Pathogenesis of Scleroderma (Systemic Sclerosis)

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Increasing interest in the vascular features of scleroderma has led to the hypothesis that the blood vessel is the major target tissue and that the endothelial cell is the principal target cell. Useful observations stemming from the vascular hypothesis include the use of microvascular abnormalities in the early detection of the patient destined to develop classical scleroderma, the discovery of a serum protease selectively cytotoxic to endothelial cells, and the study of a serum mitogenic activity for fibroblasts in scleroderma patients. Immune events related to the vascular lesions are under active study but have not as yet provided a unique immunological lesion in scleroderma patients. The possibility that immunity to basement membrane (type IV) collagen may be selective for scleroderma patients deserves further study. Persistent immunity to endothelial basement membrane structures would provide a basis for continued endothelial injury. Techniques to quantify endothelial injury are useful to assess activity of the vascular lesions and to monitor therapies designed to block further vascular injury. The definition of pre-fibrotic vascular lesions may have future therapeutic and preventive implications for scleroderma.

Scleroderma is at once the easiest connective tissue disorder to recognize and the most difficult to understand [1, 2]. This essay will discuss recent observations in our understanding of the diffuse or generalized form of scleroderma.

Scleroderma subjects demonstrate a remarkable propensity to deposit large quantities of collagenous matrix in unusual sites throughout the body, best appreciated at a glance or a touch in the skin. Analyses of this matrix suggest that it is similar to collagens deposited in other human fibrotic disorders, such as cirrhosis (liver), emphysema (lung), keloids (skin) and atherosclerosis (blood vessels), and consists of the usual 4:1 ratio of collagens types I and III [3]. The cell most likely responsible for this excess collagen is the interstitial fibroblast. The stimulus for increased collagen deposition is unknown and could represent increased synthesis per fibroblast, increased amplification or migration of fibroblasts, or decreased collagen degradation. Initial in vitro studies of scleroderma skin fibroblasts, which were not rigorously controlled for fibroblast proliferation, demonstrated increased collagen synthesis per cell [4,5]. Recent studies have confirmed this increased synthesis and have found levels of collagenase to be similar to levels in control cells [4,5]. Careful cloning studies of scleroderma and control fibroblasts might demonstrate such an increased proportion of high collagen-producing mature fibroblasts [6]. Careful cloning studies of scleroderma and control fibroblasts might demonstrate such an increased proportion of high collagen-producing mature fibroblasts [6]. The multifactal mitogen serum stimulates both equally. Recently several laboratories have reported in preliminary form that scleroderma serum contains a mitogen selective for fibroblasts [8,9]. We have confirmed the presence of a mitogen in scleroderma serum selective for healthy human skin fibroblasts and extended these observations to show that this mitogen has little to no effect on scleroderma fibroblasts and that the mitogenic effect of scleroderma serum, but not that of healthy serum, can be blocked completely by inhibitors of proteolytic enzymes such as soybean trypsin inhibitor (Kunitz type*). With the identification of a mitogen in scleroderma serum, two mechanisms by which scleroderma fibroblasts could develop an enhanced capacity to synthesize collagen must be entertained.

The simplest would be direct stimulation of the fibroblast to selectively increase collagen synthesis by an as yet unknown stimulus emanating from the early vascular and inflammatory stages of the disease. If present such a single and selective stimulus remains to be detected. The second mechanism would include increased fibroblast replication and consequently amplification of the collagen producing capacity of a given region such as the subcutaneous space by increasing the number of fibroblasts. If replication were an isolated event and each daughter fibroblast were equivalent to the parent cell in its capacity to synthesize collagen, this second mechanism would not explain the demonstrated increased capacity of scleroderma fibroblasts to synthesize collagen on a per cell basis. Furthermore, scleroderma fibroblasts studied in vitro have not shown abnormal growth characteristics [3-5,10]. They have shown a lack of response to the scleroderma serum mitogen which could be interpreted as evidence of in vivo exposure to this mitogen. The existing data are consistent with both of these mechanisms being operative, i.e., both fibroblast replication and increased collagen synthesis are mechanisms of fibrosis in scleroderma. In this situation the fibroblast would be analogous to lymphocyte populations which simultaneously replicate and increase their phenotypic expression of mediator production, such as lymphokine synthesis. It is conceivable that fibroblasts respond to similar types of stimuli as do lymphocytes. More detailed studies of the interactions between cells and modulators of the immune system and of the interstitial connective tissue are needed.

Replication is not the only mechanism by which fibroblast expression can be amplified. In vitro studies suggest that short sequences in the collagen molecule stimulate fibroblast chemotaxis in Boyden chambers [11]. Whether fibroblast migration is an important mechanism in human fibrotic disease remains to be demonstrated.

Developmental studies of fibroblast behavior suggest that enhanced levels of collagen synthesis are an inherent trait of the life history of interstitial cells in which fibroblasts mature into fibrocytes with at least three stages recognizable morphologically and with only the final stage producing substantial quantities of collagen. Thus fibroblast replication alone could, by increasing the proportion of high collagen-producing mature fibrocytes in the interstitium of scleroderma skin, lead to both fibroblast amplification and increased synthesis of collagen on a per cell basis [12]. Careful cloning studies of scleroderma and control fibroblasts might demonstrate such an increased proportion of high collagen producing fibrocytes [13]. Immune reactions to types I and IV collagens have been demonstrated in both serum and lymphocytes from scleroderma.

subjects [11,14]. A perpetuating cycle of fibrosis could develop from fibroblast proliferation → fibrocyte collagen synthesis → a host response susceptibility to develop an immune response to collagen components → activated lymphocytes-monoocytes → macrophage-mesenchymal cell fibroblast proliferation.

It has been known for several decades that the vascular abnormalities, particularly the proliferative lesion of the small arteries, are key to the ultimate prognosis in the individual patient through the mechanism of visceral insufficiency of the kidneys, lungs, heart, and gastrointestinal systems, any of which can lead to death [1,2,15]. Over the years several colleagues have attempted to study the mechanisms of vascular damage in scleroderma. Taking a clue from the careful ultrastructural studies of Fleischmajer and colleagues which demonstrated damage to the endothelial lining cell of the blood vessel [16], Kahaleh, Sherer, and LeRoy examined the effect of scleroderma serum on the growth of umbilical cord endothelial cells in vitro. They found most scleroderma sera to contain an activity which was cytotoxic to endothelial cells [17]. This endothelial cytotoxic activity could be blocked by protease inhibitors suggesting that a protease or protease-inhibitor complex could be the active endothelial cytotoxic principle. It is fascinating to realize that the same scleroderma sera contain activities which kill endothelial cells and stimulate fibroblasts and that both of these activities are blocked by protease inhibitors. It is not known whether the same protease molecules effect both endothelial cytotoxicity and fibroblast proliferation.

What could be the source of such a protease or proteases in scleroderma? The possibilities are myriad. There is a substantial amount of evidence that abnormalities of the immune system are associated with scleroderma [2]. It has been shown that activated mononuclear cells can release lykphokines and that some (interleukin 1) of these are protease in nature [18]. Mononuclear cell supernatants have been shown to stimulate fibroblasts and this stimulation is abrogated by protease inhibitors [19]. So a contender for the source of the scleroderma serum protease is activated monocytes perhaps under lymphocyte control directed against as yet unknown vascular or cutaneous antigens.

Many other possible sources of protease exist. Surface activating Hageman factor can initiate the activation of proteases via the coagulation cascade, the kinin pathway, and the activation of the complement system [20]. All cells contain proteases and their presence may mirror no more than the residue of cell damage. Rapidly dividing cells of all types secrete proteases into the medium, so that a serum protease could be the result rather than the cause of fibroblast or endothelial proliferation [21]. Platelets also contain proteases and may release portions of their protease contents by the sequence of adhesion-aggregation-and release of α granules. In scleroderma patients, platelets could be concentrated at sites of endothelial injury. It is known from the work of Kahaleh et al that a platelet α granule protein, β-thromboglobulin, is increased in the serum of scleroderma patients [22]. Thus platelets are active in scleroderma and they could represent a source of protease.

Monocyte-macrophage cells are prime suspects for the source of proteases. Very large quantities of broad spectrum proteases are secreted by activated macrophages [23]. Enzymes such as macrophage elastase have a broad spectrum of substrate affinities and could be putatively released in scleroderma lesions [24]. What would be the mechanism of activation of monocytes-macrophages? Macrophage activation is a concomitant of most immune responses. There is evidence that lymphocytes from scleroderma subjects secrete macrophage migration inhibitory factor in response to partially characterized normal cutaneous antigens [25]. These antigens could also be of vascular origin, which would attract activated immune cells to the endothelial cell or to basement membrane and provide further mechanisms for endothelial and vascular injury. Direct evidence for immu

nity against endothelial cell antigens in scleroderma has not been reported. Indirect reports of antibodies removed from scleroderma lymphocytes which recognize endothelial cells have appeared [26]. Mackel, DeLuastro and associates have observed humoral immunity to basement membrane antigens, specifically IgG antibodies to type IV collagens, in the serum of scleroderma subjects [14]. Drawing analogies from the rheumatoid arthritis-like disease which develops in experimental animals rendered immune to cartilage or type II collagen [27], it is a distinct possibility that the diffuse connective tissue diseases, specifically scleroderma, represent autoimmune disease to basement membrane antigens, specifically to type IV collagen. This is an exciting possibility which can be subjected to experimental confirmation and which holds the potential of developing an experimental model for scleroderma, a much needed model for pathogenetic and therapeutic studies. Exclusive focus on type IV collagen as the only potential basement membrane antigen would be wide; the attachment protein laminin and less well characterized basement membrane glycoproteins should also be tested as putative antigens in scleroderma.

The endothelial, intimal arterial lesion is not the only vascular abnormality in scleroderma; distinct microvascular abnormalities of morphology and function exist in the nailfold capillary bed. Maricq et al have described a widefield capillary pattern characteristic of scleroderma and scleroderma-spectrum disorders which consists of dilated, distorted capillary loops interspersed with regions of absent or diminished capillary loops. There is preliminary reason to suspect that the dilated loops are permanent features of the scleroderma capillary pattern and that the avascular areas suggest recent or “active” disease. Truly prospective testing of this “active pattern” concept is needed. On the functional side of the microvasculature, scleroderma subjects are extremely sensitive to capillary standstill, a complete cessation of capillary flow on gentle cooling. Capillary standstill should be a very useful procedure to test therapeutic agents with regard to preventing microvascular insufficiency [28]. Such agents might have a role in the management of scleroderma.

An orientation toward vascular aspects of scleroderma might substantially improve the presently dismal management situation. If one realizes that fibrotic manifestations are extremely difficult to quantify and that activity of fibrotic features is virtually impossible to assess, it is not difficult to set fibrotic features aside and focus on vascular features. Here quantification can be immediate and of many types. The most straightforward to monitor are the global assays of endothelial and vascular damage which would include serum endothelial cytoxic activity, serum factor VIII-von Willebrand factor levels (a protein complex produced only by the endothelial cell and elevated in states of endothelial perturbation; [29], serum β-thromboglobulin levels, and platelet aggregate ratios. It is premature to predict which one of these assays will provide the optimal single test for monitoring vascular injury in scleroderma. This battery of assays to monitor endothelial damage can provide quantitative measures of the degree and the activity of vascular disease in scleroderma. Therapies in this disease can be assessed over the short term by determining which agents can return to normal the abnormal levels of this battery of endothelial tests. Noncompliance and inadequate dosing can be easily detected. Once a particular therapy has been shown to normalize tests of endothelial integrity, full double-blind multicenter studies of particular agents can be undertaken to determine effects on the fibrotic features of scleroderma. Such a graduated approach to the therapy of scleroderma would seem to offer a more efficient basis for determining efficacy in scleroderma than the present empirical approach.

Using a combined endothelial and microvascular armamen-
much more than present attempts to remove fibrosis once it has occurred.

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