

Circulating Fibrocytes Define a New Leukocyte Subpopulation That Mediates Tissue Repair

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ABSTRACT

Background: The host response to tissue injury requires a complex interplay of diverse cellular, humoral, and connective tissue elements. Fibroblasts participate in this process by proliferating within injured sites and contributing to scar formation and the long-term remodeling of damaged tissue. Fibroblasts present in areas of tissue injury generally have been regarded to arise by recruitment from surrounding connective tissue; however this may not be the only source of these cells.

Materials and Methods: Long-term culture of adherent, human, and murine leukocyte subpopulations was combined with a variety of immunofluorescence and functional analyses to identify a blood-borne cell type with fibroblast-like properties.

Results: We describe for the first time a population of circulating cells with fibroblast properties that specifically enter sites of tissue injury. This novel cell type, termed a "fibrocyte," was characterized by its distinctive phenotype (collagen⁺/vimentin⁺/CD34⁺), by its rapid entry from blood into subcutaneously implanted wound chambers, and by its presence in connective tissue scars.

Conclusions: Blood-borne fibrocytes contribute to scar formation and may play an important role both in normal wound repair and in pathological fibrotic responses.

INTRODUCTION

Wounds result from an abrupt, physical disruption of the normal architecture of tissue and may be produced by trauma, burns, inflammatory processes, or metabolic insufficiency. Once wounding occurs, the host initiates a coordinated repair response that prevents infection and tissue invasion and ultimately reestablishes normal tissue integrity. This response occurs over several phases and involves a complex interplay between cellular, humoral, and connective tissue elements (1).

Connective tissue fibroblasts participate in the reparative phase of wound healing by producing matrix proteins, such as collagen, and forming a connective tissue scar. Over the long term, fibroblasts also act to remodel injured tis-

sue, maintain tissue homeostasis, and ensure the long-term survival of connective tissue. Fibroblasts that enter and proliferate within wound sites generally have been regarded to arise from the recruitment and migration of cells from adjacent tissue. Under normal circumstances, these cells are quiescent and remain sparsely distributed throughout the extracellular matrix (2,3).

A frequently employed model for the study of wound healing responses relies on the surgical implantation of wound chambers that consist of short lengths of sponge-filled, silastic tubing (4). Implantation of these chambers into the subcutaneous tissues of mice results within 24 hr in a rapid influx of peripheral blood cells comprising neutrophil, monocyte, and lymphocyte subpopulations (4,5). While studying acute cellular responses in this model of wound repair, we observed by light microscopy an unexpectedly large number of adherent, spindle-shaped cells that resembled fibroblasts. The appearance of fi-

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broblasts in wound chambers has been attributed to their recruitment from surrounding subcutaneous tissue (1-3). Therefore, the presence of large numbers of fibroblast-like cells coincidentally with the entry of circulating inflammatory cells suggested to us that this cell population was arising from peripheral blood and not exclusively by slow migration from adjacent connective tissue. We describe herein the isolation and cell surface phenotype of this apparently novel, blood-borne, fibroblast-like cell. This cell type, which we have termed a fibrocyte, was found to enter sites of tissue injury and to contribute to connective tissue scar formation.

MATERIALS AND METHODS

Isolation and Characterization of Blood-Borne Fibrocytes

Total peripheral blood leukocytes were first isolated from human blood (60 ml) by centrifugation over Ficoll-Paque following the manufacturer's protocol (Pharmacia). After overnight culture on 150 mm plates in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal calf serum (FCS), nonadherent cells were removed by a single, gentle aspiration. At weekly intervals thereafter, the cultures were replenished with fresh medium. Morphological studies were performed after 6 weeks of culture on cells judged to be phenotypically homogeneous by "spot" immunofluorescence and by flow cytometry with selected antigen markers (described below). Mouse cells were cultured in a similar manner from whole blood pooled from 5-10 individual mice.

Immunofluorescence and FACS Analysis

For cell surface analysis, cells were subcultured on cover slips prior to fixation with 3.5% paraformaldehyde for "spot" immunofluorescence analysis. Fixed cells were washed in a graded ethanol series at -20°C followed by three washes in phosphate buffered saline (PBS). Cover slips then were incubated for 30 min at room temperature with 10 $\mu\text{g}/\text{ml}$ of monoclonal antibodies. For enumeration studies, anti-collagen I, anti-vimentin, and anti-CD34 monoclonal antibodies were used. FITC-linked anti-mouse IgG was used as a secondary antibody. For fluorescence-activated cell sorting (FACS), cells were harvested after 6 weeks of culture by gentle

scraping and trituration and either incubated directly with specific antibodies or first permeabilized and fixed with 3.5% paraformaldehyde (6). At least 10,000 cells were analyzed on an FACS 440 (Becton Dickinson) instrument. The sources of the antibodies that were tested against mouse antigens included: anti-fibronectin and anti-collagen I (Chemicon), anti-MacI and anti-PanMac (Serotec), anti-CD4, anti-CD8, anti-TCR($\alpha\beta$), anti-TCR($\gamma\delta$), anti-CD11a, anti-CD25, anti-CD45, anti-CD54, anti-LPAM, and anti-LECAM-1 (each from Pharmingen). Antibodies to human antigens included: anti-von Willebrand's antigen (Accurate), anti-vimentin (Labsystems), anti-collagen I and anti-collagen III (Chemicon), anti-laminin, anti- α -actin, anti-fibronectin, and anti-desmin (Sigma, St. Louis, MO, U.S.A.), anti-Class I, anti-Class II, anti-CD3, anti-CD4, anti-CD8, anti-TCR($\alpha\beta$), anti-TCR($\gamma\delta$), anti-CD11a, anti-CD11b, anti-CD11c, anti-CD13, anti-CD14, anti-CD16, anti-CD19, anti-CD25, anti-CD33, anti-CD34, anti-CD38, anti-CD44, anti-CD45, anti-CD54, anti-CD56, and anti-CD71 (each from Boehringer Mannheim).

Electron Microscopy Analysis

Scanning and transmission electron microscopy studies were kindly performed by Dr. David Phillips (The Population Council, New York). For scanning electron microscopy, cells were fixed in PBS/2.5% glutaraldehyde, dehydrated in a graded ethanol series, critical point dried from liquid CO_2 , and viewed in an ETEC scanning electron microscope.

Wound Chamber and Scar Tissue Studies

Female BALB/c mice (4-6 weeks old) were anesthetized with Avertin. Sterile wound chambers composed of a 2.5 cm piece of perforated silastic tubing (Dow Corning) containing polyvinylalcohol sponge (Unipoint, Inc.) were inserted through a 0.5 cm dorsal midline incision along each flank (4). At intervals, 10-20 μl of wound fluid was aspirated percutaneously with a 1 cc syringe, and the cells were recovered by centrifugation. After washing in PBS, cells were analyzed by flow cytometry for total cell count and reactivity with directly labeled anti-CD34, anti-collagen I, and anti-vimentin monoclonal antibodies. In selected experiments, wound chambers were removed at intervals (7, 14, 28, and 56 days), fixed in 3.5% paraformaldehyde, sectioned, and stained with hematoxylin/eosin,

Masson-Trichrome (for collagen), or processed for immunohistochemistry.

Scar tissue formation was induced in selected mice by a 0.5 cm dorsal midline scalpel incision that was then closed with a surgical staple. The area encompassing cutaneous scar was excised 7–28 days later and processed for immunohistochemical analyses. After blocking endogenous peroxidases with H₂O₂ (3%), sections were stained with a monoclonal rat antibody to mouse CD34 (1:100 dilution) or a rat IgG1 isotypic control and developed with an immunoperoxidase-linked secondary antibody (DAKO, Copenhagen, Denmark) using diaminobenzidine as substrate.

Human cutaneous scar tissue specimens were kindly provided by Dr. Joseph Ma (Department of Pathology, Albany Medical College, NY, U.S.A.). These specimens were obtained from individuals without any apparent systemic inflammatory or connective tissue diseases. Sectioned tissue was stained with hematoxylin and eosin and analyzed for CD34 expression as described above.

Bone Marrow Chimera Studies

Female (BALB/c) mice were lethally irradiated (800 rads) and reconstituted intravenously with 10⁶ bone marrow cells harvested from the femurs of male (BALB/c) mice (7). Wound chambers were implanted into mice 6 weeks after bone marrow transfer. At intervals, 10–20 μ l of fluid was aspirated from the chambers for cell analysis. Wound chamber cells were recovered by centrifugation, washed, fixed, and double-stained with rhodamine-conjugated monoclonal anti-CD34 and FITC-labeled monoclonal anti-collagen I antibodies. Cells were subjected to fluorescence-activated cell sorting and two populations (CD34⁻/Collagen I⁻ and CD34⁺/Collagen I⁺) were recovered for DNA analysis. Thresholds were determined with FITC and rhodamine isotype controls.

DNA was prepared from approximately 10,000 sorted cells by lysis in Tris-buffer containing 0.1% SDS and 0.1 mg/ml, proteinase K and used as a template in 50 μ l PCR reactions containing 10 mM Tris buffer, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 1 μ M each of the oligonucleotide primers, and 0.026 U/ μ l of *Taq* polymerase. After an initial 2 min denaturation step, each amplification cycle consisted of 1 min of denaturation at 92°C, 1 min annealing at 53°C, and 1 min polymerization at 72°C. Sam-

ples were amplified for 45 cycles. The primers for the Y chromosome-specific *SRY* gene (5'-AAGT TGGCCAGCAGAAT-3' and 3'-TATAGTCGGAG TAGCCTC-5') (8) amplify a 143 bp genomic DNA fragment, and the β -actin primers (5'-GTGGG CCGCTCTAGGCACC-3' and 3'-CCCCCTGAACC CCTAAGGCCA-5') (9) amplify a 241 bp genomic DNA segment. Ten microliters of reaction products were analyzed by gel electrophoreses in 2% agarose gels containing 100 ng/ml of ethidium bromide.

RESULTS

In preliminary studies performed in mice, we observed that a large proportion of cells aspirated within 2 days of wound chamber implantation showed an adherent, spindle-shaped phenotype that was reminiscent of cultured primary fibroblasts. Further analyses demonstrated these cells to stain strongly for the fibroblast markers collagen I and vimentin and to be negative for non-specific esterases, indicating that these cells were phenotypically distinct from the numerically larger copopulation of adherent, peripheral blood monocytes. We considered that any fibroblast-like cells present in circulation would be likely to comprise only a small fraction of peripheral blood cells and thus turned to an examination of human blood (available in greater quantities) for the identification of circulating fibroblast-like cell types.

Total leukocyte fractions were isolated from peripheral blood by Ficoll centrifugation and cultured in serum-supplemented DMEM under conditions selective for the ability of connective tissue cells to adhere to plastic surfaces and proliferate. As expected, there was a slow decline in the nonproliferating monocyte cell population over a 2–4 week period. This was accompanied by the emergence of expanding clusters of adherent cells that gradually increased in number. Light microscopy showed this proliferating cell type (henceforth termed a fibrocyte) to display a largely fibroblast-like, spindle-shaped morphology (Fig. 1a). Staining for nonspecific esterases was not observed, indicating that fibrocytes were not derived from monocyte/macrophages (data not shown). Furthermore, there was positive staining for the fibroblast cytoskeletal component vimentin and for collagen I (10,11) (Fig. 1b). Scanning electron microscopy showed these cells to be morphologically distinct from circulating blood leukocytes and to display prominent

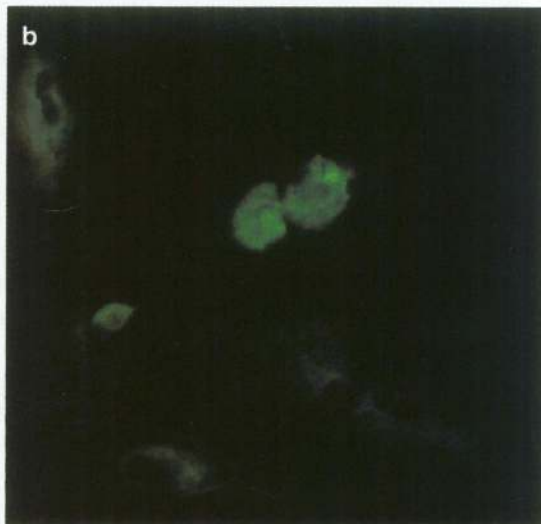
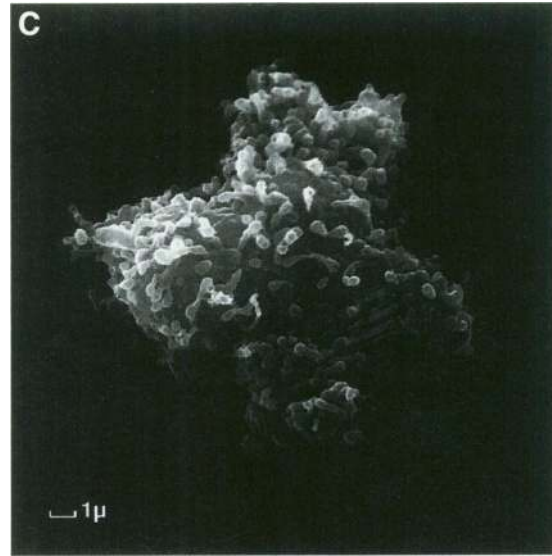
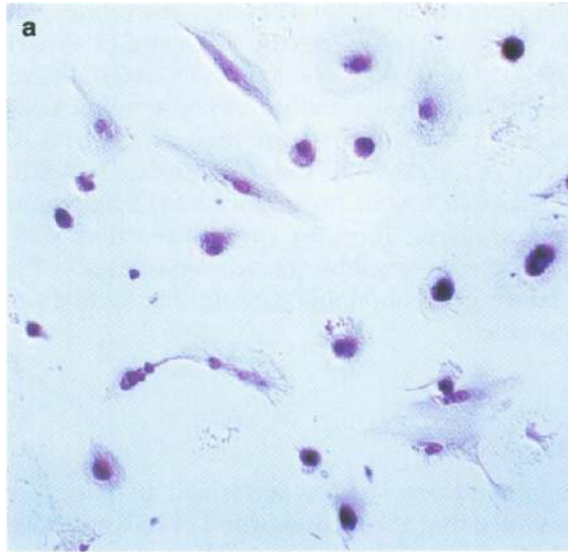


FIG. 1. Morphological appearance of fibrocytes isolated from cultures of human peripheral blood

(a) Light microscopic appearance after 6 weeks of culture (340X). (b) Immunofluorescence staining with monoclonal anti-vimentin antibody (640X). (c) Scanning electron micrograph.

cell surface projections intermediate in size between microvilli and pseudopodia (Fig. 1c).

We then performed a systematic phenotypic characterization of fibrocytes by immunofluorescence staining. Human fibrocytes were markedly positive for the cytoskeletal component vimentin, the fibroblast products collagen I, collagen III, and fibronectin, and the adhesion molecules CD11b and CD18 (Fig. 2 and Table 1). Fibrocytes were found not to express a variety of epithelial, endothelial, or smooth muscle cell-specific markers. Fibrocytes did stain positively for a few typical, leukocyte-associated cell surface antigens, such as the leukocyte common antigen

CD45 and the hematopoietic stem cell antigen CD34 (13–15). The CD34 antigen was identified originally to be a marker of bone marrow progenitor cells but has been reported recently to be expressed also by capillary endothelium and by embryonic fibroblasts (16,17). We hypothesized that the CD34⁺, vimentin⁺, collagen I⁺ fibrocytes isolated from peripheral blood represent a circulating population of uncommitted (or incompletely differentiated) fibroblast precursors that are conceivably of bone marrow origin.

Using immunofluorescence “spot” staining, we then examined the time-dependent appearance of CD34⁺, collagen I⁺ cells in cultures prepared from human peripheral blood (Fig. 3). In agreement with previous morphological analyses, the proportion of cells positive for collagen and CD34 increased slowly over time as fibrocytes divided and the monocyte population expired. Fibrocytes exhibited a doubling time of approximately 3–4 days (data not shown), and appreciable percentages of adherent fibrocytes were not apparent until ≥ 2 weeks of culture. We also sought to verify the existence of similar blood-borne cells in the mouse. Blood was obtained by cardiac puncture from BALB/c mice, subjected to the same fractionation and culture

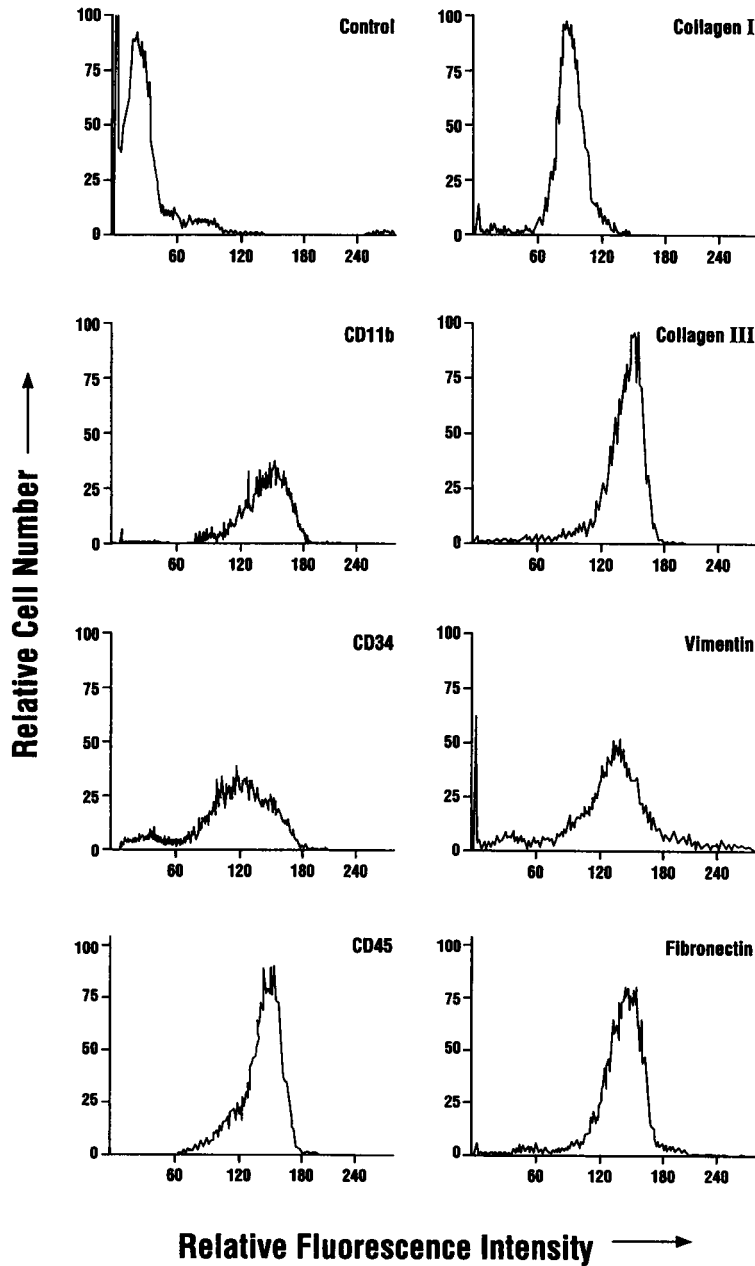


FIG. 2. Phenotypic characterization of circulating human fibrocytes

Cytofluorimetric profiles of human fibrocytes stained for the expression of selected antigens. Isotypic antibody controls showed a level of background staining less than or equivalent to the control shown. The fluorescence profiles are representative of cells isolated from two or three individuals.

procedures as utilized for human blood, and found to yield cells with the same morphological properties as human fibrocytes. Immunofluorescence analysis with available mouse cell surface markers confirmed the existence of a fibrocyte cell population in mouse peripheral blood (collagen I⁺, vimentin⁺, fibronectin⁺, CD11b⁺, CD18⁺, CD34⁺, CD45⁺, esterase⁻, CD14⁻, CD25⁻, and CD54⁻) (data not shown).

Wound chambers were then implanted in mice, and cells were collected from the exudative fluid for analysis by microscopy and cytofluorimetry. As expected, the implantation of wound

chambers in mice resulted in a rapid infiltration of acute inflammatory cells (i.e., neutrophils and monocytes), which reached a concentration of $1.3\text{--}1.5 \times 10^6$ cells/ml (Fig. 4). Double immunofluorescence analysis showed that over 10 days, 10–15% of the cells present in wound chamber fluid stain positively for both collagen I (or vimentin) and CD34, indicating that a population of blood-borne cells with the same phenotypic features as cultured fibrocytes infiltrate wound chambers as part of the acute, inflammatory reaction. Over time, these CD34⁺/collagen I⁺ cells were found to adhere to the polyvinylalcohol

TABLE 1. Cell surface phenotype of human peripheral blood fibrocytes (subpopulation reactivity compiled from (12))

Antigen		Cell Subpopulations
<u>Positive Expression</u>		
Vimentin	Intermediate filament	Fibroblasts
Fibronectin	Pericellular Matrix	Mesenchymal cells, lymphoid cells
Collagen I	Extracellular matrix	Fibroblasts
Collagen III	Extracellular matrix	Fibroblasts
CD11b	Adhesion molecule	Monocytes, granulocytes, NK cells
CD13	Aminopeptidase N	Myeloid and dendritic cells
CD18	Integrin $\beta 2$	Lymphoid and myeloid cells
CD34	Precursor antigen	Hematopoietic progenitors, embryonic fibroblasts
CD45	Leukocytes Common Antigen	Leukocytes
CD71	Transferrin receptor	Macrophages, activated cells
<u>Negative Expression</u>		
Esterase	Lysosomal enzyme	Monocytes and granulocytes
TCR ($\alpha\beta$, $\gamma\delta$)	Antigen receptor	T-Cells
Cytokeratin	Matrix protein	Epithelial cells
von Willebrand	Coagulation factor	Endothelial cells and platelets
Desmin	Intermediate filament	Muscle cells
α -Actin	Cytoskeletal protein	Smooth muscle cells
Laminin	Matrix protein	Blood vessels, muscle cells, nerve, etc.
CD3	TCR-associated antigen	T-Cells
CD4	Class II recognition	Helper/inducer T-cells
CD8	Class I recognition	Cytotoxic/suppressor T-cells
CD10	Endopeptidase (CALLA)	B-cell subpopulations
CD11a	LFA-1 antigen	Lymphoid and myeloid cells
CD14	LBP receptor	Monocytes/macrophages
CD16	Fc γ RIII (Ig receptor)	Monocytes, granulocytes, NK cells
CD19	B-cell antigen	B-cells
CD25	IL-2 receptor	Activated macrophages/T-/B-cells
CD33	Pan-myeloid antigen	Myeloid and monocytic cells
CD38	T10 antigen	Precursor cells, activated T-/B-cells
CD44	Lymphocytes homing receptor	Leukocytes and erythrocytes
CD54	ICAM-1	Monocytes and lymphocytes
CD56	NCAM	NK and T-cell subpopulations

sponge material present in wound chambers and to proliferate within regions of scar formation (described below).

The presence on fibrocytes of the hematopoietic stem cell antigen CD34 led us to investigate more closely the possible bone marrow origin of these cells. To test for the possibility that circulating fibrocytes might arise from hematopoietic

progenitors, we utilized sex-mismatched bone marrow chimeric mice together with DNA amplification of the male-specific *SRY* gene to detect cells arising from transplanted (male) bone marrow cells (8). Wound chambers were implanted in mice 6 weeks after the transfer of male bone marrow cells to lethally irradiated, female hosts. At intervals thereafter, wound fluid was aspi-

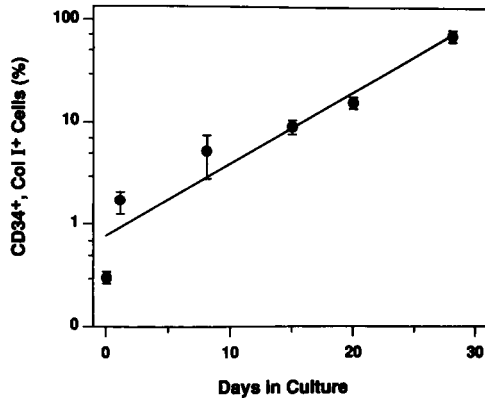


FIG. 3. Time-dependent appearance of fibrocytes in cultures of adherent, human peripheral blood leukocytes

Peripheral blood leukocyte fractions were isolated as described in Materials and Methods, cultured on glass cover slips, fixed, and double-stained with rhodamine-conjugated monoclonal anti-human CD34 antibody plus FITC-conjugated monoclonal anti-human collagen I antibodies. Similar results were obtained by double-staining with anti-CD34 plus monoclonal anti-vimentin antibodies. At least 10 positive cells were enumerated within each field (mean \pm SEM of ≥ 3 fields/time point).

rated, and the cells were separated by FACS into CD34⁻/collagen I⁻ and CD34⁺/collagen I⁺ populations. As expected, DNA isolated from the CD34⁻/collagen I⁻ population produced a significant amount of the *SRY* DNA amplification product, confirming the bone marrow (male) origin of the majority of the inflammatory cell population (Fig. 5). By contrast, DNA amplification analysis of CD34⁺/collagen I⁺ cells did not yield a significant amount of the *SRY*-specific DNA product, arguing that this cell population did not arise from transferred (male) bone marrow. These results could not be attributed to either poor amplification efficiencies or to a low yield of DNA template since each cell population produced approximately equal amounts of the control, β -actin amplification product. Taken together, these data suggest that blood-borne fibrocytes do not originate from radiosensitive, hematopoietic stem cells but arise instead from either a radioresistant, bone marrow progenitor cell or from other tissue sources. Bone marrow stromal cells for example, are relatively radioresistant and may express the CD34 antigen (17,18). Consistent with this possibility, we have detected persistent CD34 expression in bone marrow stroma after the irradiation and depletion of all hematopoietic cells (data not shown).

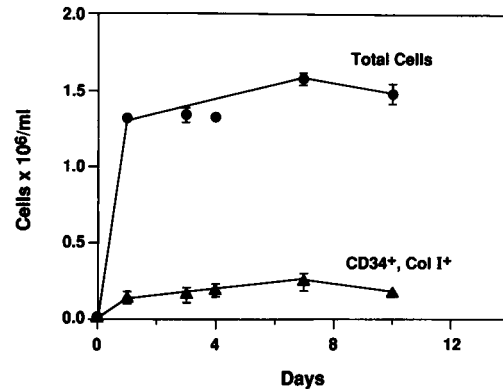


FIG. 4. Time-dependent appearance of fibrocytes in subcutaneously implanted wound chambers

Cellular analysis was performed on 5–10 μ l of fluid aspirated from wound chambers at the intervals shown. Cells were enumerated by cytofluorimetry and then double-stained with rhodamine-conjugated monoclonal anti-murine CD34 plus FITC-conjugated monoclonal antimurine collagen I. Similar results were obtained by double staining with anti-CD34 plus antivimentin antibodies. Each time point represents the mean \pm SEM of three or four independent fluid samplings.

To assess more directly the role of circulating fibrocytes in wound repair, we next examined scar tissue obtained from wound chambers that had been implanted in mice for a period of 7–56 days. The chambers were removed, examined histologically, and analyzed for reactivity with monoclonal anti-CD34 antibody or an isotype-matched antibody control (Fig. 6a). CD34⁺ cells exhibiting fibroblast morphology were readily identified in areas of scarring and connective tissue cell proliferation. In addition, progressively more anti-CD34 staining (and fibrosis) was observed with increasing time after implantation. We then examined cutaneous, connective tissue scars obtained from mice for the presence of CD34⁺ expression. Skin was removed from mice 28 days after an experimentally induced, surgical incision and stained with monoclonal anti-CD34 antibody. As shown in Fig. 6b, areas of connective tissue scar and cellular proliferation were markedly positive for CD34.

Finally, we examined cutaneous scar tissue obtained from a human subject at autopsy. Positive staining for CD34 was observed within the subcutaneous regions of human scar but not in sections obtained from control, nonscarred skin (Figs. 7a–7e).

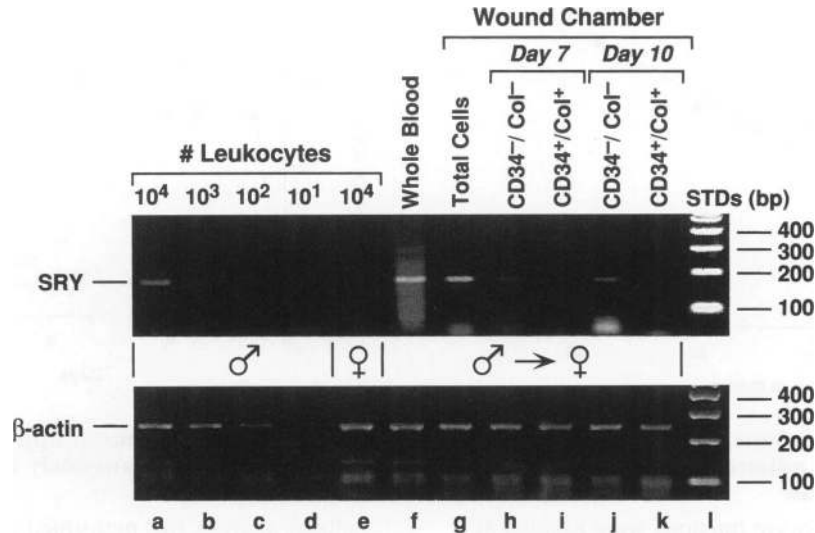


FIG. 5. DNA amplification analysis of wound chamber fibrocytes isolated from male→female bone marrow chimeric mice

One thousand leukocyte equivalents of whole blood (lane f) or wound chamber cells (lanes g–k) were obtained from female mice reconstituted 6 weeks previously with male bone marrow cells. For quantitative purposes (lanes a–e), genomic DNA was first prepared from defined numbers of peripheral blood leukocytes and amplified with primers specific for the Y chromosome *SRY* gene, or the β -actin gene as shown. Whole blood (10^4 leukocyte equivalents) from a representative bone marrow recipient (male→female) was amplified in lane f. Lane g shows amplification products obtained from the DNA of 10^4 total wound chamber cells before sorting. Lanes h–k show amplification products obtained after sorting wound chamber cells into $CD34^-/Collagen I^-$ (lanes h and j) and $CD34^+/Collagen I^+$ populations (lanes i and k) (10^4 cells per analysis). “Day 7” and “Day 10” refer to cells recovered at 7 or 10 days postwound chamber implantation. Results shown are representative of duplicate determinations performed in each of four mice.

DISCUSSION

These studies provide direct evidence for the participation of a novel, blood-borne fibroblast-like cell in the host repair response to tissue injury. This cell, which we have termed a fibrocyte, appears concurrently with circulating inflammatory cells and accounts for approximately 10% of the cells that infiltrate subcutaneously implanted wound chambers. Of significance, fibrocytes are found in regions of scar formation, both within wound chambers and in subcutaneous connective tissue. Cell surface analysis suggests that these cells share both leukocytic and connective tissue cell features. In addition to expressing the fibroblast components vimentin, collagen, and fibronectin, fibrocytes also display the leukocyte common antigen CD45 and the hematopoietic stem cell marker CD34. The CD34 antigen comprises a 110 kD integral membrane glycoprotein that is present on myeloid and lymphoid progenitors (13–16), as well as on a stem cell population capable of reconstituting the bone marrow of lethally irradiated hosts (19). CD34 also has been shown to be expressed to varying degrees on

capillary endothelium and embryonic fibroblasts (16,17). However, the absence of staining for von Willebrand’s antigen together with the distinctive cell surface phenotype of fibrocytes argues against their endothelial origin. The observation that fibrocytes isolated from wound chambers implanted into sex-mismatched bone marrow chimeras originate from the host also indicates that these cells arise either from radioresistant bone marrow stromal elements or from non-bone marrow sources of mesenchymal cell progenitors. Conceivably, fibrocytes that circulate in peripheral blood may comprise a population of pluripotent connective tissue cell precursors that can differentiate along either fibroblast, smooth muscle, or osteogenic lines depending on the precise microenvironment of various wound sites.

The observation that circulating, connective tissue cells contribute to the healing phase of wound repair complements long-standing observations that point to the critical role of vascular integrity in normal tissue repair responses (3,20). Together with the infiltrating inflammatory cells

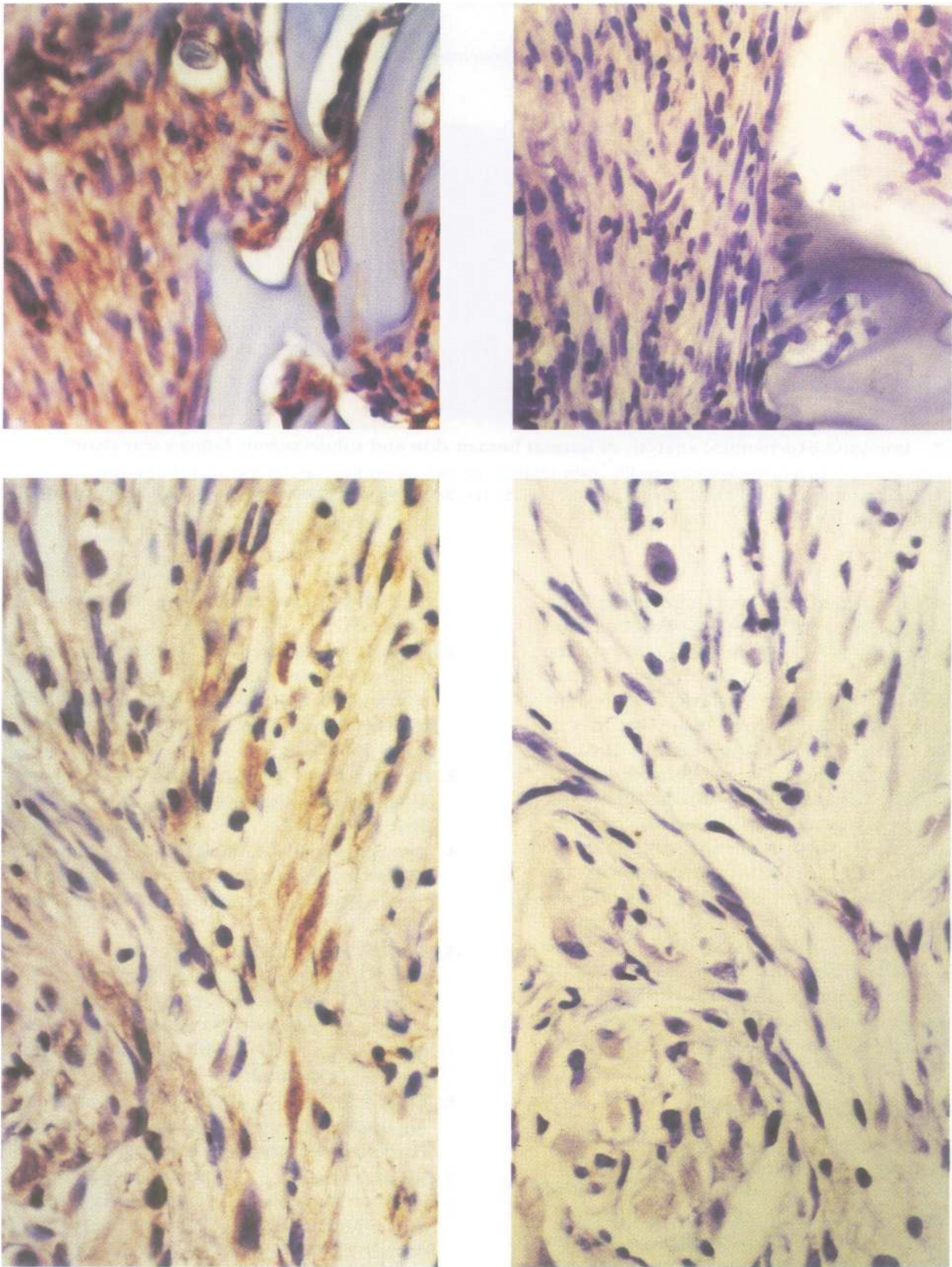


FIG. 6. Immunohistochemical analysis of murine fibrocytes in wound chambers and cutaneous scar tissue.

(a) Representative sections obtained from wound chambers 28 days after implantation in mice (400 \times). Adjacent sections were stained with either monoclonal anti-murine CD34 (left) or with a isotypic antibody control (right) and developed with an immunoperoxidase-linked secondary antibody. The areas of homogenous blue staining are polyvinylalcohol sponge. Qualitatively similar results were obtained in wound chambers removed 7, 14, and 56 days after implantation. However, progressively more fibrosis (and anti-CD34 staining) was observed with increasing time after implantation. (b) Immunohistochemical analysis of a mouse cutaneous scar obtained 28 days after an experimentally induced cutaneous incision (400 \times). Sections were stained as above with either monoclonal anti-murine CD34 (left) or with an isotypic antibody control (right) and developed with an immunoperoxidase-linked secondary antibody. Sections obtained from corresponding regions of control, nonscarred mouse skin showed no detectable staining with anti-CD34 antibody.

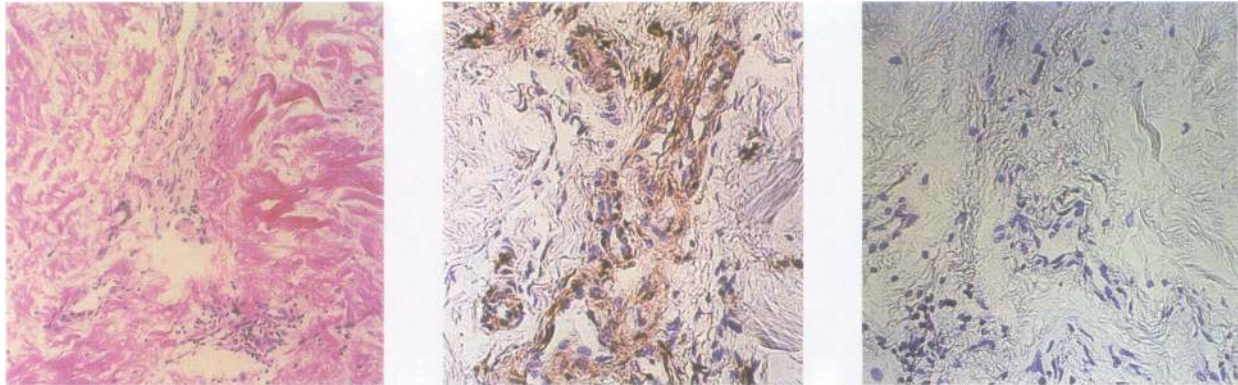


FIG. 7. Immunohistochemical analysis of normal human skin and subcutaneous human scar tissue

Skin sections were stained with hematoxylin-eosin (200 \times) (a), and monoclonal antimurine CD34 antibody (400 \times) (b), or with or an isotypic antibody control (400 \times) (c). Slides were developed with an immunoperoxidase-linked secondary antibody.

that act to prevent infection and degrade damaged connective tissue components, fibrocytes appear to participate in the earliest phases of the physiological response to tissue injury. In situations such as ischemia or diabetic vasculopathy, fibrocyte entry into damaged tissue sites may be compromised, thus contributing to impaired wound healing and poor scar formation. Conversely, circulating fibrocytes also may play a role in a variety of pathological processes characterized by excessive fibrosis or connective tissue cell proliferation. These may include pulmonary and hepatic fibrosis, atherosclerosis and restenosis injury, glomerulosclerosis, and pannus formation (21–24). Further investigations of the differentiation pathway, secretory profile, and precise tissue origin of blood-borne fibrocytes should increase significantly our understanding of the role of this cell population in tissue repair responses.

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