Inducible Expression of the Cell Surface Heparan Sulfate Proteoglycan Syndecan-2 (Fibroglycan) on Human Activated Macrophages Can Regulate Fibroblast Growth Factor Action*

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Mononuclear phagocytes are recruited from peripheral blood to the sites of tissue injury during the normal physiological processes of wound healing and inflammation as well as in pathological processes such as atherogenesis and tumor development where they play a key role in stimulating new tissue growth (1, 2). During the recruitment process, blood-derived monocytes undergo progressive differentiation to tissue macrophages characterized by changes in cell surface antigen expression, increased phagocytic activity, and most notably acquisition of the capacity to synthesize an array of different cytokines and growth factors (2). Some of the most potent of these molecules are heparin-binding growth factors such as fibroblast growth factor (FGF), vascular endothelial growth factor, and heparin binding epidermal growth factor. The action of these potent growth mediators is known to be regulated by adsorption to heparan sulfate proteoglycans (HSPGs) on the surface of both the extracellular matrix of other neighboring cells, which respectively promote or restrict interactions with their signal-transducing receptors on target cells. Here we report on the nature of HSPGs inducibly expressed on the surface of macrophages that confer these cells with the capacity to regulate endogenous growth factor activity. We reveal that activated human macrophages express only a single major 48-kDa cell surface HSPG, syndecan-2 (fibroglycan) as the result of de novo RNA and protein synthesis. In addition, we demonstrate this macrophage HSPG selectively binds the macrophage-derived growth factors FGF-2, vascular endothelial growth factor and heparin binding EGF and can present FGF-2 in a form that transactivates receptor-bearing BaF32 cells. These results define a novel and unique proteoglycan profile for macrophages and imply a key role for syndecan-2 in the delivery of sequestered growth factors by inflammatory macrophages for productive binding to their appropriate target cells in vivo.

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† The abbreviations used are: FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; hbEGF, heparin-binding epidermal growth factor; HSPG, heparan sulfate proteoglycan; HS, heparan sulfate; GAG, glycosaminoglycan; FGFR1, FGF receptor 1; mAb, monoclonal antibody; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; MDM, monocyte-derived macrophage; PAGE, polyacrylamide gel electrophoresis; IL, interleukin; TNFa, tumor necrosis factor a; LPS, lipopolysaccharide; RANTES, regulated upon activation normal T cell expressed and secreted factor; PCR, polymerase chain reaction; FACS, fluorescence-activated cell sorter.
general role for HSPGs as components of a dual receptor system for heparin-binding growth factors.

The levels of cell surface HS appear to be controlled by the regulation of HSPG core protein synthesis. To date, five major cell surface HSPG core polypeptides have been cloned and characterized including the syndecans, betaglycans (type III TGFβ receptors) glypicans (for review, see Ref. 21) and discrete isoforms of the hyaluronan receptor CD44 (22, 23). Among the most abundant HSPGs, the syndecans comprise a family of four single chain transmembrane polypeptides (24, 25), each with a distinct but partly overlapping pattern of tissue expression. For example, syndecan-1 is expressed primarily on epithelial and mesenchymal cells, syndecan-2 (fibroglycan) on endothelial cells and fibroblasts, syndecan-3 (N-syndecan) on neural cells and syndecan-4 (amphiglycan or ryudocan) on each of these cell types. The primary role of the syndecans is thought to be the anchorage of cells to the extracellular matrix (25), and in the case of syndecan-4, participation in the transduction of extracellular matrix-dependent signaling events (26). However each of the syndecans also bind growth factors including FGF-2 via their HS chains (for review, see Ref. 25), and syndecan-3 on neural cells has been shown (27) to bind the growth factor pleiotrophin (heparin-binding growth associated molecule). Such properties have led to the suggestion that the syndecans may be key regulators of heparin-binding growth factor action in vivo.

In our laboratory, we have explored the possibility that macrophages use HSPGs to regulate the biological activity of their many endogenously synthesized growth factors and chemokines. By comparison with other cell types, little is known about the nature of macrophage HSPGs (28, 29), although the synthesis of HS and its roles in regulating lipid uptake and atherogenesis have been well documented in these cells (30–32).

In this study, we reveal that primary human monocyte-derived macrophages (MDMs) inducibly express a single major core protein, syndecan-2 (fibroglycan), previously thought to be restricted to fibroblasts and endothelial cells. The functional significance of this observation is underlined by the findings that 1) macrophages expressing syndecan-2 bind FGF-2, VEGF, and hβEGF; and 2) macrophages with bound FGF-2 can promote proliferation of neighboring FGF-TR-transfected cells. Our results implicate syndecan-2 as the primary macrophage HSPG involved in regulating macrophage-derived growth factor action.

**EXPERIMENTAL PROCEDURES**

**Growth Factors, Chemokines, Cytokines, and Antibodies—**Recombinant human FGF-2 (155 residue form) was expressed in Escherichia coli from the bacterial expression vector pFC80 (kindly donated by Dr. Antonella Isacchi, Pharmacia & Upjohn, Milan, Italy) and purified by affinity chromatography on Hi-Trap heparin mini-columns (Amersham Pharmacia Biotech) using fast protein liquid chromatography. The fluororescent chemokine derivatives fluorescein isothiocyanate IL-8 and N-methyl-N-(2-N-methyl-N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)aaminomethyl)acetamido-RANTES (NBD-RANTES) described previously (33, 34) were the kind gift of Dr. Tim Wells, Cell Biology Unit, Glaxo Wellcome Medicines Research Center, Stevenage, Hertfordshire UK. The additional heparin-binding growth factors FGF-1,145, VEGF, hβEGF,145, the C-C chemokines MIP-1β and MCP-1 (all carrier-free), and the pro-inflammatory cytokines IL-1α, IL-1β, and TNFα were purchased from R&D Systems Europe, Abingdon, UK. The additional heparin-binding growth factors FGF-1,145, VEGF, hβEGF,145, the C-C chemokines MIP-1β and MCP-1 (all carrier-free), and the pro-inflammatory cytokines IL-1α, IL-1β, and TNFα were purchased from R&D Systems Europe, Abingdon, UK. The additional heparin-binding growth factors FGF-1,145, VEGF, hβEGF,145, the C-C chemokines MIP-1β and MCP-1 (all carrier-free), and the pro-inflammatory cytokines IL-1α, IL-1β, and TNFα were purchased from R&D Systems Europe, Abingdon, UK.

**Cell Lines, Transfectants, and Tissue Samples**—The human B cell line Namalwa was stably transfected with full-length human syndecan-2 cDNA in the expression vector pCDNA3 by electroporation (750 V/cm, 960 microFarads) followed by selection in RPMI 1640 10% serum containing G418 (1.25 mg/ml). Human IL-3-dependent BaF3 lymphoid cells were transfected with the human FG F receptor (FGFR1, 2 Ig domain form), termed BaF32 cells were kindly donated by Prof. John Gallagher, Christie Hospital, Manchester, UK and cultured in RPMI 1640, 10% fetal calf serum, 10% WEHI cell conditioned medium. Samples of synovial fluid were obtained with permission from patients with inflammatory arthritis attending the Nuffield Orthopaedic Center, Oxford, UK.

**Isolation and Culture of Primary Human Peripheral Blood T Cells, Monocytes, and MDMs—**Primary human T cells, monocytes, and MDMs were prepared using either peripheral blood or synovial fluid mononuclear cells as the starting material. To prepare mononuclear cells, freshly drawn (heparinized) whole blood or synovial fluid was diluted 1:1 with RPMI 1640 tissue culture medium and layered on Lymphoprep density gradient medium (Nycomed, Norway). Gradients (50 ml total volume) were centrifuged (1,200 x g, 30 min), and the mononuclear cell band was removed by aspiration. Recovered cells were then washed three times (3 times) in RPMI 1640, 10% (v/v) autologous human serum by resuspension/recentrifugation.

For the isolation of monocytes, the mononuclear cell suspensions were incubated (1 h, 37 °C) in plastic tissue culture dishes (5–15 cm in diameter, 0.5–1 x 10⁶ cells/cm²) in RPMI 1640, 10% (v/v) autologous human serum, to allow selective adherence of monocytes, followed by three successive rinses with warmed medium to remove contaminating nonadherent lymphocytes. The adherent monocytes were then either detached (1 h, 5 °C) in PBS, 5 mM EDTA, pH 7.5 and analyzed immediately (referred to as fresh monocytes) or cultured (37 °C, 5% CO₂) in RPMI 1640, 10% autologous serum either alone or supplemented with TNFα (100 ng/ml), IL-1α (1 ng/ml), or LPS (0.5 μg/ml) for 2–5 days (referred to as monocyte-derived macrophages or MDM). These preparations were at least 80% monocytes as assessed by immunofluorescent staining with antibodies to the CD14 antigen.

For the isolation of T cells, the mononuclear cell suspensions were treated with goat anti-human IgG antibodies and passed over glass bead columns (Celite®) to selectively adsorb B cells and most monocytes, respectively. The resulting purified T cells were washed, resuspended (2 x 10⁶/ml) in RPMI 1640 medium containing 10% fetal calf serum, and induced to proliferate by culture (24–120 h) with the mitogenic lectin phytohemagglutinin (5 μg/ml, Wellcome, UK). Proliferative responses were confirmed by measuring the incorporation of [³H]thymidine into genomic DNA.

**Treatment with Enzymes and Metalloproteinase Inhibitors—**For selective cleavage of cell surface heparan sulfate, adherent monocytes or monocyte-derived macrophages in RPMI 1640, 10% human serum were supplemented with heparinase I (EC 4.2.2.7, 5–20 milliunits/ml, Oxoid GlycoSciences, UK) and heparinase III (EC 4.2.2.8, 5–10 milliunits/ml, Seikagaku Corp.) and incubated for 2 h at 37 °C in a 5% CO₂ incubator before detachment with EDTA as described above. For protease treatment, cells were incubated with trypsin, chymotrypsin, papain, or pepsin (Calbiochem) (each at 100 μg/ml) for 30 min at 37 °C before the addition of the protease inhibitors antipain (60 μg/ml), bestatin (10 μg/ml), chymostatin (20 μg/ml), leupeptin (10 μg/ml), pepstatin (10 μg/ml), E-64 (60 μg/ml), phosphoramidon (60 μg/ml), Pefabloc® SC (400 μg/ml), aprotonin (10 μg/ml), EDTA (1 mM), and detachment with EDTA. For inhibition of heparan sulfate metabolism, monocytes or MDM were supplemented with 30 mM sodium chloride for 24 h before detachment.

**Immunofluorescent and Immunoperoxidase Antibody Staining, Fluorescence Microscopy, and Flow Cytometry—**For immunofluorescent antibody staining, cells (5 x 10⁶) were incubated (30 min, 5 °C) with saturating concentrations (>10 μg/ml) of the appropriate mouse antibodies against the endothelial cell surface marker von Willebrand factor (mouse antibody as appropriate). Cells were then fixed in PBS containing 2% (v/v) formaldehyde before microscopy with a Zeiss Axioskop fluorescence microscope or flow cytometry on a Becton Dickinson FACSscan.
For immunoperoxidase staining, cells were pelleted onto glass microscope slides by cytospin centrifugation (500 rpm, 5 min), dried overnight, fixed in 100% acetone, incubated with primary antibody as described above, washed, incubated (1 h room temperature) with peroxidase-conjugated goat anti-mouse IgG (DAKO EnVision kit, DAKO, UK), washed again, and developed with diaminobenzidine.

SDS-PAGE and Western Blotting—For Western blotting, samples of heparinase III-treated monocytes or MDM were electrophoresed on 12.5% polyacrylamide SDS-PAGE gels and transferred to nitrocellulose (Amersham Hybond-C Super) before blocking overnight (5% w/v dried milk powder, 0.2% Tween 20 in PBS, pH 7.5) and staining with mAbs (5 μg/ml, 1 h, 25 °C) to human syndecan-2 (10H4) or with the heparan sulfate-specific mAb 3G10. Bands were detected by autoradiography after incubation of the blots with horseradish peroxidase-conjugated rabbit anti-mouse IgG (1/1,500 dilution, 1 h, 25 °C) and luminol (Amersham ECL chemiluminescence detection kit).

Detection of mRNA by Reverse Transcriptase PCR—Reverse transcriptase PCR was carried out as described previously. Briefly, total cellular RNA was purified by extraction of cells with guanidinium thiocyanate/acid phenol extraction/ethanol precipitation (36) and used as the template for oligo-dT primed first strand cDNA synthetic reactions (3 h, 42 °C) containing 5 μg of total RNA, 0.5 μM dNTPs, 0.1 μM Tris-HCl, pH 8.3 and 2.5 million units of avian myeloblastosis virus reverse transcriptase. Samples (2 μl) of the final products were then committed to 50-μl PCR reactions (94 °C, 1 min; 55 °C, 1 min; 72 °C, 1 min; 20–30 cycles) containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.5 mM MgCl₂, 1 mM dNTPs, 1 unit of Taq DNA polymerase and the primers Syn 2 F (5'-ATCCGGAAGCTTATGCCGCGCGTGGATC-3') and Syn 2 R Bgl (5'-ATCCGGATCCCTCTTGTCCGTTTAAACAGACT-3') in a Perkin Elmer Cetus DNA thermal cycler. The PCR products were

**FIG. 1.** Cell surface heparan sulfate expression on primary human monocyte-derived macrophages and lymphocytes. Levels of cell surface HS were measured on intact fresh human peripheral blood monocytes, MDMs, and resting or mitogen-activated lymphocytes by fluorescent antibody staining and FACS analysis (see “Experimental Procedures”). Panel A shows the time courses of cell surface HS expression during culture-induced differentiation of monocytes to MDM and mitogen (phytohemagglutinin) stimulation of T lymphocytes detected by heparinase III treatment and fluorescent staining with the 3G10 HS-reactive 3G10 mAb (see “Experimental Procedures”). Full-scale values for the upper and lower histogram y axis are 50 and 200, respectively. Panel B shows the final levels of cell surface HS induced on MDM by incubation with the pro-inflammatory mediators IL-1α (1 ng/ml), TNFα (100 ng/ml), and LPS (0.5 μg/ml). Control nonheparinase treated cells were negative (not shown). Data are expressed as the mean ± S.E. for triplicate determinations of median fluorescence in each case. Panel C shows phase contrast and fluorescent micrographs of fresh monocytes (1 and 2) and IL-1α-stimulated MDM (3 and 4), stained with the mAb 10E4, which detects intact HS chains. The specificity of 10E4 for HS was confirmed in parallel cell preparations after treatment with heparinase I/III (not shown).
transferred to HYBOND N* (Amer sham, UK) and hybridized (52 °C) with the 32P-end-labeled internal probe (5′-CAGAAGACACTGCTGACACA-3′) in 6× SSC, supplemented with 20 mM HEpes, pH 7.4, 0.2% SDS, and 5× Denhardt’s solution, before washing (1× SSC, 0.5% SDS) at 57 °C (5 min). Parallel reactions were carried out using the probe hybridized with the cDNA of the macrophage 48-kDa band with syndecan-2 (fibroglycan), human Namalwa B-lymphoma cells revealed co-migration of the major fibroblast syndecan variant (Fig. 2B) and syndecan-4 (35 kDa), and glypican-1 (65 kDa) characterized (39) core proteins of syndecan-1 (85 kDa), syndecan-2—

RESULTS

Immunochemical Detection of Heparan Sulfate at the Surface of Differentiated Human Macrophages—To measure cell surface HSPG expression in primary human monocyte/macrophages, we exploited the specificity of the monoclonal antibody 3G10 to detect unsaturated Δ4,5 glucuronolipids in core HS chains generated by prior treatment of intact cells with the enzyme heparan sulfate lyase. We first questioned whether mobilization of HS to the cell surface is triggered by differentiation of blood monocytes to MDMs or by exposure to pro-

inflammatory mediators that activate macrophages and induce synthesis of heparin-binding growth factors. The results (Fig. 1, A and B) indicate cell surface HS is indeed up-regulated severalfold on the majority of MDM (characterized by elevated expression of the high affinity Fcγ receptor FcγRI, CD14 and MHC class II histocompatibility antigens, data not shown) within 24 h of differentiation from monocytes and to a further extent on MDM treated with either bacterial lipopolysaccharide or interleukin-1α. This newly mobilized HS appears to be homogeneously distributed over the surface of individual MDM as assessed by immunofluorescent staining with the mAb 10E4, which binds intact HS (Fig. 1C). Interestingly, no comparable levels of HS were detected on the surface of mitogen-activated human T cells, indicating that the capacity for extensive cell surface HS mobilization is not a general property of hemopoietic cells (Fig. 1A).

The specificity of the HS mAbs in each of these experiments was confirmed by separate controls, which showed that surface staining with the 3G10 mAb depended on pretreatment of macrophages with heparinase III and that surface staining with the 10E4 mAb was reduced by digestion with this enzyme (not shown).

The Major HSPG Core Protein on Activated Macrophages Is Syndecan-2—To identify the proteoglycan core proteins decorated with HS, we treated intact monocyte-derived macrophages with heparinase III and analyzed whole cell lysates by SDS-PAGE and Western blotting with the Δ4,5 glucuronosyl sugar-reactive mAb 3G10. The results (Fig. 2) revealed the presence of a single major 48-kDa band on MDM whose abundance was further increased by the inflammatory mediators IL-1α, TNFα, and LPS and which was completely absent from unstimulated monocytes. Size comparisons with the previously characterized (39) core proteins of syndecan-1 (85 kDa), syndecan-2 (48 kDa), syndecan-4 (35 kDa), and glypican-1 (65 kDa) in whole cell lysates prepared from cloned cDNA-transfected human Namalwa B-lymphoma cells revealed co-migration of the macrophage 48-kDa band with syndecan-2 (fibroglycan), the major fibroblast syndecan variant (Fig. 2B). The additional faint 65-kDa band visible in the MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells). Trace amounts of a further 130-kDa band in MDM samples was identified as glypican-1 by virtue of its co-migration with the authentic HSPG (note this particular HSPG is endogenously expressed by Namalwa cells).
syndecan-1, syndecan-2, syndecan-4, or glypican-1 and blotted with the 3G10 mAb. Stimulated monocytes were electrophoresed in parallel with Namalwa B-lymphoma cells transfected with cDNA encoding full-length human syndecan-1, syndecan-2, syndecan-4, or glypican-1 and blotted with the 3G10 mAb. Panel C shows a Western blot of IL-1α-stimulated MDM and syndecan-2 transfected B-lymphoma cells probed with the syndecan-2 specific mAb 10H4. Similar blots of freshly isolated monocytes were negative (not shown). The positions of the molecular mass calibration markers myosin (205 kDa), bovine serum albumin (68 kDa), ovalbumin (45 kDa), and carbonic anhydrase (29 kDa) are indicated with arrows. All SDS-PAGE gels contained 7.5% acrylamide.

The identification of the 48-kDa band as syndecan-2 was further corroborated by Western blotting of IL-1α-stimulated MDM samples with the polypeptide core-specific mAbs B-B4 (syndecan-1), 10H4 (syndecan-2), 8G5 (syndecan-4), and S1 (glypican-1), which revealed staining only with 10H4 (Fig. 2C and data not shown). In addition, syndecan-2 could be clearly visualized in IL-1α-stimulated MDM (but not in unstimulated monocytes) by immunoperoxidase staining of detergent-permeabilized cells with the mAb 6G12, which recognizes the syndecan-2 cytoplasmic tail (Fig. 3). The syndecan-2 ectodomain-reactive mAb 10H4 used in Western blotting did not yield consistent results in immunocytochemical staining because of its poor reactivity with native proteoglycan (not shown).

Next we compared syndecan-2 mRNA levels in primary human monocytes and monocyte-derived macrophages using semi-quantitative reverse transcriptase PCR amplification from total cellular RNA. The results (Fig. 4) reveal abundant levels of the 458-base pair syndecan-2 PCR product in both culture-differentiated and cytokine-stimulated (LPS, TNFα) MDM but little or no product in resting monocytes. In addition, LPS-stimulated MDM yielded higher levels of syndecan-2 product than either TNFα-stimulated or unstimulated MDM, consistent with the measurements of both core protein and HS by Western blotting and FACS analysis, respectively (Fig. 1A and Fig. 2A). Importantly the apparent differences in syndecan-2 transcript levels were detected within the exponential phase of the PCR reaction (20–30 cycles), where the level of product is proportional to the number of copies of template DNA. Finally each of the monocyte/macrophage cDNAs was found to yield similar amounts of the housekeeping gene transcript glyceraldehyde-3-phosphate dehydrogenase. Our results therefore point to a specific induction of syndecan-2 gene expression during monocyte/macrophage differentiation and activation.

**Macrophage HSPGs Sequestr Heparin-binding Angiogenic Growth Factors**—In view of the fact that monocyte/macrophages themselves actively synthesize heparin-binding growth factors, we assessed GAG-mediated binding of the macrophage-derived angiogenic growth factor FGF-2 to intact monocyes and macrophages using an iodine-125 radioligand binding assay. The results (Fig. 5) show that macrophages indeed acquire GAG-associated binding sites for FGF-2 following either differentiation or cytokine-stimulation and confirm that the extent of FGF-2 binding is broadly proportional to the level of cell-surface HS detected with the 3G10 mAb (compare with Fig. 1A). The identification of these sites as HSPGs is further supported by three lines of evidence. First, binding is sensitive to protease digestion and is inhibited by free heparin (Fig. 5, A and B). Second, lysates of LPS-stimulated MDM display syndecan-2 mediated binding to FGF-2 in a microtiter plate assay (Fig. 6). Third, binding of 125I-FGF to the surface of IL-1α-stimulated MDM is specific, saturable, of high affinity (Kd, 125 nm, 106 sites/cell), and is highly sensitive to competition by free heparin or heparan sulfate in comparison with chondroitin sulfate (IC50, 0.1, 0.5, and 50 μg/ml, respectively, Fig. 7).

To assess whether macrophage syndecan-2 displays similar affinity for other macrophage-derived ligands, we performed both radioligand and fluorescent binding analyses with a comprehensive panel including the angiogenic growth factors FGF-1 and VEGF, the vascular smooth muscle growth factor hbEGF, the C-C (β) chemokines RANTES, MIP-1β and MCP-1, and the C-K-C (α) chemokine IL-8. The results (Fig. 8) revealed high affinity heparin-inhibitable binding of 125I-FGF-1 (Fig. 8C) or to any of the chemokines, 125I-MIP-1α, 125I-MIP-1β, 125I-MCP-1, 125I-IL-8, and 125I-RANTES tested over the range 0 to 600 nM. The integrity of the radiolabeled ligands was in each case confirmed by parallel binding assays with heparin-Sepharose (not shown). Furthermore, no binding of the fluorescent derivatives, fluorescein isothiocyanate IL-8 (33) or NBD-RANTES (34), was detected in separate binding assays of IL-1α-stimulated MDM as assessed by flow cytometry. In summary, these results demonstrate that HSPGs, predominantly syndecan-2 on *in vitro* activated human
macrophages, display selective binding to the macrophage-derived growth factors FGF-2, VEGF, and hbEGF but do not appear to sequester chemokines.

**Macrophage HSPGs Can Transactivate FGFR1 on Neighbor- ing Cells**—To further assess the physiological function of syndecan-2 on MDM, we questioned whether these cells might use cell surface HSPG to promote signal transduction (in trans) through FGF receptors on neighboring cells. To explore this possibility, we assayed the capacity of glutaraldehyde-fixed (IL-1α-activated) monocytes and MDM to induce the proliferation of FGFR1-transfected BaF32 cells (which lack endogenous HSPGs) in the presence of nanomolar FGF-2 concentrations. The results (Fig. 9) show that cytokine-stimulated MDM expressing syndecan-2 are indeed efficient promoters of FGF-dependent proliferation, whereas fresh unstimulated monocytes, which express little or no syndecan-2, are not. The involvement of HS chains in this process could be clearly demonstrated by the effects of heparinase III treatment, which reduced BaF32 cell proliferation by 80%. Furthermore the capacity to stimulate proliferation is not common to all “activated” cell types because activated T-cells (which have little or no cell surface HS, see Fig. 1A) did not display such activity in our assays. Indeed comparison with syndecan-2 transfected Namalwa cells suggests activated macrophages are particularly efficient in augmenting BaF cell proliferation. Whereas the former stimulated proliferation at a 10:1 ratio of presenting cells: BaF cells, cytokine-stimulated MDM were equally active at a 1:1 ratio (data not shown). Such differences cannot be explained by higher mean cell surface syndecan-2 expression levels on macrophages, because these are lower than in the transfected cells as assessed by Western blotting (see Fig. 2B). Differences in the degree of homogeneity of cell surface syndecan-2 expression rather than the average expression level between the two cell populations, however, cannot be ruled out. In summary, our results show for the first time that HSPGs on primary human macrophages not only bind FGF-2 but can also trans-activate FGF receptors on neighboring cells.

**Syndecan-2 Expression and Macrophage Endogenous Growth Factor Presentation**—Implicit in our results is the possibility that activated macrophages may utilize syndecan-2 to present their own endogenously synthesized growth factors at the cell surface. Measurement of endogenous macrophage FGF-2 production using an ELISA indicated significant levels (394 ± 66, 65 ± 30, and 66 ± 23 pg/10^7 cells, respectively, n = 3) in cell lysates after stimulation with LPS, IL-1α, or TNFα compared with trace levels (<10 pg/10^7 cells) in fresh unstimulated monocytes. Hence the same agents we found to induce HSPG expression and FGF-2 binding in monocyte-derived macrophages in vitro also induce FGF-2 production by these cells.
cells. In all cases however the cytokine-induced growth factor remained associated with the cells and only trace levels (equivalent to 1 pg release from 10⁷ cells) could be detected in culture supernatants. Furthermore little if any cell surface bound FGF could be detected by fluorescent antibody staining, and no soluble FGF was detected after cleavage of HSPGs from the surface of cytokine-stimulated MDM with either heparinase I or heparinase III (data not shown). Thus the FGF-2 produced is likely stored within intracellular vesicles and factors other than the inflammatory cytokines tested here must be required to promote its release in vivo.

In Vivo Activated Macrophages Express Syndecan-2 and Seques ter FGF-2—To establish whether syndecan-2 expression and growth factor presentation are properties of in vivo activated macrophage populations as well as the in vitro stimulated MDM described above, we investigated the HSPG profile of macrophages isolated from the peripheral blood and synovial fluid of individuals with inflammatory joint disease. The results (Fig. 10A) revealed expression of the same predominant 48-kDa core protein identified as syndecan-2 in synovial macrophage samples in each of the inflammatory conditions (rheumatoid arthritis, systemic lupus erythematosus, and psoriatic arthritis), as confirmed by Western blotting with both the 3G10 D₄,₅ glucuronate-specific mAb and the syndecan-2 specific mAb 10H4. In contrast, no syndecan-2 was detected in parallel samples of peripheral blood monocytes from any of the individuals tested (Fig. 10A). Interestingly, synovial fluid macrophages also displayed GAG-mediated heparin-inhibitable binding of FGF-2, and this was at least twice that of peripheral blood monocytes (Fig. 10B). These results confirm that syndecan-2 is the major inducible HSPG expressed on activated macrophages in vivo and suggest that in the case of synovitis, expression is induced locally, most likely by inflammatory mediators released within the tissue itself.

DISCUSSION

Macrophages migrate to the tissues during wound healing, inflammation, and tumor angiogenesis where they play a major role in promoting cell proliferation through the production of heparin-binding growth factors (2, 40). However the specific details of how these cells regulate the bioavailability of many individual growth factors at the appropriate times and in the appropriate tissue locations are not clear. The action of heparin-binding growth factors produced by other less motile cell types is known to be regulated both positively and negatively by interactions with HSPGs within the surrounding tissues and extracellular matrix. For example FGF-2 is relatively abundant in normal un inflamed tissues where its association with matrix HSPGs appears to block its capacity to promote the proliferation of neighboring cells (42–44), despite their close proximity (45). Local release of this latent FGF in mitogenically active form is thought to be orchestrated by matrix-degrading proteinases and heparinases secreted by “activated” platelets.

![Diagram](image_url)
eq provides the specific activity of heparin. 

Fig. 7. Characterization of cell surface HSPG-mediated FGF-2 binding. The specificity and binding affinity of FGF-2 for cell surface HSPG on intact IL-1α-stimulated (1 ng/ml) MDM was measured using an iodine-125 radioligand binding assay as described under “Experimental Procedures.” Panel A shows a comparison of total 125I-FGF-2 binding (no competitor added), nonspecific binding (100 × fold molar excess unlabeled FGF-2 added) and non-HSPG mediated binding (10 μg/ml free heparin added). Panel B shows a representative specific 125I-FGF-2 binding curve and corresponding Scatchard plot (B/F, bound specific FGF-2/free FGF-2 versus bound specific FGF-2) from which the equilibrium binding constant K_d (125 nM) was estimated. Panel C compares the sensitivity of 125I-FGF-2 binding with inhibition by the free glycosaminoglycans heparin, heparan sulfate, and chondroitin 4-sulfate, calculated as the percent maximal binding (cpm bound in the presence of free glycosaminoglycan/cpm bound in the absence of free glycosaminoglycan × 100). Data in each panel is the mean ± S.E. for four replicate determinations.

and neutrophils following tissue injury (46–48). Such models, however, tend to exclude any role for macrophage-derived HSPGs in tissue growth factor regulation, despite the fact that these cells are an important and abundant source of heparin-binding growth factors. These considerations prompted us to characterize cell surface HSPG expression by human macrophages in vitro and in vivo and to investigate their capacity to regulate macrophage-derived growth factor action.

In the first part of this manuscript we showed, using the HS-specific mAbs 3G10 and 10E4, that cell surface HS expression is induced during in vitro differentiation of human monocyte-derived macrophages, conditions that give rise to cells resembling mature tissue macrophages. In addition we showed that the pro-inflammatory agents, bacterial lipopolysaccharide and IL-1α, induce further increases in HS, underlining the link between cell surface HS expression and inflammation. These results extend earlier reports of GAG biosynthesis in macrophages and macrophage-derived cell lines (49–51) based on biosynthetic labeling studies, and reveal HS as an abundant and homogeneously distributed component of the human activated macrophage cell surface. Intriguingly, the major core protein to which the HS is attached was found to be syndecan-2 (fibroglycan), an HSPG previously associated with fibroblasts and cells of endothelial origin. Syndecan-2 was also found to be the dominant cell surface HSPG on macrophages activated in vivo, underscoring the physiological relevance of the finding. In particular, samples of macrophages recovered from the synovial fluid of individuals with inflammatory arthritis (rheumatoid arthritis, systemic lupus erythematosus, and psoriatic arthritis) expressed predominantly syndecan-2. In contrast, peripheral blood monocytes isolated from the same individuals expressed little or no syndecan-2, confirming the inducibility of this HSPG in inflamed tissue in vivo. Interestingly, the pro-inflammatory cytokine IL-1α was found to induce macrophage syndecan-2 expression in vitro (see above) is a key component of the cytokine network that maintains chronic inflammation in the rheumatoid joint (52). Furthermore FGF-2, a high affinity ligand for macrophage syndecan-2, is found abundantly within inflamed synovial tissue where it stimulates the growth of fibroblast-like synoviocytes and may also promote angiogenesis (53, 54). The expression of syndecan-2 by macrophages in synovial fluid may therefore play a role in the pathology of inflammatory synovitis.

The expression of syndecan-2 on hematopoietic cells is a novel and unexpected finding. Originally named fibroglycan (55) because of its expression on human lung fibroblasts (35), syndecan-2 is in fact more widely expressed and mRNA has been detected both in endothelial and neural cell lines and to varying extents in brain, heart, muscle, kidney, and lung (56). This pattern overlaps but is distinct from those of syndecans 1, 3, and 4 and many cell types simultaneously express combinations of different syndecan. Studies of syndecan-2 expression in the mouse have revealed its localization at the sites of cell-cell and cell-matrix adhesion, notably those at the interfaces of developing epithelial and mesenchymal tissues (57). Expression has also been documented in MS-5 murine bone marrow stromal cells, prompting the suggestion that syndecan-2 may promote the differentiation of hematopoietic stem cells in vivo (58). Surprisingly, however, there have been no reports of syndecan-2 either in primary murine macrophages or macrophage cell lines, where syndecan-1 has been identified as the major cell surface HSPG (59, 60). Additionally, expression of syndecan-1 in murine macrophages is regulated translationally by changes in the level of cAMP (59), whereas expression of syndecan-2 as shown in our studies appears to be regulated at the transcriptional level. These apparent differences between
macrophages from different species are intriguing but are not presently understood. Our observations that activated MDM display heparin-mediated binding of FGF-2 ($K_d$, 125 nM), VEGF ($K_d$, 111 nM), and hbEGF ($K_d$, 90 nM) are consistent with a general role for syndecan-2 in the sequestration of heparin-binding growth factors. Although we could not demonstrate unequivocally that syndecan-2 is the primary growth factor-binding HSPG, it is difficult to conclude otherwise because it is the single most abundant core protein on the MDM cell surface. Furthermore, we confirmed, using an ELISA, that syndecan-2 present in MDM lysates displayed FGF-binding. Interestingly however, MDM cell surface HSPGs displayed no binding to FGF-1 or to any of the heparin-binding chemokines tested, which included MCP-1, MIP 1α, MIP 1β, IL-8, and RANTES. This lack of chemokine binding concurs with recent reports describing the chemokine-mediated blockade of primary human macrophage infection by monocytotropic HIV (29, 61). In these studies, no HS-mediated binding of RANTES to the cell surface was detected (29), despite the fact that RANTES HS complexes were efficient inhibitors of viral entry (61). Hence it is likely that the cell surface HSPGs of human macrophages are not promiscuous but instead display selectivity for a subset of individual growth factors.

The finding that FGF-HSPG complexes on the surface of cytokine-activated macrophages could efficiently induce proliferation of heterologous BaF32 cells lends further support to the current hypothesis that HSPGs function as co-receptors to regulate heparin-binding growth factor action. In the case of syndecan-2 reported here, this co-receptor function could operate in vivo for the presentation of growth factor either to signal macrophages from different species are intriguing but are not presently understood.

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Panel B

syndecan-2. The molecular mass calibration markers (kDa) are the same as those shown in Fig. 2. The parallel blot of syndecan-2 transfected Namalwa cells (Transfectant) indicates co-migration of the monocyte HSPG core protein with authentic syndecan-2. The molecular mass calibration markers (kDa) are the same as those shown in Fig. 2. Panel B shows the extent of heparin-inhibitable \(^{125}\)I-labeled FGF-2 binding (total binding – heparin inhibited binding) to syndecan-2 macrophages (SFM) from a patient with rheumatoid arthritis and normal unstimulated peripheral blood monocytes (PBM, equal cell numbers were used): Data are the mean ± S.E. of four replicate determinations.

transducing receptors on macrophages themselves (cis-activation) or to receptors on neighboring target cells such as fibroblasts and endothelial cells (trans-activation). Although we cannot rule out the former possibility this seems less likely as we failed to detect FGFR1 in MDMs by reverse transcriptase PCR. In addition we failed to observe proliferation of MDM even after extended culture in the presence of heparin-binding growth factors such as FGF-2, VEGF, and hbEGF.\(^3\) We therefore favor the alternative possibility that MDMs use inducible HSPGs such as syndecan-2 to transactivate FGF receptor on neighboring cells. Although such transactivatory capacity has been demonstrated recently for HSPGs expressed on transformed fibroblast (37) and lymphoma cell lines (38), this is the first report to demonstrate this phenomenon in primary human macrophages, which bear only a single major HSPG species, syndecan-2. We speculate that such an arrangement may facilitate the precise targeting of growth factors by macrophages migrating in response to chemokines while at the same time avoiding their release within inappropriate tissues. Further investigation of the potential importance of macrophage HSPGs in regulating such physiological processes is now clearly warranted.

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