Terminal Differentiation of Murine Resident Peritoneal Macrophages Is
Characterized by Expression of the STK Protein Tyrosine Kinase, a Receptor for Macrophage-Stimulating Protein

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STK, a new member of the hepatocyte growth factor receptor family, is the receptor for macrophage-stimulating protein (MSP), which acts on murine resident peritoneal macrophages. We established polyclonal and monoclonal antibodies against STK and characterized the structure of STK protein and STK expression on cells of the mononuclear phagocyte system. Western blotting showed that the STK transcript is translated into a single-chain precursor and then cleaved into a 165-kD disulfide-linked heterodimer composed of a 35-kD α-chain and a 144-kD β-chain. Western blotting detected STK protein on resident peritoneal macrophages, a target of MSP, and showed that it was autophosphorylated in cells stimulated by MSP. By flow cytometric analysis using a monoclonal anti-STK antibody, we showed that STK protein is expressed on restricted macrophage populations such as resident peritoneal macrophages, but not on exudate peritoneal macrophages or mononuclear phagocytes of the bone marrow, peripheral blood, spleen, or alveoli. Resident peritoneal macrophages were classified into two fractions according to their reactivity with an anti-STK antibody and a marker antibody for macrophages: STK(\textsuperscript{high})/F4/80\textsuperscript{low} cells and STK(\textsuperscript{negative})/F4/80\textsuperscript{low} cells. Acute exudative macrophages were all STK(\textsuperscript{positive})/F4/80\textsuperscript{low}, but they gradually became predominantly STK(\textsuperscript{high})/F4/80\textsuperscript{high} several days after entrance into the peritoneal cavity. These results showed that after monocytes migrate into the peritoneal cavity, they undergo terminal differentiation in the peritoneal microenvironment. This is the first evidence of tissue-specific terminal differentiation of peritoneal macrophages, and this terminal differentiation can be characterized by the expression of STK receptor tyrosine kinase.

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tion cascade, nerve growth factor-γ, and epidermal growth factor-binding protein. Proteins in this family are related to serine proteases, but some, like MSP and HGF, are enzymatically inactive. MSP was first shown to induce murine resident peritoneal macrophages to become responsive to the chemotactic C5a. Purified MSP also acts as a chemottractant for resident peritoneal macrophages, causes shape changes and migration of macrophages, and stimulates macrophage ingestion of complement-coated erythrocytes. However, MSP does not act on exudative peritoneal macrophages.

MATERIALS AND METHODS

Cells and mice. Cells and tissues were obtained from C57BL/6 mice. Bone marrow cells were flushed from femurs with phosphate-buffered saline (PBS). Mononuclear cells of peripheral blood were isolated by centrifugation with Lympholyte-M (Cedelane Laboratories Ltd, Hornby, Canada). A spleen cell suspension was prepared by filtering cells through mesh. Peritoneal cells were obtained by lavage of the peritoneal cavity with 5 mL of PBS. Exudative peritoneal macrophages were obtained from mice at different intervals after an intraperitoneal injection of 40 pg murine IL-3. Mature MSP was purified from human plasma as described. Mature MSP was purified from human plasma as described. Recombinant human HGF was provided by Dr M. Ueda, Snow Brand Co Ltd (Ishibashi, Tochigi, Japan). Mouse IL-3 was purified from the supernatant of COS cells transfected with murine IL-3 cDNA. IL-3 was used after titration. Sheep anti-mouse IgG, goat anti-rabbit IgG, and rabbit IgG conjugated with HRP were from Promega Corporation (Madison, WI). Monoclonal anti-phosphotyrosine antibody (clone 4G10) was from Upstate Biotechnology Inc (Lake Placid, NY). The enhanced chemiluminescence (ECL) detection system was from Amersham. Protein G-Sepharose was from Pharmacia (Uppsala, Sweden). RPMI 1640 medium and DMEM were from Life Technologies (Bovril, Canada). Lungs were lavaged with a total volume of 10 mL of PBS-0.6 mmol/L EDTA in 1-mL aliquots. Cells were centrifuged and resuspended in Dulbecco’s modified Eagle’s medium (DMEM) containing gentamicin and 10% fetal bovine serum (FBS). Macrophages were purified by adherence to plastic after an overnight incubation at 37°C. The IL-3-dependent pro-B cell line Ba/F3 was cultured in serum-free medium containing 100 U/mL of recombinant murine IL-3.

Reagents. Mature MSP was purified from human plasma as described. Recombinant human HGF was provided by Dr M. Ueda, Snow Brand Co Ltd (Ishibashi, Tochigi, Japan). Mouse IL-3 was purified from the supernatant of COS cells transfected with murine IL-3 cDNA. IL-3 was used after titration. Sheep antimouse or anti-rabbit IgG conjugated with horseradish peroxidase (HRP) was obtained from Amersham International (Amersham, UK). Goat anti-rabbit IgG conjugated with HRP was from Promega Corporation (Madison, WI). Monoclonal anti-phosphotyrosine antibody (clone 4G10) was from Upstate Biotechnology Inc (Lake Placid, NY). The enhanced chemiluminescence (ECL) detection system was from Amersham. Protein G-Sepharose was from Pharmacia (Uppsala, Sweden). RPMI 1640 medium and DMEM were from Life Technologies (Grand Island, NY).

Generation of polyclonal and monoclonal antibodies against STK. Antiserum against STK was raised in rabbits by immunization with a KLH-conjugated synthetic polypeptide corresponding to the C-terminal 17 amino acids (amino acid residues 1362 to 1378) of STK protein. A partial cDNA containing the entire extracellular domain of STK was fused in-frame to a sequence of human IgG1Fc. This STK Fc chimeric cDNA was inserted into a modified expression vector with the SREs promoter and transfected into COS7 cells using diethyl aminoethyl (DEAE)-dextran. The STK Fc chimeric protein was purified from the serum-free conditioned medium of transfected COS7 cells. Hybridomas were produced by fusion of P3U1 mouse myeloma cells with spleen cells from a Wistar rat immunized with purified STK Fc chimeric protein. Positive hybridomas were selected by fluorescence-activated cell sorting (FACS), using STK-transfected NIH3T3 cells (see below) as indicators.

FACS. Before staining, Fc receptors on cells from tissues were blocked with 0.5 μg/mL of mouse anti-mouse CD32/16 (FcγII/III receptor) monoclonal antibody (PharMingen, San Diego, CA). The cells were stained with 5 μg/mL of biotinylated anti-STK monoclonal antibody, followed by allaphorphococyanin (APC)-conjugated streptavidin (Becton Dickinson Immunocytometry Systems, San Jose, CA) and fluorescein isothiocyanate (FITC)-conjugated F4/80 monoclonal antibody (Serotec Ltd, Oxford, UK). Dead cells were excluded by propidium iodide staining. Stained cells were analyzed and sorted on a FACStar® plus (Becton Dickinson Immunocytometry Systems). Monocytcs and macrophages in the cell suspensions were gated by forward and side scatter, and then their staining profiles were analyzed.

Establishment of STK-overexpressing cell lines. Full-length STK cDNA was subcloned into the eukaryotic expression vector, pRC/RSa, derived from pRC/CMV (Invitrogen, San Diego, CA), and introduced into Ba/F3 cells and NIH3T3 cells by electroporation. Cells were selected in the presence of G418. Among several resistant clones, clones with high STK expression, designated Ba/F3 STK and 3T3/STK, were selected for this study. Ba/F3 cells transfected with vector alone (Ba/F3 vector cells) served as negative controls.

Immunoprecipitation and Western blotting. Cells were solubilized with lysis buffer (10 mmol/L Tris [pH 7.8], 1% Triton X-100, 150 mmol/L NaCl, 1 mmol/L EDTA, 50 μg/mL aprotinin, 1 mmol/L phenylmethylsulfonyl fluoride [PMSF]). Protein in cell lysates was immunoprecipitated with rabbit antisera against the C-terminus of STK or anti-STK monoclonal antibodies. Immune complexes were collected on protein G-Sepharose (Pharmacia). Cell lysates and immune complexes were boiled for 3 minutes at 100°C in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer with or without 5% 2-mercaptoethanol (2-ME; reduced or non-reduced condition), resolved by SDS-PAGE with 6.5% or 10% polyacrylamide as described by Laemmli. Among several resistant clones, clones with high STK expression, designated Ba/F3 STK and 3T3/STK, were selected for this study. Ba/F3 cells transfected with vector alone (Ba/F3 vector cells) served as negative controls.

Cell surface biotinylation. Ba/F3/vector and Ba/F3/STK cells were biotinylated using the ECL protein biotinylation system (Amersham). Briefly, 5 × 10⁶ cells in PBS were labeled with biotinylation reagent on ice for 30 minutes. The reaction was stopped by removing the biotin solution, washing two times with PBS, and incubating for 10 minutes with Tris-buffered saline (20 mmol/L Tris-HCl [pH 7.6], 150 mmol/L NaCl). Cells were solubilized with lysis buffer, and cell lysates were preclarihed with Pansorbin Cells (Calbiochem Co, La Jolla, CA) and protein G-Sepharose. Protein in preclarihed cell lysates was immunoprecipitated with anti-STK monoclonal antibodies. Immune complexes were collected and resolved by SDS-PAGE with 10% polyacrylamide, as described above. After electrophoresis, proteins were transferred to nitrocellulose membranes (Nihon Millipore Ltd, Yonezawa, Japan), which were then probed with the indicated antibodies, and specific binding was detected using the enhanced chemiluminescence system (ECL; Amersham).

Cell surface biotinylation. Ba/F3/vector and Ba/F3/STK cells were biotinylated using the ECL protein biotinylation system (Amersham). Briefly, 5 × 10⁶ cells in PBS were labeled with biotinylation reagent on ice for 30 minutes. The reaction was stopped by removing the biotin solution, washing two times with PBS, and incubating for 10 minutes with Tris-buffered saline (20 mmol/L Tris-HCl [pH 7.6], 150 mmol/L NaCl). Cells were solubilized with lysis buffer, and cell lysates were preclarihed with Pansorbin Cells (Calbiochem Co, La Jolla, CA) and protein G-Sepharose. Protein in preclarihed cell lysates was immunoprecipitated with anti-STK monoclonal antibodies. Immune complexes were collected and resolved by SDS-PAGE with 10% polyacrylamide, as described above. After electrophoresis, proteins were transferred to nitrocellulose membranes, probed with streptavidin-HRP, and visualized with the ECL system.

In vitro tyrosine phosphorylation assays. Ba/F3/STK cells, incubated for 12 hours in the absence of IL-3, were used for the phosphorylation assay. Ba/F/STK cells at a concentration of 1 × 10⁴/mL were incubated with 1.3 mmol/L human MSP, 1.3 mmol/L human HGF, or 10% fetal calf serum (FCS; Summit, Ft. Collins, CO) at 37°C for 10 minutes. The cells were then solubilized with lysis buffer (50 mmol/L HEPES [pH 7.4], 1% Triton X-100, 10% glycerol, 10 mmol/L sodium pyrophosphate, 100 mmol/L sodium fluoride, 4 mmol/L EDTA, 2 mmol/L sodium orthovanadate, 50 μg/mL aprotinin, 1 mmol/L PMSF, 100 μmol/L leupeptin, 25 μmol/L pepstatin A), and STK was immunoprecipitated with rabbit antisera against
STK. Tyrosine phosphorylation of STK β-chain was evaluated by Western blotting with the anti-phosphotyrosine antibody, 4G10. To detect the STK β-chain, the membrane was stripped and reprobed with rabbit antiserum against STK. MSP-stimulated murine peritoneal cells were evaluated by the same protocol.

Assays for change in cell morphology and erythrophagocytosis. Peritoneal macrophages and BaF3/STK cells were suspended in serum-free RPMI 1640 medium and incubated in 24-well tissue culture plates at a concentration of 5 × 10⁵/mL in the presence or absence of 1.3 mmol/L purified human plasma MSP for 1 hour. Thereafter, the cell morphology was observed under a phase contrast microscope at a magnification of 200X. The effect of MSP on erythrophagocytosis was evaluated as previously described. Sheep erythrocytes coated with IgM anti-Forssman antibody and complement C3bi pro-

Fig 1. FACS analysis of STK expression on BaF3/STK cells with monoclonal antibodies (clone 1B and 2B) against the extracellular domain of STK: (A) BaF3/vector cells; (B) BaF3/STK cells.

Fig 2. Detection of STK protein in BaF3/STK cells. (A) Western blots of STK expression in BaF3/STK cell lysates. Proteins were separated on SDS-PAGE with 6.5% polyacrylamide under reducing (lane 1) and nonreducing (lane 2) conditions, and then transferred and probed with rabbit antiserum against the C-terminus of STK. (B) Detection of STK protein by cell surface biotinylation. Proteins in cell lysates of surface-biotinylated BaF3/vector (lanes 1 and 3) and BaF3/STK (lanes 2 and 4) cells were immunoprecipitated with anti-STK monoclonal antibodies 1B (lanes 1 and 2) and 2B (lanes 3 and 4). Immune complexes were separated on SDS-PAGE with 10% polyacrylamide under reducing conditions, transferred, and probed with streptavidin-HRP. (C) Western blots of STK expression on immunoprecipitates from lysates of BaF3/vector cells (lanes 1, 3, and 5) and BaF3/STK cells (lanes 2, 4, and 6) with rabbit antiserum against STK (lanes 1 and 2), monoclonal antibody 1B (lanes 3 and 4), and monoclonal antibody 2B (lanes 5 and 6). Proteins were separated on SDS-PAGE with 6.5% polyacrylamide under reducing conditions, transferred, and probed with rabbit antiserum against STK.
tein (ElgMC3bi) were added to macrophage monolayers in tissue culture wells at an ElgMC3bi-to-macrophage ratio of 25:1. After a 1-hour incubation at 37°C in the presence or absence of 1 mmol/L MSP, wells were observed for the presence of macrophages surrounded by rosettes of erythrocytes. Ammonium chloride erythrocyte lysis buffer was then added to lyse rosetted erythrocytes, leaving ingested erythrocytes morphologically intact. Macrophage monolayers were stained with Diff-Quick (American Scientific Products, McGaw Park, IL) and examined at a 1,000× magnification for the presence of ingested erythrocytes.

Ultrastructural peroxidase cytochemistry. For cytochemical demonstration of peroxidase activity, STK<sup>h-F480</sup> peritoneal macrophages in the steady state and STK<sup>h-F4/F480</sup> peritoneal macrophages 6 hours after an intraperitoneal injection of Con A were collected by cell sorting using a FACStar<sup>TM</sup> (Becton Dickinson Immunocytometry Systems). The cells were fixed with 1% glutaraldehyde for 30 minutes. After washing in 0.05 mol/L cacodylate buffer, the cells were incubated for 30 minutes according to the method of Graham and Karnovsky. Ultrathin sections were observed in an H-12A electron microscope (Hitachi, Tokyo, Japan) without staining.

RESULTS

Production of anti-STK monoclonal antibodies and characterization of STK protein in BaF3/STK cells. Monoclonal antibodies were raised against the extracellular domain of STK, and two clones, designated 1B (IgG2a κ) and 2B (IgG1 κ), were isolated. FACS analysis showed that both monoclonal antibodies reacted with BaF3/STK cells, a cell line expressing STK, but not with BaF3/vector control cells (Fig 1). Rabbit antisera raised against a synthetic polypeptide corresponding to the STK C-terminus was used in the Western blots. The rabbit antisera detected a 165-kD protein (p165<sup>STK</sup>) under nonreducing conditions, and 144- and 160-kD proteins under reducing conditions (Fig 2A). Rabbit preimmune serum did not detect any STK proteins (data not shown). On the other hand, 35-kD and 144-kD proteins, but not 160-kD protein, were detected by cell surface biotinylation followed by immunoprecipitation with monoclonal antibodies (Fig 2B). These data show that p165<sup>STK</sup> is a disulfide-linked heterodimer composed of a 35-kD α-chain and a 144-kD β-chain and that the 160-kD protein detected under reducing conditions may be the cytoplasmic uncleaved single-chain precursor protein. A faint band at 37 kD in Fig 2B may be a degradation product, because this band was detected only in BaF3/STK cells but not in BaF3/vector cells. Both polyclonal and monoclonal antibodies immunoprecipitated STK from lysates of BaF3/STK cells, but not from BaF3/vector cell lysates (Fig 2C).

MSP induces autophosphorylation of STK β-chain in resident peritoneal macrophages. The STK β-chain of BaF3/STK cells was phosphorylated in response to purified human plasma MSP, but not to recombinant human HGF, a protein with 45% sequence identity to MSP (Fig 3A). FCS also induced STK β-chain phosphorylation, suggesting the presence of bovine pro-MSP or MSP in the serum. The basal level of autophosphorylation of the STK β-chain in BaF3/STK cells may be due to the overexpression of STK (Fig 3B). The other protein in Fig 3A that was immunoprecipitated with rabbit antiserum against STK is the 160-kD putative cytoplasmic uncleaved single-chain STK precursor, which becomes functional only after cleavage to the disulfide-linked heterodimer. Predictably, addition of MSP did not cause phosphorylation of this protein (Fig 3A). In murine resident peritoneal macrophages, which express physiological amounts of STK, which becomes functional only after cleavage to the disulfide-linked heterodimer. Predictably, addition of MSP did not cause phosphorylation of this protein (Fig 3A).

MSP causes shape changes in STK-expressing cells. MSP causes shape changes and increased motility in resident peritoneal macrophages. Sixty percent of the resident peritoneal macrophages showed elongated shapes 1 hour after the addition of MSP (Fig 4B). Sixty-eight percent of BaF3/STK cells also responded to MSP with shape changes, including ruffling, budding, and elongation (Fig 4D). These changes were not detected in BaF3/vector cells (data not shown).

Fig 3. Induction of autophosphorylation of STK protein in response to MSP. (A) BaF3/STK cells were stimulated with medium only (none), 1.3 nmol/L human MSP, 1.3 nmol/L human HGF, or 10% FCS. (B) Peritoneal cells were stimulated with medium only (none) or 1.3 nmol/L human MSP. STK protein in cell lysates was immunoprecipitated with rabbit antiserum against STK. Immunoprecipitates were Western-blotted and probed with an anti-phosphotyrosine (anti-P-tyr) antibody (4G10; upper panels) and with rabbit antiserum against STK (lower panels).
STK protein is detected on resident peritoneal macrophages but not on blood monocytes or other tissue macrophages. We investigated STK expression on monocytes and various tissue macrophages by FACS using a monoclonal anti-STK antibody (2B) and an antibody (F4/80) specific for mouse mononuclear phagocytes. To confirm the correlation between STK expression and MSP-responsiveness of different macrophage populations, we analyzed the capacity of MSP to stimulate the ingestion of ElgMC3bi.

FACS analysis showed that STK protein is expressed on resident peritoneal macrophages, but not on mononuclear phagocytes from bone marrow, peripheral blood, spleen, or alveoli (Figs 5 and 6). The percentage of positive cells in the bone marrow, peripheral blood, spleen, and alveoli was within the background level. As expected from these results, MSP stimulated the uptake of ElgMC3bi by resident peritoneal macrophages, but not by alveolar macrophages (Fig 7) or by splenic macrophages (data not shown). The percentage of peritoneal macrophages that ingested at least one erythrocyte was 2% in the absence of MSP and 27% in the presence of MSP.

Resident peritoneal macrophages showed two distinct staining patterns (Fig 5, Table 1): STK^{high}, F4/80^{high} cells and STK^{negative}, F4/80^{low} cells. Morphological analysis revealed that all of the STK^{high}, F4/80^{high} cells were typical macrophages, and half of the STK^{negative}, F4/80^{low} cells were also typical macrophages (Table 1). Although the number of STK^{negative}, F4/80^{low} macrophages was much less than STK^{high}, F4/80^{high} macrophages, the combination of anti-STK and F4/80 antibody clearly showed heterogeneity of resident peritoneal macrophages. Inasmuch as F4/80 staining intensity is lower in blood monocytes and exudative macrophages than in resident macrophages, 23 our results suggested that the STK^{negative}, F4/80^{low} macrophages might be recent arrivals from the circulation that had not yet matured into the STK^{high}, F4/80^{high} phenotype. This hypothesis was tested in the experiment described in the following section.

The peritoneal macrophage expression of STK is upregulated during terminal differentiation. To test our hypothesis that the maturation of peritoneal macrophages is associated with an increase in F4/80 antigen and the appearance of STK, we induced an acute inflammation in the peritoneal cavity by an intraperitoneal injection of CoA and analyzed the peritoneal cells by FACS (Fig 8). At 1 hour after injection, there was a marked decrease in the number of peritoneal cells recovered by lavage (below 10% of that before injection). At 3 hours after injection, there was a large influx of granulocytes (74.2% of the peritoneal cells recovered by lavage) that were not stained with F4/80 or anti-STK antibody. At 6 hours after injection, macrophages were found in the peritoneal lavage (59.5%). They were stained with F4/80 at low intensity but did not react with anti-STK. Thereafter, there was a progressive increase in the number of cells that reacted with both antibodies, as well as the fluorescence intensity per cell for both antibodies. On day 6, the profile was comparable with that of the preinjection control.

Ultrastructural peroxidase cytochemistry of STK^{high}, F4/80^{high} and STK^{negative}, F4/80^{low} macrophages. Etudative and resident macrophages are easily distinguished by the different localization patterns of endogenous peroxidase activity. Resident macrophages show peroxidase activity in the nuclear envelope and rough endoplasmic reticulum, whereas exudative macrophages show activity in the primary lysosomes but not in the nuclear envelope or the rough endoplasmic reticulum. 11,13 Most of the STK^{high}, F4/80^{high} macrophages in the steady state showed peroxidase activity in the nuclear envelope and rough endoplasmic reticulum (Fig 9A). Some macrophages showed weak peroxidase activity, probably because of the process of cell sorting. STK^{high}, F4/80^{high} macrophages revealed ultrastructural characteristics of mature macrophages such as well-developed rough endoplasmic reticulum and prominent cytoplasmic projections and infoldings (Fig 9A). On the other hand, most of the STK^{negative}, F4/80^{low} macrophages (>95%) after an eliciting stimulus showed peroxidase activity in the lysosomal granules but not in the nuclear envelope or rough endoplasmic reticulum (Fig 9B). Cytoplasmic processes of these cells were less prominent than those of resident macrophages. Localization of endogenous peroxidase and ultrastructural characteristics of these cells correspond to those of exudative macrophages recently migrated from the blood stream.

DISCUSSION

The gene for the novel receptor tyrosine kinase, STK, was first isolated from mouse hematopoietic stem cells, and its mRNA has been detected at various differentiation stages
EXPRESSION OF STK IN MONONUCLEAR PHAGOCYTES

Fig 5. Detection of STK expression on resident peritoneal macrophages by FACS analysis with monoclonal anti-STK antibody 2B. Peritoneal macrophages were selected by gating cells characterized by high forward and side scatter and were then analyzed for their staining pattern. (A) Peritoneal cells, and (B) the percentage of each cell population of panel A. R2 (region 2), STK<sup>−</sup>-F4/80<sup>−</sup> cells; R3 (region 3), STK<sup>+</sup>-F4/80<sup>−</sup> cells; R4 (region 4), STK<sup>−</sup>-F4/80<sup>−</sup> cells; and R5 (region 5), STK<sup>−</sup>-F4/80<sup>−</sup> cells.

Fig 6. FACS analysis of STK expression on mononuclear phagocytes using monoclonal anti-STK antibody 2B. Mononuclear phagocytes were selected by gating cells characterized by high forward and side scatter and were then analyzed for their staining pattern. (A) Bone marrow cells, (B) peripheral blood cells, (C) spleen cells, and (D) alveolar macrophages.
Fig 7. MSP-induced stimulation of ElgMC3bi ingestion by murine resident peritoneal macrophages, but not by alveolar macrophages. Peritoneal macrophages without (A) and with (B) MSP; alveolar macrophages without (C) and with (D) MSP. The percentage of peritoneal macrophages that ingested at least one erythrocyte was 2% in the absence of MSP and 27% in the presence of MSP.

of hematopoietic cells by reverse transcription-polymerase chain reaction (RT-PCR) analysis. However, Northern blotting revealed that STK mRNA was not preferentially expressed in the bone marrow. Recently, we and others have identified STWRON as the receptor for MSP. The biological effects of MSP on resident peritoneal macrophages suggested the presence of STK protein on these cells. To identify the expression of STK protein on hematopoietic cells, we generated polyclonal and monoclonal antibodies against STK.

Table 1. Antibody Reactivity and Morphology of Resident Peritoneal Cells

<table>
<thead>
<tr>
<th>Antibody Reactivity</th>
<th>Morphology</th>
<th>% of Gated Cells</th>
<th>% of Total Cells</th>
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<tbody>
<tr>
<td>STK&lt;sup&gt;+&lt;/sup&gt;-F4/80&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Macrophages</td>
<td>73.4</td>
<td>20.3</td>
</tr>
<tr>
<td>STK&lt;sup&gt;-&lt;/sup&gt;-F4/80&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Macrophages (48%), eosinophils (52%)</td>
<td>13.0</td>
<td>4.5</td>
</tr>
<tr>
<td>STK&lt;sup&gt;-&lt;/sup&gt;-F4/80&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Lymphocytes</td>
<td>8.4</td>
<td>70.0</td>
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Morphology data of resident peritoneal cells gated as in Fig 5 and sorted by criteria of antibody reactivity and then characterized by May-Grünwald-Giemsa staining. The percentage of each cell population was determined by FACS analysis within the gated cells, as described in Fig 5, or the total cell population.
promoter, the subcellular machinery responsible for proteolytic cleavage is overloaded. This may lead to the abnormal expression of uncleaved STK protein in BaF/STK cells.

Western blotting also demonstrated the presence of STK protein on resident peritoneal macrophages, a target of MSP (Fig 3B). The MSP-induced stimulation of motile (Fig 4B) and phagocytic activity (Fig 7A) of resident peritoneal macrophages were associated with autophosphorylation of the STK \( \beta \)-chain (Fig 3B). These data confirmed that STK transduces the MSP signal.

As it does in resident peritoneal macrophages, MSP caused autophosphorylation of the STK protein and shape changes in STK-transfected Ba/F3 pro-B cells (Figs 3 and 4). MSP also stimulated the cell migration of STK-transfected NIH/3T3 fibroblasts.\(^{17}\) These data suggested that the STK receptor activated by MSP can transduce signals for shape changes and cell motility in various types of cells. Moreover, in STK-transfected Ba/F3 or erythroid cells, activated STK receptor could transduce pleiotropic biological effects such as the stimulation or suppression of cell proliferation (A.I., T.S., unpublished data, February 1995).

Among the many macrophage populations surveyed in our FACS analysis, STK expression was detected on resident peritoneal macrophages. Our preliminary analysis showed that osteoclasts, members of the mononuclear phagocyte system, also express STK and that MSP stimulates bone resorbing activity of osteoclasts.\(^{14}\) However, STK was not expressed on exudative peritoneal macrophages or mononuclear phagocytes of the bone marrow, peripheral blood, spleen, or alveoli (Figs 5 through 7). On the other hand, F4/80 antigen, a marker cell surface antigen of monocytes and macrophages, is expressed on all mononuclear phagocytes.\(^{10}\) M-CSF receptor, a receptor tyrosine kinase, is also expressed on almost all mononuclear phagocytes we examined (A.I., T.S., unpublished data, January 1995). Thus, the restricted expression of STK receptor tyrosine kinase in the mononuclear phagocyte system is very unique, and STK could be a good marker discriminating the heterogeneity of the mononuclear phagocyte system.

MSP stimulates resident, but not exudative, peritoneal macrophages.\(^{26}\) The FACS analysis presented here clarified that STK, the MSP receptor, is present on resident peritoneal macrophages, but not on exudative macrophages or peripheral blood monocytes (Figs 5, 6, and 8; Table 1). By determining the STK and F4/80 phenotype of exudative macrophages on successive days after the eliciting stimulus, we established that the profile of the recent arrivals from the circulation into the peritoneal cavity is STK\(^{\text{negative}}\)-F4/80\(^{\text{low}}\).
and that within 6 days, they differentiate into STK$_{\text{high}}$-F4/80$_{\text{high}}$ macrophages (Fig 8). Ultrastructural peroxidase cytochemistry confirmed these findings (Fig 9). STK$_{\text{high}}$-F4/80$_{\text{high}}$ macrophages showed a resident pattern of peroxidase localization, whereas STK$_{\text{negative}}$-F4/80$_{\text{low}}$ macrophages showed an exudative pattern. Moreover, STK$_{\text{high}}$-F4/80$_{\text{high}}$ macrophages exhibited ultrastructural characteristics of mature macrophages, while STK$_{\text{negative}}$-F4/80$_{\text{low}}$ macrophages exhibited those of exudative macrophages recently migrated from the blood stream. The upregulation of STK during differentiation is the first evidence of tissue-specific terminal differentiation of peritoneal macrophages. STK could be a good marker antigen to characterize this terminal differentiation.

Even in the absence of an acute inflammatory stimulus, there is a steady-state influx of STK$_{\text{negative}}$-F4/80$_{\text{low}}$ blood monocytes into the peritoneal cavity, which accounts for our detection of this phenotype in addition to the terminally differentiated STK$_{\text{high}}$-F4/80$_{\text{high}}$ macrophages in the resident peritoneal cell population (Fig 5, Table 1). During an acute inflammation, MSP, one of the serum factors, might diffuse into the inflammation site of the peritoneal cavity and form a local MSP gradient. This gradient may mediate the focal accumulation of resident macrophages and activate these cells at the site of inflammation. During the early stage of inflammation, resident peritoneal macrophages may be activated by MSP to oppose pathogens, and subsequently, exudative peritoneal macrophages may undergo terminal differentiation and take their place.

The tissue-specific terminal differentiation of macrophages is believed to be mediated by the microenvironments into which they migrate. However, the specific factors that account for this differentiation have not been identified. We tried using in vitro culture of peripheral blood monocytes in the presence of various cytokines, such as M-CSF, GM-CSF, IL-3, and IL-6; however, we could not derive STK-positive macrophages in vitro (data not shown). Considering that STK is expressed on restricted macrophage populations, upregulation of STK on macrophages is thought to be regulated by some unknown, tissue-specific microenvironments. Therefore, STK could be a good marker also in investigating microenvironments critical to terminal differentiation of macrophages.

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REFERENCES


Fig 9. Ultrastructural peroxidase cytochemistry. (A) STK$_{\text{high}}$-F4/80$_{\text{high}}$ peritoneal macrophages in the steady state. Endogenous peroxidase is localized in the nuclear envelope and rough endoplasmic reticulum (arrows). (B) STK$_{\text{negative}}$-F4/80$_{\text{low}}$ peritoneal macrophages 6 hours after an intraperitoneal injection of Con A. Endogenous peroxidase is localized in the lysosomal granules.
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Terminal differentiation of murine resident peritoneal macrophages is characterized by expression of the STK protein tyrosine kinase, a receptor for macrophage-stimulating protein

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