49 Kanner, S. B., Grosmaire, L. S., Ledbetter, J. A. and Damle, N. K. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 7099–7103
50 Vuori, K., Hirai, H., Aizawa, S. and Ruoslahti, E. (1996) Mol. Cell Biol. **16**, 2606–2613
51 Schaller, M. D., Hildebrand, J. D., Shannon, J. D., Fox, J. W., Vines, R. R. and Parsons, J. T. (1994) Mol. Cell Biol. **14**, 1680–1688

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Peripheral blood fibrocytes: novel fibroblast-like cells that present antigen and mediate tissue repair

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Introduction

Wounding is defined as a physical disruption of the normal architecture of a tissue and may be caused by trauma, burns, inflammatory processes or metabolic insufficiency. The host initiates a co-ordinated repair response to wounding that serves to prevent infection and ultimately re-establish normal tissue integrity. This response to injury involves a complex interplay of cellular, humoral and connective tissue elements [1,2]. Platelets play an early role by releasing factors that are required for both clotting and the recruitment of peripheral blood leucocytes to the injured site. Peripheral blood leucocytes then produce mediators that combat infection and co-ordinate successive steps of the tissue repair response [1–3]. Connective-tissue cells play a critical role in the reparative phase of wound healing by secreting extracellular matrix proteins. The usual outcome of this cascading series of cellular events is elimination of the invasive stimulus followed by connective tissue scar formation and, over time, remodelling of the injured site.

Connective-tissue fibroblasts are a quiescent cell population that under normal circumstances remain sparsely distributed throughout the extracellular matrix [4]. As a consequence of injury, fibroblasts enter and proliferate within the injured site [5]. The precise origin of the fibroblast-like cells within wounds has been controversial since the original microscopic studies of developing connective tissue performed by Paget in 1863 [6,7]. That wound fibroblasts appeared by migration from adjacent tissue was supported by experiments showing the apparent ingrowth of fibroblasts from local areas, and by the observation that India Ink-tagged monocytes failed to develop into tissue fibroblasts *in vivo* [7,8]. Other studies, however, reported evidence for the differentiation of leucocytes into fibroblasts within subcutaneous diffusion chambers and the apparent *in vitro* transformation of peripheral blood mononuclear cells into collagen-producing cells [9,10].

Abbreviations used: APC, antigen-presenting cell: TGF-β1, transforming growth factor β1; M-CSF, macrophage colony-stimulating factor; MIP, macrophage-inflammatory protein; IL, interleukin; TNF-α, tumour necrosis factor α.

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Discovery of peripheral blood fibrocytes

Several years ago, investigations into the cell population present in experimentally implanted subcutaneous wound chambers led to the discovery of an adherent proliferating cell type that displayed fibroblast properties yet expressed distinct haemopoietic/leucocyte cell-surface markers [11]. Wound chambers consist of short lengths of sponge-filled silastic tubing and are a frequently employed model for the study of tissue-reparative responses *in vivo*. Implantation of these chambers into the subcutaneous space of mice results in a rapid infiltration of peripheral blood inflammatory cells, including neutrophils, monocytes and lymphocytes [12,13]. Large numbers of adherent spindle-shaped cells that resemble fibroblasts were unexpectedly observed to infiltrate wound chambers soon after implantation and coincidentally with the appearance of circulating inflammatory cells. Double-immunofluorescence studies showed that, within 24 h of implantation, as many as 10–15% of the cells present in wound-chamber fluid stain posi-

tively for both type-1 collagen and CD34 [11]. At the time, the cell-surface marker CD34 was considered to be expressed exclusively by haemopoietic stem cells, and the combination of CD34 and collagen had not previously been described on any cell type. These studies suggested the presence of wounds of a previously uncharacterized cell type displaying fibroblast-like features but expressing markers for bone-marrow-derived cells. Follow-up immunohistochemical analysis of wound chambers that had been implanted in mice confirmed the presence of $CD34^+$ spindle-shaped cells in areas of collagen-matrix deposition. These cells were also identified to be present in areas of scarring [11].

More detailed investigations have since demonstrated that peripheral blood fibrocytes comprise approx. 0.5% of circulating leucocytes (J. Chesney, unpublished work). Fibrocytes obtained from blood constitutively express in culture the fibroblast products collagen I, collagen III and fibronectin, as well as the leucocyte common antigen CD45RO, the pan-myeloid antigen CD13 and the haemopoietic stem-cell antigen CD34 [11]. Fibrocytes do not synthesize epithelial (cytokeratin), endothelial (von Willebrand factor VIII-related protein) or smooth-muscle (α-actin) cell markers and are negative for non-specific esterases as well as the monocyte/macrophage-specific markers, CD14 and CD16 [11]. Fibrocytes also do not express proteins produced by dendritic cells or their precursors (CD25, CD10 and CD38) or the pan-B-cell antigen CD19 [11,14–16]. Scanning electron microscopy has shown these cells to be morphologically distinct from blood-borne leucocytes and to display unique cytoplasmic extensions intermediate in size between microvilli and pseudopodia. Of note, studies employing sex-mismatched bone-marrow chimaeric mice and sensitive DNA-amplification techniques for the male-specific *SRY* gene have demonstrated that peripheral blood fibrocytes arise from either a radioresistant bone-marrow progenitor cell population or other tissue sources [11].

Fibrocytes secrete a distinct profile of cytokines and matrix molecules

Given that fibrocytes rapidly enter sites of tissue injury, it is not surprising that these cells produce a variety of cytokines that serve to co-ordinate the successive inflammatory and reparative responses. DNA-amplification analysis of mRNA obtained from fibrocytes in wound chambers has shown that these cells are an especially abundant source of growth factors, cytokines and matrix components [17]. Fibrocytes express high levels of mRNA for the fibrogenic growth factors, platelet-derived growth factor A and transforming growth factor β1 (TGF-β1), the haemopoietic growth factor macrophage colony-stimulating factor (M-CSF) and the chemokines macrophage inflammatory protein (MIP)-1α and MIP-2. Fibrocytes also express detectable mRNAs for the proinflammatory cytokines, interleukin (IL)-1β and tumour necrosis factor α (TNF-α). Finally, of the various cell populations present in wound chambers, only fibrocytes express mRNA for type-I collagen.

Recent studies suggest that fibrocyte collagen production is tightly regulated in the context of the inflammation and wound-healing responses. For instance, addition of the critical wound-healing mediator IL-1β to fibrocytes in culture suppresses type-I collagen production (J. Chesney, C. M. Metz, A. B. Stavitsky, M. Bacher and R. Bucala, unpublished work). Conversely, IL-1β induces fibrocyte secretion of the inflammatory chemokines MIP-1α, MIP-1β, monocyte chemoattractant protein-1, IL-8, and GROα; the haemopoietic growth factors M-CSF and IL-6 regulate macrophage differentiation and lymphocyte proliferation respectively, and are known to be released during the early phase of tissue repair [18–20]. TNF-α and TGF-β1 have also been found to be present in the tissue repair microenvironment and have been implicated in the stimulation of connective-tissue cell migration, proliferation and matrix production *in vivo* [5]. Thus peripheral blood fibrocytes may play an essential role in the recruitment and activation of both inflammatory and connective-tissue cells during the tissue-repair response. Furthermore IL-1β may function to maintain peripheral blood fibrocytes in a proinflammatory state early in tissue repair, resulting in the increased production of molecules that recruit and expand the inflammatory cell population within the wound environment.

Peripheral blood fibrocytes are potent antigen-presenting cells (APCs)

The skin is a vital barrier to infection or tissue invasion and plays a major role in host immunity [21]. When injured by physical trauma, burns or vascular insufficiency, the skin can become a significant portal of entry for pathogenic microorganisms. Resident APCs such as the Langer-

hans cell initiate antigen-specific immune responses by processing and presenting microbial antigens to $CD4^+$ T-cells by an MHC class-II dependent pathway [22]. Recently, human peripheral blood fibrocytes were found to express each of the known surface components required for antigen presentation, including MHC class-II molecules (HLA-DP, -DQ and -DR), the co-stimulatory molecules CD80 and CD86, and the adhesion molecules CD11a, CD54 and CD58 (J. Chesney, M. Bacher, A. Bender and R. Bucala, unpublished work). Human fibrocytes were also found to be able to induce APC-dependent T-cell proliferation when cultured with specific antigen. This proliferative activity was significantly higher than that induced by monocytes and nearly as high as that induced by purified dendritic cells. Mouse fibrocytes also express the surface components required for antigen presentation (I-A, I-E, CD11a, CD54 and CD86) and function as potent APCs *in vitro*. In addition, mouse fibrocytes pulsed *in vitro* with antigen and delivered to a site of cutaneous injury were found to migrate to proximal lymph nodes and to specifically prime naive T-cells. These studies suggest that fibrocytes may play an early and important role in the initiation of antigen-specific immunity.

The constitutive expression by fibrocytes of the surface proteins known to be necessary for antigen presentation contrasts with what has been described for tissue fibroblasts, which require activation by interferon γ to express measurable quantities of HLA-DR [24]. Although several tissue-derived cells have been shown to be capable of presenting antigen to memory T-cells, including dermal fibroblasts, endothelial cells and melanocytes [24–26], the sensitization of naive T-cells has been considered to be a particular function of dendritic cells [27,28]. Fibrocytes are distinct from dendritic cells and their precursors not only in their growth properties (fibrocytes are an adherent proliferating cell population whereas dendritic cells are non-adhering and poorly proliferating) but also in their surface protein expression ($collagen^+/CD13^+/CD34^+/CD25^-/CD10^-/CD38^-$). That fibrocytes also have a specialized and potent antigen-presenting activity suggests that they may play a critical role in the initiation of immunity during tissue injury and repair.

In mice, an appreciable portion (5%) of fibrocytes have been found to home to regional lymph nodes after intradermal injection into skin (J. Chesney, M. Bacher, A. Bender and R. Bucala, unpublished work). Fibrocytes may function *in vivo* to capture foreign proteins at sites of tissue injury and to migrate into regional lymph nodes for the purpose of sensitizing naive T-cells and/or activating memory T-cells. Fibrocytes are a particularly abundant source of the potent $CD4^+$ T-cell chemoattractants, MIP-1α and -1β, and the entry of $CD4^+$ T-cells into areas of tissue damage is considered to be an essential requirement for the generation of an antigen-specific immune response [1,29]. Fibrocytes thus may not only activate but also recruit $CD4^+$ T-cells into the tissue-repair microenvironment.

Peripheral blood fibrocytes participate in disorders of excessive fibrosis

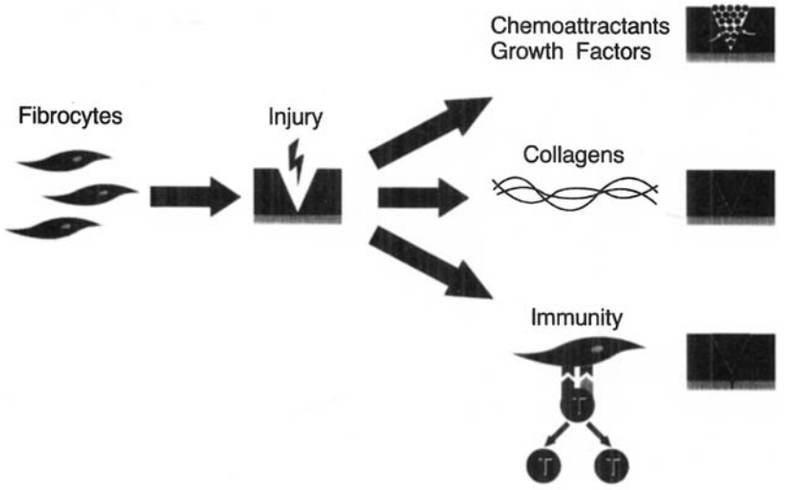
The ability of fibrocytes to both recruit and activate T-cells and to secrete type-I collagen suggests that these cells may play a critical role in certain connective-tissue disorders. We hypothesize that a persistent fibrocyte/T-cell activation response may lead to pathologically significant fibrosis in a variety of disease states. We have recently had the opportunity to examine fibrocyte function in schistosomiasis, a parasitic disease characterized by a fibrosing T-cell-mediated reaction directed against parasite eggs that become entrapped in the hepatic and pulmonary circulations [30]. Immunohistochemical studies of livers obtained from *Schistosome japonicum*-infected mice have shown that numerous spindle-shaped $CD34^+$ cells co-localize to areas of connective-tissue matrix deposition [17]. These data are the first to suggest that fibrocytes contribute to fibrotic pathology. Fibrocytes may also participate in the generation of excessive fibroses associated with various autoimmune disorders involving persistent T-cell activation, such as scleroderma or graft versus host disease.

Conclusions

The peripheral blood fibrocyte is a novel cell type that has been shown to rapidly enter sites of tissue injury and contribute to connective scar formation (Figure 1). Fibrocytes express a distinct profile of cytokines and growth factors and may function to attract and activate inflammatory and connective-tissue cells. Fibrocytes are also specialized to present antigen and may play a critical role in the initiation of cognate immunity during the earliest phases of tissue injury. In situations such as ischaemia or diabetic vasculopathy, fibrocytic entry into damage tissue sites

Figure 1

Role of fibrocytes in the wound-healing and inflammatory responses *in vivo*



may be compromised, thus contributing to poor scar formation. Conversely, peripheral blood fibrocytes may play a role in various pathological processes characterized by excessive fibrosis, including pulmonary and hepatic fibrosis, atherosclerosis, glomerulosclerosis and pannus formation. Further investigation into the function of this novel cell population may provide important insights into the regulation of host wound-healing and fibrotic responses.

1 Wahl, L. M. and Wahl, S. M. (1992) in Wound Healing: Biochemical and Clinical Aspects (Cohen, I. K., Diegelman, D. F. and Lindblad, W. J., eds.), pp. 40–62, Saunders, Philadelphia
2 Clark, R. A. F. (1996) in the Molecular and Cellular Biology of Wound Repair (Clark, R. A. F., ed.), pp. 3–35, Plenum Press, New York
3 Rappolee, D. A., Mark, D., Banda, M. J. and Werb, Z. (1988) Science **241**, 708–712
4 Morgan, C. J. and Pledger, W. J. (1992) in Wound Healing: Biochemical and Clinical Aspects (Cohen, I. K., Diegelman, D. F. and Lindblad, W. J., eds.), pp. 40–62, Saunders, Philadelphia
5 Kovacs, E. J. and DiPietro, L. A. (1994) FASEB J. **8**, 854–861
6 Paget, J. (1863) Lectures on Surgical Pathology Delivered at the Royal College of Surgeons of England, 848 pp., Longmans, London
7 Dunphy, J. E. (1963) N. Engl. J. Med. **268**, 1367–1377
8 Jackson, D. S. (1961) in The Biology of the Connective Tissue Cells (Jackson, D. S., ed.), pp. 172–178, Arthritis and Rheumatism Foundation, New York
9 Petrakis, N. L., Davis, M. and Lucia, S. P. (1961) Blood **17**, 109–118
10 Labat, M. L., Bringuier, A. F., Arys-Philippart, C., Arys, A. and Wellens, F. (1994) Biomed. Pharmacother. **48**, 103–111
11 Bucala, R., Spiegel, L. A., Chesney, J. A., Hogan, M. and Cerami, A. (1994) Mol. Med. **1**, 71–81
12 Diegelmann, R. F., Lindblad, W. J. and Cohen, I. K. (1986) J. Surg. Res. **40**, 229–237
13 Diegelman, R. F., Lindblad, W. J., Smith, T. C., Harris, T. M. and Cohen, I. K. (1987) J. Leukoc. Biol. **42**, 667–672
14 Freundenthal, P. S. and Steinman, R. M. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 7698–7702
15 Galy, A., Travis, M., Chen, D. and Chen, B. (1995) Immunity **3**, 459–473
16 Barclay, A. N., Beyers, A. B., Birkeland, M. L., Brown, M. H., Davis, S. J., Somoza, C. and Williams, A. F. (1993) The Leukocyte Antigen Facts Book, pp. 142–143, Academic Press, New York
17 Reference deleted
18 Kishimoto, T., Akira, S. and Taga, T. (1992) Science **258**, 593–597
19 Callard, R. and Gearing, A. (1994) The Cytokine Facts Book, pp. 174–178, Academic Press, New York
20 Ford, H. R., Hoffman, R. A., Wing, E. J., Magee, M., McIntyre, L. and Simmons, R. L. (1989) Arch. Surg. **124**, 1422–1428
21 Mast, B. (1992) in Wound Healing: Biochemical and Clinical Aspects (Cohen, I. K., Diegelman, R. F. and Lindblad, W. J., eds.), pp. 344–355, Saunders, Philadelphia
22 Chu, T. and Jaffe, M. B. (1994) Br. J. Cancer Suppl. **23**, S4–S10

23 Reference deleted
24 Geppert, T. D. and Lipsky, P. E. (1985) J. Immunol. **135**, 3750–3762
25 Pober, J. S., Gimbrone, M. A., Cotran, R. S., Reiss, C. S., Burakoff, S. J., Fiers, W. and Kenneth, A. (1983) J. Exp. Med. **157**, 1339–1353
26 Poole, I. C., Mutis, T., Rene, M., Wijngaard, G. J., Westerohof, W., Ottenhoff, T., Vries, R. and Das, P. K. (1993) J. Immunol. **12**, 7284–7292
27 Inaba, K., Metlay, J. P., Crowley, M. T. and Steinman, R. M. (1990) J. Exp. Med. **172**, 631–640
28 Levin, D., Constant, S., Pasqualini, T., Flavell, R. and Bottomly, K. (1993) J. Immunol. **12**, 6742–6750
29 Schall, T. J., Bacon, K., Camp, R. D. R., Kaspari, J. W. and Goeddel, D. V. (1993) J. Exp. Med. **177**, 1821–1825
30 Warren, K. S., Domingo, E. O. and Cowan, R. B. T. (1967) Am. J. Pathol. **51**, 735–756

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Expression of extracellular matrix molecules, proliferation markers and cyclin-dependent kinase inhibitors in inflamed tissues

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A variety of factors, including transforming growth factor β (TGF-β), platelet-derived growth factor and tumour necrosis factor, are produced in inflamed tissues which are able to promote and/or regulate fibroblast proliferation and accumulation of various extracellular matrix (ECM) molecules, allowing injured tissues to be repaired. In particular, TGF-β (different isoforms have been described with similar biological activities), a key member of a family of growth and differentiation factors, has pleiotropic functions, actively participating in embryonic development, cell growth, immune functions and tissue repair by providing specific fibrogenic and inhibitory signals to different cell types [1–4]. These signals are necessary for the regulation of immune responses in inflammatory processes as well as for the maintenance of balanced tissue growth, avoiding excessive production of different cell components after tissue damage. The anti-proliferative effects of TGF-β are particularly pronounced in epithelial, endothelial and haematopoietic cells [5]. Circumstantial evidence for the important regulatory role of TGF-β in immunological and inflammatory conditions is also provided by the occurrence of multifocal inflammatory disease in many organs in TGF-β-1-null (knock-out) mice [6].

Fibrogenic signals

ECM plays an important role in determining cellular behaviour, influencing the migration of different cell types, cell–cell adhesion, proliferation and differentiation. TGF-β plays a critical role in the synthesis and degradation of ECM, regulating its deposition and composition in embryogenesis, tissue repair and inflammatory lesions. The range of ECM molecules with expression regulated by TGF-β includes different types of collagen, fibronectin, thrombospondin, tenascin (TN) and others [3]. Lymphocytes interact with various molecular components of the ECM through a repertoire of receptors precisely regulated according to microenvironmental stimuli to allow rapid interconversion between adhesive and non-adhesive states [7]. Many components of the integrin family are in fact receptors which are expressed after activation and can specifically recognize and bind discrete sequences on different ECM macromolecules [8]. In turn, some ECM molecules such as fibronectin cannot be merely regarded as cell-adhesion substrata, since they have diverse important roles including mitogenic effects on immune and haemopoietic cells in co-operation with a number of cytokines and growth factors

Abbreviations used: TGF-β, transforming growth factor β; ECM, extracellular matrix; TN, tenascin; CDK, cyclin-dependent kinase; TUNEL, TdT-mediated dUTP–biotin nick end labelling.

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Fibroblast-mediated formation of basement membrane (composed of a layer of basal lamina and a layer of reticular lamina) serve as structural scaffolds critical for tissue regeneration in wound healing; cell barriers segregating epithelial from endothelial tissues; barriers preventing malignant escape or invasion of cancerous cells; filtration devices found in the glomerular filtration of blood in the kidney; and filtration of the.Â Fibroblasts also play a role in blood clotting, such as in the production of urokinase plasminogen activators (PAs) and their inhibitors (PAIs).Â Fibroblasts have close interactions with endothelial cells and facilitate angiogenesis into tissues beyond the reach of existing blood vessels.