Stimulatory Autoantibodies to the PDGF Receptor in Systemic Sclerosis

Silvia Svegliati Baroni, Ph.D., Mariarosaria Santillo, Ph.D., Federica Bevilacqua, M.D., Michele Luchetti, M.D., Tatiana Spadoni, B.Sc., Matteo Mancini, B.Sc., Paolo Fraticelli, M.D., Paola Sambo, M.D., Ada Funaro, Ph.D., Andrius Kazlauskas, Ph.D., Enrico V. Avvedimento, M.D., Ph.D., and Armando Gabrielli, M.D.

ABSTRACT

BACKGROUND
Systemic sclerosis (scleroderma) is characterized by immunologic abnormalities, injury of endothelial cells, and tissue fibrosis. Abnormal oxidative stress has been documented in scleroderma and linked to fibroblast activation. Since platelet-derived growth factor (PDGF) stimulates the production of reactive oxygen species (ROS) and since IgG from patients with scleroderma reacts with human fibroblasts, we tested the hypothesis that patients with scleroderma have serum autoantibodies that stimulate the PDGF receptor (PDGFR), activating collagen-gene expression.

METHODS
We analyzed serum from 46 patients with scleroderma and 75 controls, including patients with other autoimmune diseases, for stimulatory autoantibodies to PDGFR by measuring the production of ROS produced by the incubation of purified IgG with mouse-embryo fibroblasts carrying inactive copies of PDGFR α or β chains or the same cells expressing PDGFR α or β. Generation of ROS was assayed with and without specific PDGFR inhibitors. Antibodies were characterized by immunoprecipitation, immunoblotting, and absorption experiments.

RESULTS
Stimulatory antibodies to the PDGFR were found in all the patients with scleroderma. The antibodies recognized native PDGFR, inducing tyrosine phosphorylation and ROS accumulation. Autoantibody activity was abolished by preincubation with cells expressing the PDGFR α chain or with recombinant PDGFR or by PDGFR tyrosine kinase inhibitors. Stimulatory PDGFR antibodies selectively induced the Ha-Ras-ERK1/2 and ROS cascades and stimulated type I collagen–gene expression and myofibroblast phenotype conversion in normal human primary fibroblasts.

CONCLUSIONS
Stimulatory autoantibodies against PDGFR appear to be a specific hallmark of scleroderma. Their biologic activity on fibroblasts strongly suggests that they have a causal role in the pathogenesis of the disease.
Systemic sclerosis (scleroderma) is a disorder characterized by fibrosis of the skin and visceral organs. Although some features of the scleroderma phenotype are well established and represent the hallmarks of the disease, the primary cause has not been fully delineated; endothelial-cell damage, immunologic abnormalities, and excessive extracellular-matrix production are well documented associations. The scleroderma phenotype at the cellular level is characterized by oxidative stress associated with the accumulation of large amounts of reactive oxygen species (ROS) in fibroblasts. ROS are key cell transducers of fibroblast proliferation and collagen-gene expression. We recently observed that a pathway linking the signaling proteins Ha-Ras, growth-factor–activated extracellular-signal–regulated kinases 1 and 2 (ERK1/2), and ROS is amplified in fibroblasts from patients with scleroderma.

Since platelet-derived growth factor (PDGF) can induce ROS and Ras-ERK1/2 signaling and since IgG derived from patients with scleroderma reacts with human fibroblasts, we sought Ha-Ras, ERK1/2, and ROS stimulatory molecules in the serum of patients with scleroderma. We hypothesized that serum from patients with scleroderma may contain stimulatory IgG autoantibodies directed toward the PDGF receptor (PDGFR). These autoantibodies may trigger PDGFR, which induces ROS by way of Ha-Ras and ERK1/2 signaling. This cascade is thought to be ultimately responsible for fibroblast activation, which represents a distinctive feature of scleroderma.

Methods

Patients

Forty-six consecutive white patients with scleroderma (8 men and 38 women; median age, 58 years [range, 35 to 77]) were studied. We also included 10 recipients of allogeneic bone marrow transplants who presented with graft-versus-host disease and scleroderma-like skin lesions. The demographic and clinical characteristics of the study groups are presented in Table 1.

The study was approved by the institutional ethics committee (Università Politecnica delle Marche, Ancona, Italy). After oral and written informed consent had been obtained, a blood sample was taken from the patients and controls (after acclimatization at 21°C for 30 minutes), and the sample was spun in a refrigerated centrifuge after clot formation. The supernatants were collected and stored at −30°C until they were assayed, usually within four weeks.

Bioassay for Anti-PDGFR Autoantibodies

The serum samples from patients with scleroderma and from controls were tested for the presence of PDGFR-activating autoantibodies in a functional bioassay. In brief, mouse-embryo fibroblasts expressing PDGF, α subunits, β subunits, or both (Fα, Fβ, and Fαβ) were exposed in vitro to immunopurified IgG. Mouse F−/− cells, derived from PDGFR-knockout embryos and devoid of PDGFR, were used as control cells (as described in the Supplementary Appendix). Cells were plated in duplicate at a density of 20,000 per 1.83-cm² well, cultured for 24 hours at 37°C in 0.2 percent fetal-calf serum, and incubated in the presence of 1 ml of immunopurified IgG (200 μg per milliliter) from a patient or a normal control for 15 minutes at 37°C before ROS production was determined.

Fluorimetric determination of intracellular ROS generated by adherent fibroblasts was carried out after the cells had been loaded with 2′,7′-dichlorofluorescein diacetate (DCF) (10 μM, Molecular Probes) as previously described. Each IgG sample was tested in duplicate. The results were expressed as a stimulation index, which corresponds to (S−C)/(P−C), where S is the DCF fluorescence intensity of the test IgG, C is the DCF fluorescence intensity of a negative control obtained by culturing cells without IgG, and P is the DCF fluorescence intensity of a positive control.
obtained by incubating cells with PDGF (15 ng per milliliter for 15 minutes). The intraplate variation was less than 3 percent. The samples were recorded as positive if the stimulation index was greater than the 95th percentile among the normal controls. In some experiments, ROS generation was evaluated in cells exposed to several inhibitors: the PDGFR tyrosine kinase inhibitor (AG 1296; 2 μM for two hours), the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (AG 1478; 2 μM for two hours), a chemical inhibitor of ERK1/2 signaling (PD 98059; 40 μM for two hours), and a farnesyl transferase inhibitor, which inhibits Ras farnesylation (FTI-277; 20 μM for two hours) (Calbiochem).

STATISTICAL ANALYSIS
The stimulation index is expressed as a median value and a range. Comparisons of the levels of anti-PDGFR autoantibodies between the group with scleroderma and the control groups were performed with the nonparametric Kruskal–Wallis test. Other data are expressed as means ±SE. Mean values were compared with the use of Student’s paired t-test. All reported P values are two-sided. Data were analyzed with the use of SAS software (SAS Institute).

RESULTS
Since PDGF induces the accumulation of ROS,8,9 we hypothesized that stimulatory serum antibodies targeting PDGFR were present in patients with scleroderma. To test this hypothesis and to eliminate the confounding effect of PDGF or other cytokines present in serum, we purified total IgG from patients with scleroderma and determined its biologic activity. As target cells we used a mouse-embryo cell line carrying inactive copies of PDGFR α and β subunits as a reference, as well as the same line of cells expressing recombinant and functional PDGFR α or β subunits (Fα, Fβ, and Fαβ). These cells were exposed to the IgG fractions isolated from patients’ serum specimens. To determine ROS levels, cells were starved and incubated with the peroxide-sensitive fluorophore DCF before treatment with purified IgG.

IgG from patients with scleroderma stimulated ROS production in Fα, Fβ, and Fαβ cells in a dose-dependent manner. ROS rapidly increased to the maximum level 15 minutes after IgG exposure and returned to baseline 40 to 120 minutes later. The best discrimination in ROS-stimulation activity between normal IgG and IgG from patients with systemic sclerosis was obtained in Fα cells and with IgG at a concentration of 200 μg per milliliter. These conditions were followed in all subsequent experiments unless otherwise specified.

| Table 1. Demographic Characteristics of the Patients and Controls.† |
|------------------|------------------|------------------|------------------|------------------|------------------|
| Characteristic   | Systemic Sclerosis (N = 46)† | Primary Raynaud’s Phenomenon (N = 15) | Systemic Lupus Erythematosus (N = 14) | Rheumatoid Arthritis (N = 15) | Idiopathic Pulmonary Fibrosis (N = 10) |
| Male sex (no.)   | 8                | 2                | 1                | 2                | 6                |
| Age (yr)         | 58               | 42               | 36               | 65               | 64               |
| Range            | 35–77            | 22–70            | 26–50            | 22–91            | 56–79            |
| Duration of Raynaud’s phenomenon (yr) | NA               | NA               | NA               | NA               | NA               |
| Median           | 14               | 6                | NA               | NA               | NA               |
| Range            | 2–50             | 2–25             | NA               | NA               | NA               |
| Duration of disease (yr) | 7               | 6                | 7                | 11               | 0.5              |
| Median           | 1–48             | 2–25             | 1–23             | 1–28             | 0–4              |
| Range            | 2–50             | 2–25             | NA               | NA               | NA               |

† NA denotes not applicable.
†† Of the 46 patients with systemic sclerosis (scleroderma), 24 had limited disease and 22 diffuse disease. Nine of the patients with systemic sclerosis had anticentromere antibodies, and 26 had anti–Scl-70 antibodies.
The concentration of 200 μg per milliliter for 15 minutes per 20,000 cells, expressed as the stimulation index (median, 0.86; range, 0.31 to 3.82) were significantly higher than the levels of ROS generated by normal IgG (median, 0; range 0 to 0.02) or by IgG from patients with primary Raynaud’s phenomenon (median, 0; range, 0 to 0.19), systemic lupus erythematosus (median, 0; range, 0 to 3.0).
autoantibodies in systemic sclerosis

n engl j med
354;25
www.nejm.org
june
22, 2006
2671

0.22), rheumatoid arthritis (median, 0; range, 0 to 0.13), or interstitial lung disease (median, 0; range, 0 to 0.17) (P<0.001 for all comparisons). Using the 99th percentile as the upper limit of normal, antibodies stimulating ROS production were found in all patients with scleroderma and in none of the controls (Fig. 1A).

To prove that ROS-inducing activity was mediated by the activation of PDGFR, we performed several additional experiments. First, the ROS accumulation induced in Fα cells exposed to sclerodermal IgG was inhibited by preincubating the cells with the PDGFR tyrosine kinase inhibitor AG 1296 (Fig. 1A). Sclerodermal IgG did not stimulate ROS in F−/− cells (Fig. 1A). In addition, sclerodermal IgG, and not IgG from controls, immunoprecipitated the PDGFR α and β subunits (Fig. 1B). PDGFR-interacting antibodies in sclerodermal IgG were completely removed by preabsorption with Fα cells but not with F−/− cells (Fig. 1C). Moreover, the supernatant after absorption with Fα cells did not stimulate ROS production (data not shown). Conversely, when IgG from patients with scleroderma was incubated with F−/− cells, PDGFR-interacting antibodies were not removed (Fig. 1C). Recombinant PDGFR receptor, preincubated with sclerodermal IgG, inhibited its ROS-inducing activity completely and in a dose-dependent manner (Fig. 1D).

To purify these antibodies, we immortalized the lymphocytes derived from two randomly selected patients with scleroderma and screened isolated clones for the presence of ROS-inducing antibodies in the cell system shown in Figure 1A. Figure 2A shows the results of fluorescence-activated cell-sorting analysis of two of these antibodies purified from lymphocytes from the same patient. These antibodies reacted with Fα cells but not with F−/− cells (Fig. 2A). They also stimulated ROS production in Fα cells but not in

Figure 2. Isolation of Stimulatory Antibodies Derived from Patients with Scleroderma.
As shown in Panel A, Fα and F−/− cells were incubated with medium (0.1 ml) obtained from clones 4G11 and 1F5 before fluorescence-activated cell-sorting analysis. These clones were obtained by immortalizing lymphocytes from patients with scleroderma with Epstein–Barr virus. Shaded profiles are those of the isotype-negative control antibody. The results are representative of seven experiments with clones from two patients with scleroderma. The concentration of IgM was approximately 1 to 2 μg per milliliter. Panel B shows the levels of ROS (expressed as the stimulation index) in Fα and F−/− cells incubated with 1 ml of medium obtained from clones 4G11 and 1F5. Results are the means of the results of three experiments. T bars represent the SE. The concentration of IgM was approximately 1 to 2 μg per milliliter. As shown in Panel C, Fα cells were starved for 48 hours in 0.2 percent fetal-calf serum, trypsinized, and incubated with PDGF (green) and without PDGF (black) (at 15 ng per milliliter for 30 minutes at 4°C) before incubation with 100 μl of medium from clone 5B2, which reacts with F−/− cells and does not induce ROS, and from ROS-inducing clones (4G11 and 1F5). Shaded profiles are those indicated in Panel A. The concentration of IgM was approximately 1 to 2 μg per milliliter.
F−/− cells (Fig. 2B). PDGF could competitively inhibit the binding to Fα cells (Fig. 2C).

With respect to the relation between the ROS-inducing activity of sclerodermal IgG and the patients’ clinical features, we found that activity (measured as ROS induction) was higher in patients with early, limited scleroderma (duration, less than 5 years) than in patients with late disease (duration, more than 10 years) (P<0.01). In the subgroup of patients with scleroderma whose disease was diffuse, there was a trend toward higher values among patients with early disease (less than three years) than among patients with disease of more than six years’ duration. The difference, however, was not statistically significant, possibly because of the small number of patients (four) with early diffuse disease. No correlation was found with other clinical and serologic features. Recently, we found similar agonistic auto-antibodies in 10 recipients of allogeneic bone marrow transplants who presented with graft-versus-host disease and had scleroderma-like skin lesions (unpublished data).

**Triggering of the Ha-Ras-ERK1/2-ROS Cascade in Normal Fibroblasts**

To dissect the signaling cascade triggered by sclerodermal IgG, we analyzed the ROS-generating activity of IgG from three patients with scleroderma as compared with three normal controls in the presence of specific inhibitors: inhibitors of EGFR and PDGFR signaling (AG 1478 and AG 1296, respectively); FTI-277, an inhibitor of farnesyl transferase, an enzyme required for Ras attachment to the plasma membrane; and a MEK inhibitor, PD 98059, which is a kinase located upstream from EGFR and PDGFR (not ERK), Ras, and MEK prevented ROS induction by sclerodermal IgG in normal fibroblasts (P<0.001 for the comparison with scleroderma, one patient with systemic lupus erythematosus, and two normal controls at the same protein concentration (200 μg per milliliter). Figure 3C shows the immunoblot with commercial anti-PDGFRα, anti-PDGFRβ, or anti-EGFR antibodies. Sclerodermal IgG efficiently immunoprecipitated the PDGFR α and β subunits but not EGFR (Fig. 3C). We noticed that IgG isolated from the serum of 10 tested patients with scleroderma did not recognize recombinant α and β subunits of PDGFR on Western blotting, indicating that these antibodies recognize conformations present only in the native receptor (data not shown).

To delineate the stimulatory nature of the antibody–receptor interaction, we challenged normal fibroblasts for 15 minutes with increasing concentrations of IgG derived from a patient with scleroderma and evaluated the tyrosine phosphorylation of the PDGFR. Sclerodermal IgG induced tyrosine phosphorylation of the PDGFR in a dose-dependent manner (Fig. 3D). The time course of tyrosine phosphorylation was longer than that induced by PDGF (data not shown).

**Biologic Consequences of Anti-PDGFR Agonistic Antibodies from Patients with Scleroderma**

To determine the biologic effects induced by sclerodermal IgG, we assayed the expression of two genes that characterize scleroderma fibroblasts — the genes encoding α–smooth-muscle actin and type I collagen — in normal human fibroblasts exposed to sclerodermal IgG. α–Smooth-muscle actin is a distinctive marker of myofibroblasts, which are mesenchymal cells that are derived from fibroblasts and that possess characteristics of both smooth-muscle cells and fibroblasts. α–Smooth-muscle actin was induced by sclerodermal IgG in normal human fibroblasts but not by normal IgG (P<0.001), according to immunohistochemical assays and immunoblotting (Fig. 4A). Messenger ribonucleic acid encoding the type I collagen α1 and α2 chains was robustly induced by sclerodermal IgG and not by normal IgG, as shown by Northern blot analysis and real-time quantitative polymerase-chain-reaction analysis (Fig. 4B). In addition, the PDGFR tyrosine kinase inhibitor AG 1296 down-regulated expression of the type I collagen gene. To demonstrate that a single antibody was able to induce expression of the genes encoding collagen and α–smooth-
muscle actin, we tested purified clonal antibodies. These antibodies induced the expression of these genes with higher specific activity than did total sclerodermal IgG (1 to 2 μg of protein per milliliter for 200 μg per milliliter for 15 minutes) and preincubated with selective inhibitors of epidermal growth factor receptor (EGFR) and PDGFR (AG 1478 and AG 1296, respectively; 2 μM for two hours) and with FTI-277 (20 μM for two hours) and PD 98059 (40 μM for two hours). FCS denotes fetal-calf serum. Panel B shows fluorescence microscopical images and immunoprecipitation immunoblots of Ha-Ras protein in normal human fibroblasts incubated with normal (N) and sclerodermal (SSc) IgG (200 μg per milliliter for 15 minutes) in the presence and absence of selective inhibitors (AG 1478 and AG 1296; 2 μM for two hours). The slight inhibition of the Ha-Ras band by AG 1478 was dependent on the high concentration used in this specific experiment (50 percent inhibitory concentration of EGFR, 3 nM). Representative results from one of five experiments are shown. NIS denotes nonimmune serum, and pERK1/2 phosphorylated ERK1/2. As shown in Panel C, cell lysates were immunoprecipitated with IgG purified from two normal controls (N), two patients with systemic sclerosis (SSc), and one patient with systemic lupus erythematosus (SLE) and developed with specific antibodies against PDGFR and EGFR (with the use of an immunoblot technique). The first two lanes on the left are immunoblots of total proteins extracted from sclerodermal or normal fibroblasts and probed with the antibodies against PDGFR and EGFR. The representative results of one of three experiments are shown. As shown in Panel D, normal human fibroblasts were stimulated with sclerodermal (SSc) IgG (100, 200, and 300 μg per milliliter for 15 minutes) or PDGF (15 ng per milliliter for 15 minutes) or grown in 0.2 percent fetal-calf serum. Cell lysates were immunoprecipitated with a polyclonal antibody against PDGFR (subunit β), and the immunoblots were developed with a specific antibody against phosphorylated tyrosine (pTyr).

**Figure 3. Stimulation of Ha-Ras-ERK1/2-ROS Signaling by Sclerodermal Antibodies to the α and β Subunits of PDGFR in Normal Fibroblasts.**

Panel A shows the levels of ROS, evaluated as the mean (±SE) 2′,7′-dichlorofluorescein diacetate (DCF) fluorescence intensity, in normal human fibroblasts incubated with IgG from three normal controls (N) and IgG from three patients with systemic sclerosis (SSc) (at 200 μg per milliliter for 15 minutes) and preincubated with selective inhibitors of epidermal growth factor receptor (EGFR) and PDGFR (AG 1478 and AG 1296, respectively; 2 μM for two hours) and with FTI-277 (20 μM for two hours) and PD 98059 (40 μM for two hours). FCS denotes fetal-calf serum. Panel B shows fluorescence microscopical images and immunoprecipitation immunoblots of Ha-Ras protein in normal human fibroblasts incubated with normal (N) and sclerodermal (SSc) IgG (200 μg per milliliter for 15 minutes) in the presence and absence of selective inhibitors (AG 1478 and AG 1296; 2 μM for two hours). The slight inhibition of the Ha-Ras band by AG 1478 was dependent on the high concentration used in this specific experiment (50 percent inhibitory concentration of EGFR, 3 nM). Representative results from one of five experiments are shown. NIS denotes nonimmune serum, and pERK1/2 phosphorylated ERK1/2. As shown in Panel C, cell lysates were immunoprecipitated with IgG purified from two normal controls (N), two patients with systemic sclerosis (SSc), and one patient with systemic lupus erythematosus (SLE) and developed with specific antibodies against PDGFR and EGFR (with the use of an immunoblot technique). The first two lanes on the left are immunoblots of total proteins extracted from sclerodermal or normal fibroblasts and probed with the antibodies against PDGFR and EGFR. The representative results of one of three experiments are shown. As shown in Panel D, normal human fibroblasts were stimulated with sclerodermal (SSc) IgG (100, 200, and 300 μg per milliliter for 15 minutes) or PDGF (15 ng per milliliter for 15 minutes) or grown in 0.2 percent fetal-calf serum. Cell lysates were immunoprecipitated with a polyclonal antibody against PDGFR (subunit β), and the immunoblots were developed with a specific antibody against phosphorylated tyrosine (pTyr).

**DISCUSSION**

Five independent experiments in the current study document the presence of PDGFR stimulatory antibodies in the serum of patients with scleroderma. First, purified immunoglobulin fractions from patients with scleroderma induced ROS levels in cells
Figure 4. Induction of the Expression of Type I Collagen and α–Smooth-Muscle Actin by Autoantibodies against PDGFR.

Panel A (top) shows α–smooth-muscle actin (α-SMA) induction in normal human fibroblasts stimulated with PDGF (15 ng per milliliter for 30 minutes), normal (N) human IgG (200 μg per milliliter for 30 minutes), and scleroderma (SSc) IgG (200 μg per milliliter for 30 minutes) in the presence and absence of AG 1296 (2 μM for 1 hour before treatment). The experiment shown represents a typical blot obtained in three independent experiments. The results of densitometric analysis of three independent experiments are also shown in Panel A (bottom). The data shown represent the means (±SE) of the results of three independent experiments. FCS denotes fetal-calf serum. Panel B (top) shows type I collagen–gene expression by normal human fibroblasts after incubation with 0.2 percent fetal-calf serum (for 48 hours), PDGF (15 ng per milliliter for 15 minutes), normal (N) human IgG (200 μg per milliliter for 15 minutes), and scleroderma (SSc) IgG (200 μg per milliliter for 15 minutes) in the presence and absence of AG 1296 (2 μM for 1 hour before treatment). Northern blot analysis was used to detect messenger RNA encoding the α1 and α2 chains of type I collagen. Representative results from one of three experiments are shown. The results of real-time quantitative polymerase-chain-reaction analysis of the transcripts of genes encoding the α1 and α2 chains of type I collagen are also shown in Panel B (bottom).
expressing PDGFR but not in cells from which PDGFR was absent. Second, immunoglobulin fractions from patients with scleroderma recognized and immunoprecipitated PDGFR (α and β chains) in its native configuration. Third, PDGFR binding and the ROS-generating activities of sclerodermal IgG were removed by preadsorption to recombinant PDGFR or to PDGFR-expressing cells and not to cells without PDGF. Similarly, PDGF competitively inhibited the binding of purified antibodies to PDGFR-expressing cells. Fourth, immunoglobulin fractions or purified antibodies derived from patients with scleroderma induced myofibroblast conversion, type I collagen expression, and ROS production in normal fibroblasts. Finally, we detected these antibodies in all 46 tested patients with scleroderma and in none of the controls. The ROS-inducing activity of the sclerodermal immunoglobulins was inhibited by PDGFR inhibitors. These antibodies were not detected in patients with primary Raynaud’s phenomenon, systemic lupus erythematosus, rheumatoid arthritis, or interstitial lung disease. The current in vitro data indicate that these antibodies recognize and activate PDGFR as a primary target. The data also might be taken to imply that fibrosis in scleroderma is triggered by the accumulation of these activating autoantibodies in the blood.

In dissecting the cascade initiated by PDGF in vitro, we recently observed that growth factors and ROS regulate Ras proteins in a seemingly novel manner. In normal primary cells, Ras proteins are maintained at low levels by continuous proteasomal degradation. In that setting, PDGF transiently induces ROS through ERK1/2 and ultimately prevents Ras degradation by proteasomes. Sclerodermal IgG activates PDGFR but replicates the action of PDGF only in part. We monitored the response of sclerodermal cells in culture under serum-deprived conditions. The cells maintained high ROS production, but after a day or two, the rate of ROS production slowly decreased. We hypothesize that antibodies to PDGFR remain in the membrane for a longer time than PDGF and generate a more persistent stimulus. Figure 5 is a schematic diagram of the pathway that we consider to be activated by antibodies by way of PDGFR.

We also analyzed the short-term and long-term biologic effects of sclerodermal IgG in normal cells — namely, ROS production and collagen-gene expression, respectively. The phenotypes we describe here closely replicate the features of sclerodermal fibroblasts. Activation of the transcription of collagen genes and the gene encoding α-smooth-muscle actin results in the appearance of myofibroblasts and fibrosis. We previously noted that fibroblasts derived from patients with scleroderma undergo rapid senescence and accumulate DNA and chromosomal aberrations.
— a process that may explain the loss of cells in chronic lesions. In addition, these data indicate that early, limited scleroderma (duration, less than 5 years) can be distinguished from late disease (duration, more than 10 years) on the basis of the ROS-inducing activity of antibodies. It remains to be seen whether ROS-inducing activity can be detected before the appearance of clinical symptoms.

In conclusion, we have identified agonistic antibodies against PDGFR in patients with scleroderma. These antibodies appear to trigger an intracellular loop that involves Ha-Ras, ERK1/2, and ROS and that leads to increased collagen-gene expression. The purification of anti-PDGFR ROS-stimulating antibodies and the results of tests of their biologic activity as purified clones strongly argue that these antibodies have a causal role in the pathogenesis of scleroderma. However, direct proof awaits in vivo studies and validation in larger numbers of patients from other populations.

Supported in part by Società Italiana di Reumatologia, Associazione Italiana per la Lotta alla Sclerodermia, Ministero Italiano per l’Università e la Ricerca Scientifica (2003), Associazione Italiana Ricerca sul Cancro, and the Naples Oncogenomic Center, Fondazione Italiana Ricerca Cancro, Biotecnologie Avanzate, and by a grant (EV012509, to Dr. Kazlauskas) from the National Institutes of Health.

No potential conflict of interest relevant to this article was reported.

This article is dedicated to the memory of Dr. E. Carville LeRoy and Stelio Varrone.

We are indebted to Dr. Marcello Melone (Dipartimento di Neuroscienze–Istituto di Fisiologia, Università Politecnica delle Marche, Ancona, Italy) for the help with confocal microscopy; to Prof. Pier Paolo Roggero (Dipartimento di Scienze Ambientali e delle Produzioni Vegetali–Università Politecnica delle Marche, Ancona) for help with statistical analysis; and to Drs. Stefano Gasparini and Piergiorgio Zitti (Dipartimento di Medicina Interna, Pneumologia ed Immunologia Clinica–Azienda Ospedali Riuniti, Ancona) for allowing us to study their patients with idiopathic pulmonary fibrosis.

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