Increased Plasma Levels of Platelet-Derived Growth Factor Activity in Patients with Progressive Systemic Sclerosis (42880)

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Abstract. **We measured mitogenic activity of whole blood serum and platelet-poor plasma-derived serum of a group of 10 patients with progressive systemic sclerosis and of 8 controls.Mitogenic activity of plasma-derived serum was greater in patients than in controls, in the absence of other signs of platelet activation. This increased activity was inhibited by specific antibodies, anti-platelet derived growth factor, suggesting that circulating levels of platelet-derived growth factor may be present in progressive systemic sclerosis patients. Platelet-derived growth factor, released either by platelets or by monocytes, might play a role in the pathogenesis of scleroderma.**

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Progressive systemic sclerosis, or scleroderma, is a connective tissue disorder characterized by vascular lesions, fibrosis, inflammation, and diffuse sclerosis in various districts. Abnormal proliferation of dermal fibroblasts and excessive deposition of collagen are characteristic features of this disorder (1, 2). Endothelial cell damage triggered by deposition of immune complexes or cytotoxic factors has been proposed as the initial step of the pathogenetic process underlying this disorder. Following the damage, circulating cells, such as monocytes or platelets, activated by contact with the subendothelial surface would release mitogenic factors which, in turn, might be responsible for the hyperproliferative reaction and increased collagen deposition (3, 4). Platelet involvement in scleroderma has been suggested on the basis of a few clinical observations (5). Activated platelets might release peptides contained in their α-granules, such as platelet-derived growth factor (PDGF) and transforming growth factor β (6, 7). The two substances have opposite effects on fibroblast proliferation. However, both of them stimulate secretion of components of the extracellular matrix (mucopolysaccharides, collagen, thrombospondin) and activation of collagenolytic enzymes, so that they may play a role in the pathogenesis of scleroderma (8–11).

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Abnormal response to PDGF has been described in dermal fibroblasts of progressive systemic sclerosis patients. Indeed, growth proliferation rate and secretion of collagen and glycosaminoglycans are lower in fibroblasts of patients than in controls. *In vivo* exposure to abnormally high levels of PDGF might explain the behavior of fibroblasts of the patients, through a mechanism of down-regulation (12, 13). Following this hypothesis, we measured mitogenic activity of whole blood serum (WBS) and platelet-poor plasma-derived serum (PDS) of a group of progressive systemic sclerosis patients in comparison to control subjects. The activity was tested in the presence and the absence of anti-PDGF IgG to discriminate between PDGF and other mitogenic factors.

Materials and Methods

Patients. Patients were diagnosed according to clinical (taut skin proximal to the metacarpophalangeal joints), instrumental (Doppler flowmetry, x-ray screening), and laboratory (indirect immunofluorescence for antinucleus antibodies) criteria (14). All patients were positive for Scl-70-specific antigen (15).

Ten women (aged 24–64 years) with clinical symptoms of progressive systemic sclerosis and 8 normal women (aged 48–63 years) were examined. Pharmacologic treatment was withheld from patients 15–30 days before the study. All patients had normal platelet count and renal function, the latter indicated by normal plasma creatinine levels. All patients were at an intermediate level of the disease, as indicated by similar oesophageal complications.

Sample Collection. WBS was prepared from ve-

nous blood taken without anticoagulants and allowed to clot at 37°C for 2 hr. PDS was prepared from blood taken on 0.126 *M* sodium citrate (10%). Anticoagulated blood was centrifuged at 3000 rpm for 20 min; the supernatant was recalcified by incubation with 20 m*M* $CaCl_2$ at 37°C for 2 hr. After clot formation, both WBS and PDS were centrifuged at 3000 rpm for 20 min, heated at 56°C for 30 min, and centrifuged again. The supernatants were stored at −20°C. Platelet number was determined by visual counting with a hemocytometer. Levels of β-tromboglobulin (βTG) and platelet factor 4 were determined by commercially available radio immunoassays (Amersham, England).

Mitogenic Activity. Serial dilutions of WBS and PDS were tested for their ability to stimulate [^{3}H] thymidine ([^{3}H]TdR) incorporation into NIH 3T3 cells. Cells were plated in Costar 96-well plates at the density of 2×10^3 cells/well in Dulbecco's modified Eagle's medium supplemented with 10% calf serum. After 4 days, cells were rinsed twice with phosphate-buffered saline; fresh medium (200 μl/well) was added along with various dilutions of serum samples (2.5, 5, 10, 20 μl/well). After an additional 15 hr, [^{3}H]TdR (0.5 μCi/well; 6.7 Ci/mmol; New England Nuclear, Boston, MA) was added and pulsed for 2 hr. Trichloroacetic acid-precipitable radioactivity was then collected and counted in a beta counter (16).

Effect of anti-PDGF IgG (α-PDGF). PDS samples (25 μl) were incubated at 37°C for 2 hr with or without 100 μl of goat anti-human PDGF IgG (1 mg/ml), and the volume was adjusted to 500 μl with Dulbecco's modified Eagle's medium. Two-hundred microliters of the incubation mixture were subsequently transferred to 3T3 cells and [^{3}H]TdR incorporation was tested as described above. The concentration of IgG used (40 μg/well) was previously found to inhibit the mitogenic activity of highly purified human PDGF (1 ng/well) by 50% in the same assay.

Results

Serial dilutions of WBS and PDS from control subjects and sclerodermic patients were tested on [^{3}H] TdR incorporation by NIH 3T3 cells. PDS from sclerodermic patients showed greater mitogenic activity than PDS from control subjects at each concentration tested (Fig. 1A). The difference was statistically significant at the concentrations of 5% ($P < 0.05$) and 10% ($P < 0.01$, Duncan's test). WBS from sclerodermic patients had greater stimulant activity than controls at low concentrations (2.5%, $P < 0.05$, Duncan's test), but had normal or lower than normal activity at higher concentrations (5–10%) (Fig. 1B). Incubation with anti-PDGF IgG inhibited the stimulatory activity of PDS from patients (42.1 ± 13% inhibition, mean ± SD, of five results), but did not affect mitogenic activity of PDS of controls (Fig. 2).

Finally, βTG levels in PDS samples were 26 ± 12 ng/ml in sclerodermic patients and 19 ± 6 ng/ml in controls (mean ± SD of 8–10 subjects); PF4 levels were 9 ± 5 ng/ml in sclerodermic patients and 8 ± 4 ng/ml in controls (mean ± SD of 8–10 subjects).

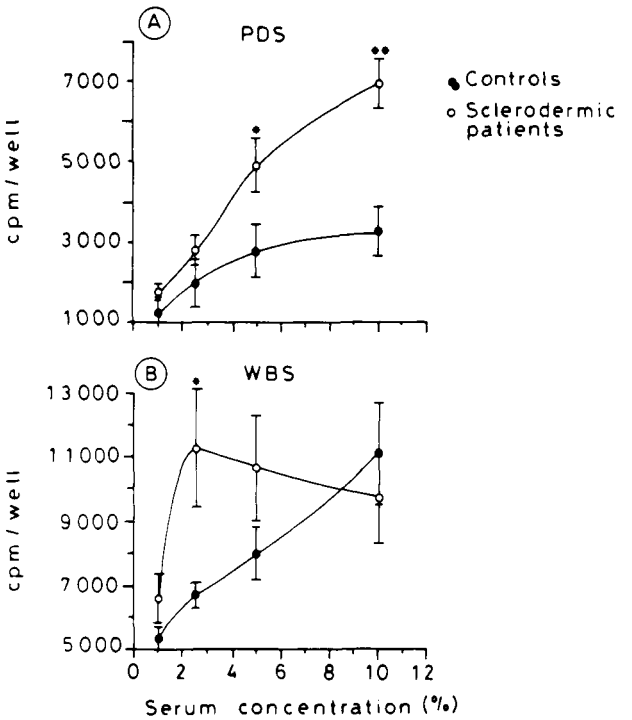

Figure 1. Effect of various concentrations of PDS and WBS of control subjects and sclerodermic patients on [^{3}H]TdR incorporation by NIH 3T3 cells. Mean ± SD of 8–10 subjects. $^*P < 0.05$; $^{**}P < 0.01$, Duncan's test.

Discussion

We measured the ability of serum samples of 10 sclerodermic patients and 8 matched controls to stimulate [^{3}H]TdR incorporation by NIH 3T3 cells in the presence or the absence of specific anti-PDGF antibodies. Two different preparations, i.e., whole blood-derived serum and platelet-poor plasma-derived serum were tested. The activity of WBS of sclerodermic patients was dual; it was greater than that of controls at low concentrations (2.5%) and lower than that of controls at higher concentrations (5–10%). We concluded that WBS contained both a stimulatory and an inhibitory activity. Contrasting data have been obtained by evaluating mitogenic activity of scleroderma serum; indeed an activity either higher or lower than normal has been described (3, 4, 17, 18). An inhibitory effect of scleroderma serum on endothelial cells was first discovered by Kahaleh and LeRoy (4). More recently, Takehara *et al.* (19) reported an association between serum's inhibitory activity and previous treatment of sclerodermic patients with Dypiridamole, a known antiaggregating agent. Since mitogenic activity of serum of both sclerodermic and control subjects was abolished by preincubation with specific anti-PDGF IgG, it was

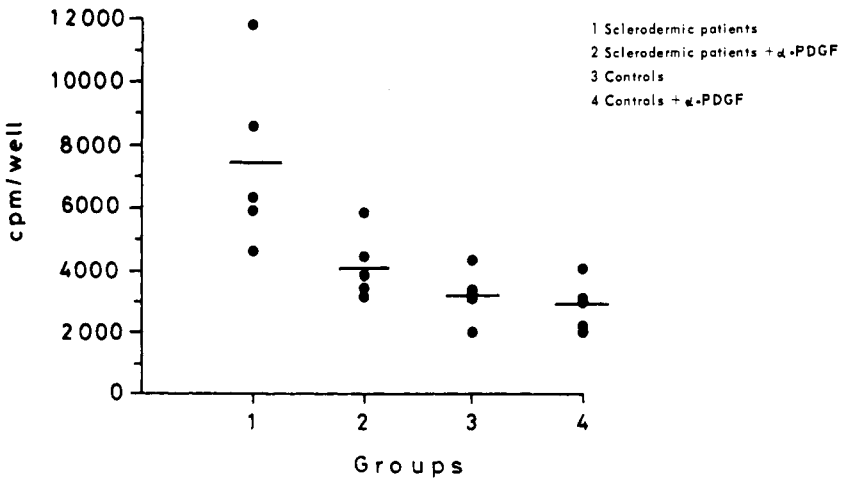

Figure 2. Effect of preincubation with anti-PDGF IgG on the ability of PDS (5%) to stimulate [^{3}H]TdR incorporation by NIH 3T3 cells. Five control subjects and five sclerodermic patients were tested.

argued that the inhibitory activity of scleroderma serum was attributable to a reduced platelet secretion of PDGF and not to the presence of a cytotoxic factor. A similar explanation can be ruled out in our case, since sclerodermic patients were not on drug treatment. Alternatively, a release of transforming growth factor β, a platelet-derived protein endowed with inhibitory activity for fibroblast proliferation, might be suggested (20).

In our experience, WBS of scleroderma patients contained also a stimulatory factor. Accordingly, Potter *et al.* (21) recently described an enhanced mitogenic activity for human fibroblasts in WBS of a group of sclerodermic patients; this increase was attributed to a partially characterized factor, named "fibroblast growth factor" (21). In addition, we also found that PDS of the patients had an increased mitogenic activity as compared with matched controls. The rise was completely reversed by incubation of PDS with anti-PDGF IgG, suggesting that it was due to release of PDGF. Similarly, an increased mitogenic activity, inhibited by incubation with anti-PDGF antibodies, has been described in plasma samples of young patients with coronary atherosclerosis (22).

No data were available until now on the circulating levels of platelet-derived mitogens in scleroderma patients. The presence of PDGF-like material in plasma appears to be due to a specific release of PDGF, since the levels of other proteins released by platelet α-granules, such as βTG and platelet factor 4, were not increased in parallel. Different authors found increased plasma levels of βTG in a group of scleroderma patients; this apparent discrepancy might be related to different stages of the disease (5).

The involvement of PDGF in the pathogenesis of scleroderma has been suggested on the basis of abnormalities in the proliferation rate and secretion of collagen and glycosaminoglycans by skin fibroblasts of sclerodermic patients (13, 23, 24). Our observation that a group of sclerodermic patients had circulating levels of a mitogen similar to PDGF supports the hypothesis that the abnormal connective tissue reaction typical of scleroderma is, at least in part, due to circulating hormones of platelet origin. A better knowledge of those factors might constitute the basis of an innovative therapeutical approach to this disease.

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