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Circulating Progenitor Cells and Scleroderma

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Abstract

Scleroderma (systemic sclerosis) is a disease of unknown origins that involves tissue ischemia and fibrosis in the skin and internal organs such as the lungs. The tissue ischemia is due to a lack of functional blood vessels and an inability to form new blood vessels. Bone marrow–derived circulating endothelial progenitor cells play a key role in blood vessel repair and neovascularization.

Scleroderma patients appear to have defects in the number and function of circulating endothelial progenitor cells. Scleroderma patients also develop fibrotic lesions, possibly as the result of tissue ischemia. Fibroblast-like cells called fibrocytes that differentiate from a different pool of bone marrow–derived circulating progenitor cells seem to be involved in this process. Manipulating the production, function, and differentiation of circulating progenitor cells represents an exciting new possibility for treating scleroderma.

Introduction

Scleroderma involves both reduced blood flow in tissues (ischemia) and the inappropriate formation of scar tissue (fibrosis). Patients also have 1) altered levels of several proteins including cytokines and other signaling molecules, 2) autoantibodies, and 3) altered number and activity of many cell types encompassing the innate and adaptive immune systems as well as other cell types [1•]. Although there does not appear to be Mendelian inheritance of scleroderma, women develop the disease more often than men, and there is an abnormally high incidence among Native American Choctaw Indians [2], suggesting a genetic susceptibility component. Despite impressive efforts by researchers in the field, the disease's underlying cause is unknown, and experts do not yet understand which of the many observed alterations are caused by other observed alterations. In addition, no available therapies are 100% effective.

As one can imagine, much of the morbidity and mortality of scleroderma is caused by tissue ischemia and fibrosis. Ischemia causes a decrease in tissue oxygenation, which causes tissue damage; fibrosis then alters tissue structure and function. The ischemia is due to defects in blood vessel formation, function, and repair. Recent work has indicated that bone marrow–derived circulating progenitors exist in the blood and that under certain conditions, these progenitors can differentiate into neurons, hepatocytes, adipocytes, epithelial cells, osteoblasts, chondrocytes, and—most importantly for scleroderma—endothelial cells and fibroblast-like cells [3,4]. This review focuses on the role of circulating endothelial and fibroblast progenitors in scleroderma.

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Vasculogenesis and Circulating Endothelial Cell Progenitors

Key features of scleroderma are the reduced ability of existing blood vessels to constrict or dilate and the reduced ability to form new blood vessels [1•,5]. There are two main ways endothelium can repair itself or form new blood vessels in response to tissue ischemia. First, in a process called angiogenesis, mature endothelium can partly regenerate itself and form new blood vessels [6]. Second, in a process called vasculogenesis, endothelium repair and new blood vessel formation is mediated by bone marrow–derived progenitor cells that circulate in the blood, home to a repair site or a nascent blood vessel, and differentiate into endothelial cells [6,7]. At least two different types of circulating progenitors appear able to become endothelium [8]. One type of progenitor displays the markers CD133, CD34, and vascular endothelial growth factor receptor 2 (VEGFR2) [6,9]; most studies (and this review) focus on this population. A second progenitor population is a subset of CD14⁺ monocytes distinguishable from the conventional endothelial progenitor cells by the fact that they are CD34[−] [4,6]. Both circulating progenitor cell types can differentiate into mature endothelium in culture. During neovascularization in, for instance, granulation tissue, previously circulating endothelial progenitor cells contribute up to 25% of new endothelium [10]. However, endothelial progenitor cells are rare, representing less than 0.01% of circulating peripheral blood mononuclear cells (PBMCs) [8]. In addition to circulating cells, endothelial cells can also differentiate from stem cells in the heart and neural stem cells [8]. The data indicate that although they are rare and seem to have diverse lineages, bone marrow–derived circulating endothelial progenitor cells significantly contribute to neovascularization.

Wounding or tissue ischemia increases the number of circulating CD34⁺ endothelial progenitor cells, suggesting that signals from a tissue induce their proliferation or release from the bone marrow into the blood. A wide variety of factors or conditions affect the number of circulating endothelial progenitor cells [6]. For instance, vascular endothelial growth factor (VEGF) is a peptide that induces endothelial progenitor cell proliferation and recruitment [11]. The number of endothelial progenitor cells is also increased by estrogen, exercise, granulocyte colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), myocardial infarction, and vascular injury, whereas the number is decreased by smoking, hypertension, and other risk factors [6]. However, much remains to be understood about what normally regulates endothelial progenitor cell mobilization from the bone marrow into the circulation. Once in the circulation, endothelial progenitor cells home to sites of vessel repair or neovascularization. As will be described later, endothelial progenitor cell function seems altered in scleroderma patients.

Endothelial Progenitor Cells and Scleroderma

On average, studies have found an increased number of circulating endothelial progenitor cells in patients who had developed scleroderma within 3 to 5 years, whereas patients who had the disease for more than 5 years usually have a normal or somewhat reduced number of endothelial progenitor cells [12,13•,14]. During periods of disease activity, the number of circulating endothelial progenitor cells tends to increase [14]. VEGF is a key signal that regulates the number of circulating endothelial progenitor cells. There are significantly increased levels of plasma VEGF in scleroderma patients compared with controls, indicating that the impaired neovascularization in scleroderma patients is due to something other than an abnormally low level of VEGF [12,13•,15]. However, in an interesting new observation, scleroderma patients tend to have abnormally low plasma levels of the proangiogenic signal plasmin and abnormally high levels of the antiangiogenic signal angiostatin [16•]. Endothelial progenitor cell proliferation is strongly inhibited by angiostatin [17]. This suggests the intriguing possibility that high levels of angiostatin might be responsible for the decreased number of endothelial progenitor cells sometimes observed in scleroderma patients. Because the number of

circulating endothelial progenitor cells is sometimes increased and sometimes decreased in scleroderma patients, it is unclear if a decrease in the number of endothelial progenitor cells is responsible for scleroderma-associated defects in neovascularization.

Bone marrow appears to be the source of endothelial progenitor cells [7]. When researchers cultured bone marrow cells from scleroderma patients, they found that these cells were less able to produce nonadherent cells and stroma; they found fewer mesenchymal stem cells, and these cells had a much poorer ability to proliferate [13••]. In scleroderma patient bone marrow, there also tends to be a decreased number of cells positive for the endothelial progenitor cell marker CD133 and an increased number of cells positive for the mature endothelial cell marker P1H12 [13••]. These results suggest that scleroderma patients have an altered bone marrow environment and that this bone marrow has a decreased ability to produce normal endothelial progenitor cells.

CD133⁺ endothelial progenitor cells isolated from scleroderma patient blood or bone marrow had a very poor ability to differentiate into endothelial cells compared with progenitor cells from controls [12,13••]. Bone marrow cells from scleroderma patients also have a decreased propensity to chemotax toward a source of the endothelial cell chemoattractants VEGF or stromal cell–derived factor 1 (SDF-1) [18••]. VEGF and SDF-1 also potentiate the differentiation of endothelial progenitor cells into capillary-forming endothelium. In the presence or absence of VEGF or SDF-1, scleroderma patient bone marrow cells had a decreased ability to form tubular structures [18••]. These results indicated that scleroderma patient endothelial progenitor cells have a decreased ability to differentiate into functional endothelium. Recently, the signal transduction pathway through which SDF-1 regulates endothelial progenitor cell chemotaxis and differentiation was found to require heme oxygenase [19•]. Interestingly, scleroderma patient fibroblasts may have defects in their ability to regulate this enzyme [20]. Examining whether SDF-1 regulation of heme oxygenase is defective in scleroderma patient endothelial progenitor cells might yield insight into the reasons for reduced vasculogenesis in scleroderma.

Fibrosis and Circulating Fibroblast Progenitors

A key characteristic of scleroderma is the formation of fibrotic lesions in the skin and other organs [1••]. Healing wounds and fibrotic lesions in diseases such as hypertrophic scarring and asthma contain fibroblast-like cells that arise from circulating bone marrow–derived progenitor cells [3,21,22]. The circulating progenitors appear to be a subset of CD14⁺ monocytes, but markers defining this subset have not been identified [23••]. Some authors refer to the circulating progenitors as fibrocytes; this review refers to these as circulating fibrocyte progenitors and uses “fibrocytes” to describe the fibroblast-like cells into which these circulating cells differentiate. In culture, fibrocytes have a spindle shape, similar to the morphology of some endothelial progenitor cells in culture [24]. Whereas the endothelial progenitors express CD133; von Willebrand factor; and kinase domain receptor (KDR), which is also called VEGFR2; fibrocytes do not (Gomer et al., unpublished data) [3]. This finding indicates that although both fibrocyte progenitors and some endothelial progenitors share some features (eg, bone marrow origin, circulation in the blood, and form a spindle shape in culture), they are distinct cell types. Fibrocytes express a variety of markers, including CD34, the leukocyte marker CD45, collagen I, and collagen III [3]. There does not appear to be a unique marker expressed by fibrocytes, so these cells are always identified by dual staining, typically CD34 and collagen I or CD45 and collagen I.

Fibrocytes can further differentiate into myofibroblasts, cells that resemble smooth muscle and can secrete collagen to form scar tissue. Signals from real or perceived wounds cause the fibrocyte progenitor cells to extravasate at the wound site [23••]. For instance, in mice, cardiac

ischemia appears to recruit fibrocyte progenitors to the heart, where they differentiate into fibrocytes [25]. An essentially unknown set of conditions causes the progenitors to differentiate into fibrocytes. Interestingly, extravasation of monocytes through an apparently damaged endothelium occurs in patients with recently diagnosed scleroderma [26].

Fibrocytes promote wound healing in three key ways. First, fibrocytes can themselves differentiate into myofibroblasts [21,23••]. Interestingly, the differentiation of fibrocytes into myofibroblasts is potentiated by endothelin-1 [21], a factor that is elevated in scleroderma patients [27]. Second, fibrocytes secrete proangiogenic factors, and the resulting capillaries can bring nutrients and cells to the wound site [28,29]. Third, fibrocytes secrete a variety of cytokines [28]. In a recent significant discovery, investigators found that fibrocytes secrete cytokines that induce resident fibroblasts to differentiate into myofibroblasts [30•]. This suggests that fibrocytes can have a multiplicative effect on fibrosis.

The role of fibrocytes in scleroderma remains unclear. One report suggested that scleroderma patients may have increased circulating CD14⁺ cells that can differentiate into CD34⁺ fibroblast-like cells [31]. In a recent study of eight scleroderma patients and eight controls, no difference was seen in the percent of circulating CD14⁺/CD45⁺ monocytes expressing several cell surface markers, but these authors did not examine the expression of collagen on these cells or their differentiation into fibrocytes [32]. In a very exciting new finding in the fibrosis field, patients with pulmonary fibrosis were found to have an increased number of circulating CD45⁺/collagen I⁺ cells [33••]. Thus, determining these cell levels in scleroderma patients would be very useful. Although fibrocytes tend to lose expression of CD34, this antigen is a marker of early fibrocytes [23••]. In the skin lesions of scleroderma patients, there were abnormally low numbers of CD34⁺ cells compared with normal skin [34], suggesting that these lesions do not tend to contain early fibrocytes. However, fibrocytes are present in fibrotic lesions in patients with nephrogenic systemic fibrosis (also known as nephrogenic fibrosing dermopathy), a recently characterized fibrosing disease with similarities to scleroderma [35]. In a puzzling contradiction, fibrocytes secrete proangiogenic factors, but scleroderma patients have defective blood vessel formation. Thus, if fibrocytes are involved in the fibrotic lesions in scleroderma, something in the patients must block the ability of fibrocytes to promote blood vessel formation.

New Treatment Possibilities

Statins

Statins are normally used to lower cholesterol levels, but a pleiotropic effect of these drugs seems to be an increase in the number of circulating endothelial progenitor cells, possibly by stimulating the Akt pathway in endothelial progenitor cells [36,37]. In a study of 13 female scleroderma patients treated with 10 mg atorvastatin once a day for 12 weeks, in addition to the expected decrease in serum cholesterol, researchers found a 3.8 ± 19 -fold increase in the number of circulating CD133/VEGFR2 double positive endothelial progenitor cells, raising the count to approximately one third of that seen in healthy controls [38]. There were decreases in the serum levels of the endothelial injury markers vascular cell adhesion molecule 1 and E-selectin, as well as a decrease in the extent of Raynaud's phenomenon (discoloration and pain in the fingers induced by exposure to cold and caused by reduced blood flow). All of the above changes returned to baseline 4 weeks after the patients stopped taking atorvastatin. However, the atorvastatin treatment did not affect the patients' circulating endothelial progenitor cells' ability to differentiate into endothelium in vitro. An important recent observation might limit the use of statins: in patients with coronary artery disease, long-term statin use decreases the number of circulating endothelial progenitor cells [39•]. Because GM-CSF and G-CSF also stimulate endothelial progenitor cell production, Kuwana et al. [38] suggested these as possible treatments for the decreased number of endothelial progenitor cells in scleroderma.

Autologous stem cell transplantation

A second possible treatment method for scleroderma is autologous stem cell transplantation therapy, which is an established method to treat leukemia. Clinical trials using autologous stem cell transplantations focusing on endothelial progenitor cells have also been used to successfully treat critical leg ischemia, whereas mixed results have been observed in similar trials to treat acute myocardial infarction and chronic heart disease [8]. Autologous stem cell transplantations appear to “reset” the immune system and the source of progenitor cells. Despite impressive advances, much remains to be understood about how the therapy resets the immune system and what specific cell types are involved [40].

For scleroderma, there are two ongoing phase 3 clinical trials, the Autologous Stem cell Transplantation International Scleroderma (ASTIS) trial in Europe and the Scleroderma Cyclophosphamide or Transplant (SCOT) trial in the United States. Both trials compare stem cell therapy to monthly treatment with cyclophosphamide. Of the approximately 100 scleroderma patients receiving autologous stem cell transplants in phase 1 and 2 trials, about 70% have an improvement in skin hardness, and approximately 33% show sustained remission [41,42].

To collect autologous stem cells, patients are treated with the nitrogen mustard cyclophosphamide and injections of G-CSF, which increase the number of circulating stem cells [43]. Circulating CD34⁺ stem cells are then collected and stored. The cells can also be grown and expanded in vitro [8]. A high dose of cyclophosphamide combined with antithymocyte globulin is used for immunodepletion (the SCOT trial also uses whole body irradiation), and then the stem cells are injected back into the patient. The relative success of the stem cell transplantation trials suggests that a key causative factor in scleroderma is an autoimmune disorder or at least a defect in a cell type that is killed during the immunodepletion step. In the future, gene-modified endothelial progenitor cells may improve the results of stem cell transplants. For instance, in animal models, using genetically modified adenovirus with a VEGF expression construct to transfect endothelial progenitor cells resulted in cells with better neovascularization properties [44].

Cyclophosphamide alone

Cyclophosphamide kills lymphocytes and most hematopoietic cells but spares primitive progenitor cells [45]. As with the stem cell therapy regimen, this appears to reset the immune system. High doses of cyclophosphamide have been used to treat aplastic anemia and several autoimmune diseases [46,47••]. In a recent pilot study, six patients with diffuse scleroderma were given 50 mg/kg cyclophosphamide each day for 4 days as part of a treatment regimen [47••]. One patient died from an infection, probably due to a weakened immune system. Of the remaining five patients, four showed an improvement in the modified Rodnan skin score at 1 month, and all showed an improvement at 3 months. At 12 to 16 months, two of the patients had relapsed, whereas three showed sustained remission [47••]. Thus, cyclophosphamide is an exciting new potential treatment for scleroderma.

Serum amyloid P

While looking for cell density-sensing factors secreted by PBMCs, my group noticed that fibrocytes rapidly differentiate in culture in the absence of serum and that serum inhibits this process [48]. We purified the factor in human serum that inhibits the differentiation of human PBMCs into fibrocytes and identified it as serum amyloid P (SAP), a pentameric protein secreted by liver cells. In a small sample, we observed that some scleroderma patients appeared to have abnormally low serum SAP levels [48], although another study did not observe low serum SAP levels from scleroderma patients [49]. In a murine model of cardiac fibrosis, and in rat and murine models of pulmonary fibrosis, we found that SAP injections prevented the

appearance of fibrocytes in the affected tissue and prevented fibrosis [25,50]. There are no effective therapies for fibrosing diseases; SAP injections may be useful as a therapy.

Conclusions

Although scleroderma is a frustratingly complex disease, two of its key components are tissue ischemia and fibrosis. A combination of basic and applied research focusing on scleroderma and fields that initially seemed far removed from scleroderma suggests that circulating progenitors of both endothelial cells and fibroblast-like cells might be involved in the abnormal decrease in endothelium formation and the abnormal increase in connective tissue formation in scleroderma. New therapies aimed at increasing circulating endothelial cell progenitors, improving their function, and decreasing circulating fibroblast progenitor cell differentiation hold promise as possible therapies for scleroderma.

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