoferrin were very low, and there was no difference in the intracellular levels of lactoferrin between wild-type and me/me PMN (Fig. 5c). From these observations, we conclude that the absence of SHP-1 does not alter the intracellular stores of CD11b or the secondary granules but rather enhances their mobilization to the plasma membrane after agonist stimulation.

Cell motility is defective in motheaten PMN

To assess whether SHP-1 deficiency alters cell motility, we assessed chemotaxis using a Boyden-type chamber with methylcellulose/nitrocellulose filters. As illustrated in Fig. 6, the results of this analysis revealed that chemotaxis in response to zymosan-activated serum was severely diminished in me/me relative to wild-type cells. This defect was also apparent in the motheaten cells when fMLP (90 ± 6% decrease at 10⁻⁶ M) or recombinant KC (88 ± 7% decrease at 10⁻⁶ M) was used as the chemoattractant (not illustrated).

To further investigate the basis for this chemotactic defect, the ability of me/me and me/me cells to move along a surface (chemokinesis) was studied using time-lapse video microscopy. As illustrated in Fig. 7, movement of me/me and me/me PMN across a fibrinogen-coated surface was markedly diminished, whether measured as the total distance migrated or as the net distance from the origin. Visual inspection of the time-lapse images indicated that the me/me and me/me PMN behaved as if their uropod was “tethered” to the substratum; these cells were frequently observed to move around a point representing the site of uropod attachment to the substratum. These observations are consistent with a defect in deadhesion, a process necessary for forward movement.

**FIGURE 6.** PMN from motheaten mice have diminished chemotaxis. Chemotaxis of bone marrow PMN isolated from wild-type, me/me, and me/me mice was measured in a micro-Boyden chamber using a trap filter method as described in Materials and Methods. The chemoattractant was zymosan-activated murine serum. Similar responses were observed with other chemoattractants including fMLP, fNLP, and KC but were more variable. Values represent the mean ± SEM of three separate experiments. Data were analyzed by ANOVA with correction for multiple comparisons (Sheffé); **, significance at p < 0.01.

**FIGURE 7.** Analysis of the movement of PMN from wild-type and motheaten mice using a chemokinetic assay. a and b, The locomotion of PMN from wild-type (a) and me/me (b) mice in the presence of chemoattractant (fMLP 10⁻⁶ M) was measured using time-lapse video microscopy as described in Materials and Methods. Filled circles represent the starting point and lines represent the path of individual cells (the position of the cell centroid at 1-s increments from beginning to end of the period of observation) as determined by computer analysis (Track Objects, Metamorph, Universal Imaging). The bar indicates 10 μm. c, Quantitative analysis of the movement of bone marrow PMN from wild-type and me/me mice in the presence of chemoattractant (fMLP 10⁻⁶ M). Data are displayed as the total distance moved and the net distance from the origin. The total distance represents the sum of the distances between the position of the cell centroid between frames (accumulated every second) over the period of observation. The distance from origin (net distance moved) represents the distance between the cell centroid at the beginning and end of the period of observation. Values represent the mean ± SEM of three separate experiments. Data were analyzed by ANOVA with correction for multiple comparisons (Sheffé); *, significance at p < 0.05.

SHP-1 deficiency results in abnormal cytoskeletal regulation

One possible contributing factor to abnormal cell motility in motheaten PMN is defective regulation of the actin cytoskeleton. To investigate this possibility, we assessed the amount of F-actin in control and motheaten PMN before and after agonist stimulation. This analysis revealed that in quiescent PMN, the amount of F-actin was 25% higher in me/me as compared with wild-type cells (Fig. 8a). At early time points (30 s) after addition of chemoattractant, the amount of F-actin remained higher in me/me than in wild-type PMN, whereas at later time points, the amount of F-actin was similar in wild-type and me/me cells. Stimulation of both wild-type and me/me PMN with fMLP induced shape change and a rapid redistribution of F-actin (Fig. 8b).

SHP-1 deficiency does not alter PMN deformability

Another possible contributing factor to reduced movement of motheaten PMN through the pores of polycarbonate filters is decreased cellular deformability. To investigate this possibility, we compared the deformability of wild-type and motheaten PMN by monitoring the pressure required to pass a cell suspension through polycarbonate filters with 6.5-μm pores. This analysis revealed
that there was no difference in the pressure required to pass quiescent wild-type or me/me PMN through the filters (filtration pressure for wild-type PMN, 2.1 ± 0.2 cm H₂O, and for me/me PMN, 2.2 ± 0.3 cm H₂O). Additionally, there was no difference in the pressure required to pass fMLP-stimulated wild-type and me/me PMN through the filters (filtration pressure for wild-type PMN, 4.1 ± 0.3 cm H₂O, and for me/me PMN, 4.2 ± 0.5 cm H₂O).

**SHP-1 deficiency does not impair phagocytosis**

Phagocytosis of microbial pathogens represents another important microbicidal function of PMN and a prerequisite for efficient killing. To evaluate the relevance of SHP-1 to this function, me/me, me'/me', and wild-type PMN were compared with respect to their capacities to internalize serum- or IgG-opsonized zymosan. The results of this analysis revealed that the phagocytic ability of moth-eaten cells was comparable with that of wild-type PMN with respect to serum-opsonized (Fig. 9) or IgG-opsonized (not illustrated) zymosan.

**Discussion**

The primary purpose of the current study was to assess the contribution of SHP-1 to the regulation of PMN function. The data reported herein reveal roles of SHP-1 in modulating PMN adhesion, actin assembly, motility, and production of reactive oxygen intermediates, all essential properties of the microbicidal function of these phagocytic cells. The data suggest that SHP-1 facilitates down-regulation and/or termination of PMN activation cascades. Although the magnitude of SHP-1 effects on some PMN behaviors (e.g., adhesion and oxidant production) appears relatively small, the cumulative physiological effect may be greater when exerted in vivo over prolonged periods of time. This conclusion is consistent with the profound inflammation and severe tissue injury manifested by SHP-1-deficient mice (37, 74, 83, 84), animals that die prematurely as a consequence of a hemorrhagic interstitial pneumonia associated with intra-alveolar accumulation of macrophages and PMN (85, 86) and with enhanced production of TNF-α by the alveolar macrophages (39, 86). Our recent data revealing that levels of SHP-1 expression are high in human peripheral blood PMN (35) suggest a potential role for this signal-terminating molecule in the regulation of inflammatory tissue damage that may occur in pathologic contexts in humans.

The mechanisms by which SHP-1 exerts such diverse influence on PMN functions remain to be determined. SHP-1 has been shown to bind to a plethora of signaling effectors including growth factor receptors and cytosolic signaling molecules. Examples of the latter include Grb-2; Cbl; STAT3; STAT5a; STAT5b; Shc, the p85 subunit of PI 3-kinase; Vav, the Ras-GTPase-activating protein; and p62DOK (43, 87, 88). SHP-1 has also been recently shown to associate with a 130-kDa tyrosyl-phosphorylated species (P130) in macrophages comprising two transmembrane glycoproteins, PIR-B/p91A and the signal-regulator protein family member BIT (31). These latter proteins are hyperphosphorylated in macrophages from me'/me' mice and may therefore represent SHP-1 substrates (as already shown with respect to PIR-B in B cells). Our observations that there is an enhanced respiratory burst in response to agonists that act via plasma membrane receptors (fMLP and zymosan-activated serum), as well as by agents such as PMA (a direct activator of PKC) that bypass surface receptors, indicate that there are likely additional targets of SHP-1 situated downstream in the signaling pathway leading to NADPH oxidase activation. Additional studies are required to determine the extent to which
SHP-1 interactions with these or other proteins account for SHP-1 effects on myeloid cell behavior.

In concert with our previous observations, the current studies indicate that SHP-1 is important in the regulation of tyrosine phosphorylation-dependent signaling pathways in myeloid leukocytes. Our previous studies suggested a role for SHP-1 in regulation of tyrosine phosphorylation in human peripheral blood PMN (35). In these cells, activation by distinct agonists led to a time-dependent decrease in the activity of SHP-1 that correlated with an increase in whole-cell tyrosine phosphorylation. Based on these observations, a prediction would be that cells deficient in SHP-1 would have enhanced levels of tyrosine phosphorylation. Indeed, increased levels of tyrosine phosphorylation of several polypeptides were observed in PMN from motheaten mice (Fig. 2). However, it is also apparent that other factors are important in the regulation of tyrosine phosphorylation in myeloid cells, because despite the absence of SHP-1, stimulation of motheaten PMN led to a further increase in levels of cellular tyrosine phosphorylation. One interpretation of these observations is that agonist-induced increases in tyrosine kinase can be effected despite deficiency in SHP-1 and that increased activity of these kinases contributes significantly to the enhanced levels of tyrosine phosphorylation after agonist exposure. Additionally, in the absence of SHP-1, alternate tyrosine phosphatases could contribute to dephosphorylation of these same phosphoproteins and the activity of these phosphatases could be modulated in agonist-stimulated cells.

The hyperadhesiveness of motheaten PMN suggests that SHP-1 is also involved in regulating the adherence properties of leukocytes. Enhanced adhesiveness of these cells appears to reflect in part an effect of SHP-1 on β2 integrin function because surface expression of the CD11b isoform of the α-chain is increased in me/me PMN and the enhanced adhesion is abrogated by anti-CD11b mAbs. PMN hyperadhesiveness may represent a contributory factor in the massive myeloid cell accumulation of observed tissues of motheaten mice (37, 83, 84), particularly in view of data revealing that the inflammatory infiltration is partially ameliorated by treatment with anti-CD11b (5C6) Ab (40).

At present the mechanism(s) whereby SHP-1 influences CD11/CD18 function and cell adhesion remain unclear, as is the role of tyrosine phosphorylation in modulating β2 integrin function (89, 90). It is noteworthy that SHP-1 associates with tyrosine phosphorylated platelet endothelial cell adhesion molecule-1 (91) and with several molecules found in adhesion complexes including paxillin, vimentin, and F-actin in CSF-1-stimulated macrophages (87), and the relevance of these interactions with SHP-1 in modulation of cell adhesion needs to be investigated. Interestingly, several other protein tyrosine phosphatases have also been implicated in the regulation of myeloid cell-cell and cell-substrate adhesion (92, 93). For example, the closely related PTP, SHP-2, appears to play an important role in β3 integrin-mediated activation of mitogen-activated protein kinase (94), and the leukocyte tyrosine phosphatase CD45 is required for the maintenance of integrin-mediated adhesion in murine bone marrow macrophages (95). Importantly, SHP-1-deficient macrophages were reported to have defective de-adhesion attributable to an increase in d-3 phospholipids as a consequence of an increase in membrane-associated PI 3-kinase activity (33). By analogy, the hyperadhesiveness and the impaired motility in motheaten PMN observed in our study might be related to aberrant PI 3-kinase regulation.

With respect to cell motility, for cells to migrate through a chemotaxis filter or along a surface, repetitive cycles of adhesion and de-adhesion are required (96, 97), and if the latter is impaired, the net effect would be diminished vectorial movement. There is a precedent for the involvement of phosphatases in regulation of deadhesion and cell motility. Maxfield and colleagues reported that chemokinesis of human PMN along a vitronectin-coated surface required the calcium/calmodulin-dependent serine/threonine phosphatase, calcineurin (96, 98). Interestingly, these experiments revealed that effective forward motion required calcium/calcineurin-dependent release of adhesion followed by internalization and recycling of these integrins to the leading edge of migrating PMN (98). Although not investigated directly, similar mechanisms might account for defective chemotaxis and chemokinesis in motheaten PMN.

The significance of the chemotactic defect observed in vitro is uncertain because in the intact animal, circulating PMN are apparently able to emigrate from the vascular space into the tissues. It is likely, however, that multiple ligands for adhesion molecules are available in vivo that enable effective leukocyte motility despite a defective β2 integrin function. It should be noted that bone marrow myeloid progenitors and macrophages have recently been reported to have enhanced chemotactic responses to SDF-1, a CXC chemokine (58). One potential explanation for the apparent discrepancy with our observation that mature PMN exhibited diminished chemotaxis is that chemotactic signaling pathways might have unique aspects that are dependent on the particular chemotactic ligand (C5a, FMLP, and KC vs SDF-1).

The relative contribution of SHP-1 and other PTP to adhesion and motility requires further investigation. To date, a number of PTP have been suggested or proven to play a role in the regulation of cell migration and adhesion. The tyrosine phosphatase LAR has been localized to focal adhesions (99), and cells from mice deficient in LAR or the closely related PTPα demonstrate impairment of cell migration (100–102). LAR and the tyrosine phosphatase PTP1A appear to regulate cell motility through interactions with the Rho and Rac G proteins (103, 104). SHP-2 and PTEN have been shown to negatively regulate focal adhesion signaling by mediating FAK dephosphorylation (105–108), while PTP-PEST has been shown to mediate p130Cas dephosphorylation (109, 110) and associate with paxillin (111). While most studies suggest a down-regulatory role for PTP in cell migration, PTPα directly activates c-Src (112, 113) and positively regulates focal adhesion signaling pathways (114, 115).

Regulation of chemoattractant-induced actin assembly was abnormal in motheaten PMN. The primary defect was that unstimulated PMN from motheaten mice had higher levels of F-actin when compared with wild-type controls. Consequently, chemoattractant-induced increases in F-actin were proportionately less in the motheaten cells. Although the mechanisms underlying this defective cytoskeletal regulation are not known, actin-associated proteins are known to be phosphorylated on tyrosine residues (87). Abnormalities in the regulation of tyrosine phosphorylation of proteins involved in the control of actin assembly could be disrupted in motheaten cells. This defective cytoskeletal regulation could contribute to the defective cell motility observed in motheaten PMN.

Oxidant production was also increased in motheaten PMN, an observation that suggests the involvement of SHP-1 in regulating the phagocyte NADPH oxidase. This latter protein is part of a multicomponent enzyme complex that transfers a single electron from NADPH to molecular oxygen, resulting in the production of superoxide (O2·-) (4, 75). Although the signaling pathways leading to activation of the NADPH oxidase remain to be clarified, tyrosine phosphorylation may be relevant to the process because increases in tyrosine phosphorylation correlate temporally with activation of the oxidase (11). Additionally, inhibitors of PTK block production of reactive oxygen intermediates (11, 116), and inhibition of PTP with vanadate or its peroxides has been shown to potentiate FMLP-induced superoxide production in whole cells and...
to activate a respiratory burst in electroporated cells (25–27). Taken together, these observations suggest that PTP negatively regulates NADPH oxidase activation and suggest that this role is mediated at least in part by SHP-1.

It is possible that the increased plasma membrane levels of CD11b and enhanced oxidant production could be a manifestation of enhanced mobilization of secondary granules that contain intracellular stores of the β2 integrin and the flavoprotein component of the NADPH oxidase. However, this possibility seems unlikely based on our studies demonstrating that the amounts of CD11b and lactoferrin (also contained in the secondary granules) in intracellular stores was comparable between wild-type and motheaten cells.

One potential explanation for the apparent state of hyperresponsiveness of the motheaten PMN described in our studies is that the cells are primed or activated by a systemic inflammatory response as cytokines.

Ischemia/reperfusion-induced microvascular dysfunction: role of oxidants and lipid mediators.

References

tyrosine Cγ1 induced by cross-linking of the high-affinity or low-affinity Fc receptor for IgG in U937 cells. Proc. Natl. Acad. Sci. USA 89:3659.
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