Scleroderma fibroblasts constitutively express the long pentraxin PTX3

M.M. Luchetti, P. Sambo, P. Majlingová, S. Svegliati Baroni, G. Peri¹, P. Paroncini, M. Introna¹, A. Stoppacciaro², A. Mantovani³, A. Gabrielli

ABSTRACT

Objective. PTX3 is a secreted molecule which consists of a C-terminal domain similar to classical pentraxins (e.g. C-reactive protein) and of an unrelated N-terminal domain. Unlike the classical pentraxins, PTX3 is expressed in response to IL-1β and TNF-α but not to IL-6. The present study was designed to investigate the expression of PTX3 in normal and scleroderma fibroblasts.

Methods. Normal and SSc fibroblasts were cultured in the presence and absence of inflammatory cytokines. PTX3 mRNA expression in fibroblasts was evaluated by Northern analysis. PTX3 protein levels in fibroblast culture medium were estimated by ELISA.

Results. Normal fibroblasts were induced to express high levels of PTX3 mRNA by IL-1β and TNF-α but not by other cytokines or growth factors. Scleroderma fibroblasts, unlike normal fibroblasts, constitutively expressed high levels of PTX3 in the absence of deliberate stimulation. The constitutive expression of PTX3 in SSc fibroblasts was not modified by anti-TNF-α antibodies or IL-1 receptor antagonist. In contrast, IFN-γ and TGF-β inhibited the constitutive but not the stimulated expression of PTX3 in SSc fibroblasts.

Conclusions. PTX3 is a main feature of activated scleroderma fibroblasts.

Introduction

Systemic Sclerosis (SSc, scleroderma) is an autoimmune connective tissue disease characterized by fibrosis of the skin and/or internal organs (1). The mechanisms involved in the development of fibrosis in scleroderma are still obscure, but it is well established that stromal fibroblasts play a pivotal role in the development of the disease, over-producing in vitro extracellular matrix (ECM) proteins, mainly type I collagen, through an augmented transcription rate of the corresponding genes (2-4). The role of fibroblast proliferation in the pathogenesis of SSc is less well defined but some reports have suggested that fibroblasts from scleroderma patients exhibit abnormal cell growth, as manifested by increased proliferation under serum-free culture conditions (5). Furthermore, the c-myc proto-oncogene, an essential element of the response to growth factors in normal cells, is higher in SSc fibroblasts when compared to controls (5), suggesting an abnormal proliferative pathway in scleroderma fibroblasts. Lastly, Piccinini et al. demonstrated that the proto-oncogene c-myb, normally involved in the proliferation of hematopoietic cells, is also over-expressed in resting scleroderma fibroblasts and that c-myb antisense oligonucleotides down-regulate fibroblast proliferation (6).

Thus, fibroblasts from scleroderma patients are activated in vitro but the exact mechanism of this sustained activation is unknown, although recently it has been ascribed, at least partly, to an excessive generation of reactive oxygen species (7-11 and manuscript submitted). However, the relationship of fibroblast activation to the autoimmune features of scleroderma are still ill defined.

PTX3 is a member of the pentraxin family and it is the first long pentraxin identified. The gene was cloned from IL-1β (12) and TNF-α (13) treated human umbilical vein endothelial cells. It encodes a 381 amino acid, a 42 kDa secretory protein with a COOH-terminal domain showing up to 28% sequence identity to the short pentraxins human C-reactive protein (CRP) and serum amyloid P-component (SAP) (12, 13), including the presence of the pentraxin signature and two cysteine

Istituto di Clinica Medica Generale, Ematologia ed Immunologia Clinica, Università Politecnica delle Marche, Ancona; ¹Istituto di Ricerche Farmacologiche “Mario Negri”, Milano; ²Servizio di Anatomia Patologica, Ospedale S. Andrea, Università “La Sapienza”, Roma, Italy.

Address correspondence and reprint requests to: Armando Gabrielli, MD, Istituto di Clinica Medica Generale, Ematologia ed Immunologia Clinica, Polo Didattico, Via Tronto no. 10, 60020 Ancona, Italy.

© Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2004.

Key words: Pentraxin, scleroderma, autoimmunity, fibroblast, inflammation, cytokine.
residues found in all the pentraxins cloned so far (14). The inducibility by two important inflammatory cytokines, such as IL-1β and TNF-α, makes attractive the hypothesis that PTX3 may be an important molecule in the inflammation processes. So far it has been demonstrated that it participates in the acute phase response that begins in the aftermath of injury, trauma, and infections (15), appearing in the serum of mice and humans after LPS injection and, unlike CRP and SAP, the major site of synthesis is extrahepatic (14, 16).

In view of its role in inflammation, PTX3 has been investigated in autoimmune diseases included rheumatoid arthritis (17), systemic lupus erythematosus and small vessel vasculitis (18). In small vessel vasculitis, PTX3 serum levels correlated with disease activity suggesting that it could reflect inflammatory involvement of the vascular tree. Since sclerodema is characterized by widespread microvascular injury, fibrosis and autoimmune features, we elected to investigate the constitutive and inducible expression of PTX3 in fibroblasts and monocytes obtained from SSc patients.

Materials and methods

Reagents

Collagenase, Ficoll-Hypaque, Actinomycin D, Cycloheximide and the E-Toxate kit were obtained from Sigma Chemical Co. (St. Louis, MO). Percoll was purchased from Pharmacia (Uppsala, Sweden). RPMI 1640 and L-glutamine were from BioWhittaker, (Walkersville, MD), whereas penicillin-streptomycin and FCS were obtained from Gibco (Milan, Italy). Mycoplasma-detection kits were purchased from Boheringer Mannheim (Milan, Italy). IL-1β and TNF-α cDNA probes were a kind gift of Dr. A. Rodriguez, Hospital de La Princesa, Madrid, Spain. IFN-γ, TGF-β, IL-4, IL-1β, TNF-α, FGF, and IL-6 were purchased from Genfyme Diagnostic (Cambridge, MA). IL-1 receptor antagonist (IRA) and anti-TNF-α monoclonal antibody (B154.2 mAb) were a kind gift of Dr. G. Trinchieri (Wistar Institute, Philadelphia, PA).

Cells

After obtaining their informed consent, human skin fibroblasts were grown from punch biopsies taken from the forearms of normal volunteers and from the involved skin of patients who fulfilled the preliminary criteria of the American Rheumatism Association for the diagnosis of systemic sclerosis (19). Primary explant cultures were established in 25 cm² culture flasks in MEM containing 10% FCS, 2 mM glutamine, penicillin (100 U/ml) and streptomycin (100 µg/ml). MEM with these supplements is hereafter referred to as “culture medium”. The culture medium was tested periodically with specific commercial kits for mycoplasma contamination and for the presence of bacterial endotoxins. Monolayer cultures were maintained at 37°C in 5% CO₂.

Monocytes from peripheral blood of SSc patients were purified as previously described (10). Briefly, mononuclear cells were collected by centrifugation at 400 g for 20 min at room temperature (RT) on a gradient of Ficoll-Hypaque, and mononuclear cells collected at the interface were separated into lymphocytes and monocytes by 47% Percoll gradient centrifugation at 600 g for 30 min at 4°C. The cells were immediately collected and washed with ice-cold PBS, and the pellet was harvested with guanidine-isothiocyanate for the RNA extraction.

Experimental fibroblast cultures

Fibroblasts at the fourth subpassage were used for all experiments unless otherwise specified. Briefly, fibroblasts were harvested from confluent cultures with trypsin and plated in culture medium at the concentration reported below. After attachment for 48 hr, the culture medium was discarded and changed to MEM containing 0.2% FCS. After a further 48 hr period of incubation, the cells were harvested with guanidine isothiocyanate or incubated with the appropriate cytokines for 6 hr. Cytokines and growth factors were used at the following final concentrations: IL-1β: 20 ng/ml, TNF-α: 20 ng/ml, PDGF-BB: 20 ng/ml, IFN-γ: 100 U/ml, TGF-β: 1 ng/ml, IL-6: 50 U/ml, IL-4: 280 U/ml, and FGF-β: 50 U/ml. Actinomycin D and cycloheximide were used at a concentration of 2 mg/ml and 10 µg/ml, respectively. IL-1 receptor antagonist (IL-1Ra) at a final concentration of 300 ng/ml and anti-TNF-α MoAb at a 1:2000 final dilution.

Probes

The probe used for the detection of the PTX3 mRNA was an insert of 1300 bp containing the entire coding region of the human PTX3 gene, cloned into a BamHI restriction site of a BlueBac plasmid. The probes used for the detection of the IL-1β and TNF-α mRNAs were an insert of 350 bp and an insert of 600 bp, respectively, cloned in a PTZ-19 plasmid and containing the 5’-coding regions of the genes. For Northern blot analysis the inserts were labeled with ³²P-dCTP by the random priming standard procedures to specific activities of 5 x 10⁶ cpm/mg DNA.

RNA isolation and analysis

Total cellular RNA was extracted according to the guanidine isothiocyanate-cesium chloride method. Fifteen micrograms of total RNA were loaded in each lane of a 1% agarose formaldehyde gel and transferred to GeneScreen membranes by standard blotting procedures and blots were then baked at 80°C for 2 hr. Prehybridizations were carried out for 7 h at 60°C in 1M NaCl, 1% SDS, 10% Dextran Sulfate and 100 mg/ml denatured salmon sperm DNA. Hybridizations were performed overnight at 60°C in the same buffer by adding 1.3 x 10⁶ cpm/ml of the labeled probe. Thereafter, the filters were washed twice with 2X SSC/1% SDS at 60°C for 10 min, twice with 0.5X SSC/0.5% SDS for 15 min, and finally twice with a large volume of 0.5X SSC at room temperature. The blots were dried and exposed to Kodak X-Omat X-ray film with two intensifying screens.

RT-polymerase chain reaction (PCR) for PTX3 expression analysis

Two micrograms of total RNA, extracted as above described, were directly reverse-transcribed in 5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl pH 8.3,
M-MLV reverse transcriptase. Samples were incubated for 10 min at room temperature and then for 45 min at 42°C. 2 µl of the reverse transcription reactions were amplified by polymerase chain reaction PCR using the following primers specific for PTX3 (P-forward: GAAGATTTTTGGAAGCTGC, P-backward: CCACTCTCAACAGTGTTAGCC) or β-actin cDNA as control gene (a-forward: CCTTCCAAGCTTGATCTTC, a-backward: GAGGCAATGATCTCGTTC) and the following reaction conditions: 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, 0.2 mM each dNTP, 2.5 U/ml Taq DNA polymerase and 5 ng/ml of each primer. PCR conditions were set to avoid the reaching of the plateau state, performing several PCRs at a different number of cycles (data not shown). The better PCR conditions for PTX3 were: 94°C, 5 min, 1 cycle; thereafter 15 cycles at 94°C, 1 min; 58.5°C, 1 min; 72°C, 1 min. An aliquot of the PCR products (spanning 300 bp) was run in a 1.5% ultra-pure agarose gel (BRL) and transferred to nylon membranes by standard Southern blotting procedures. Pre-hybridizations, hybridizations, and the exposure of the membranes were carried out as described above.

**PTX3 protein determination by ELISA**

PTX3 was measured using a sandwich ELISA (17, 18). In brief, 96-well ELISA plates (Nunc, Roskilde, Denmark) were coated with 70 ng of rat mAb MNB4 anti-PTX3 in 100 µl of coating buffer and incubated overnight at 4°C. After incubation, the plates were extensively washed with Dulbecco’s phosphate buffered saline (pH 7.2) containing 0.05% Tween 20 (washing buffer), and 300 µl of 5% dry milk in washing buffer were then added to block the non-specific binding sites. After 2 h incubation at room temperature, the plates were again washed three times with washing buffer, and 50 µl of either purified human recombinant PTX3 standards (100 pg/ml to 2 ng/ml, diluted in RPMI 1640/2% BSA), cell supernatants, or biologic fluids were added in triplicate to each well and incubated for 2 h at 37°C. The plates were washed three times with washing buffer, and 100 µl of biotin conjugated anti-PTX3 rabbit polyclonal diluted 1:2000 in washing buffer was added. The plates were incubated for 1 h at 37°C, and then washed three times with 200 µl of washing buffer. One hundred microliters per well of peroxidase-streptavidin conjugated to dextran back-bone (AMDEX, Copenhagen, Denmark) diluted 1:4000 was subsequently added and the plates were incubated for 1 h at room temperature. After incubation, the plates were washed four times before the addition of 100 µl of the chromogen substrate ABTS. Plates were read at 405 nm in an automatic ELISA reader. The ELISA assay did not cross-react with CRP.

**Immunohistochemical staining**

Five micrometer thick paraffin-embedded synovial tissue sections were rehydrated and pre-treated in a 750 W microwave oven in 0.01 M sodium citrate buffer pH 7.3 for 5 min and allowed to cool for 20 min. Endogenous peroxidase activity was blocked in 0.3% alcoholic hydrogen peroxide for 30 min. Sections were treated with 3% normal rabbit serum for 30 min at RT and then incubated overnight at 4°C with mouse mAb 1C8 anti-PTX3 diluted 1:20 in PBS. After washing with PBS, the sections were incubated with an appropriate biotinilated rabbit anti mouse Ig antibody for 30 min at RT and then with peroxidase-avidin-biotin complex (Vectastain Elite kit, Vector Lab, Burlingame, CA, USA) for 30 min at RT. 0.05% 3’-diaminobenzidine (Sigma) in 0.05 M Tris-HCl (pH 7.6) containing 0.01% H₂O₂ was used as the chromogen and sections counterstained using Mayer haematoxylin. Appropriate negative controls were performed omitting the primary antiserum and replacing it with normal rabbit serum or with supernatant fluid from CHO 2.1 cell cultures.

**Statistical analysis**

Data are expressed as a mean ± 1 SD, and were compared by Mann-Whitney-U-test. A p value of less than 0.05 was considered statistically significant.

---

**Fig. 1. Panel A.** Northern blot analysis showing PTX3 expression in fibroblasts from 6 scleroderma (SSc) patients and 6 normal controls. Fibroblasts were made quiescent by serum-deprivation for 48 h before total RNAs was prepared and analysed by Northern blot analysis as described in the Materials and Methods. Expression of GAPDH is shown for normalization of the quantities of RNA loaded in gel lanes. Panel B. PTX3 protein levels in supernatants from normal and scleroderma fibroblasts. Supernatants were collected after 48 h of quiescence in serum-deprived culture medium and PTX3 levels were determined by ELISA. Panel C. PTX3 expression by RT PCR in monocytes from 6 scleroderma (SSc) patients and 6 normal controls. Representative results from 1 of 3 experiments are shown. Monocytes were purified as described in the Materials and Methods. Expression of GAPDH is shown for normalization of the quantities of RNA loaded in the gel lanes.
Results

**Scleroderma (SSc) fibroblasts and monocytes constitutively express PTX3 mRNA**

Scleroderma skin fibroblasts cultured in serum-deprived conditions for 48 h display an activated phenotype, over-expressing collagen genes (1-3), c-myb and c-myc proto-oncogenes (5, 6), and spontaneously releasing free radical species (11). We used these experimental conditions to evaluate PTX3 mRNA expression. Thus, normal and SSc skin fibroblasts were kept in 0.2% FCS for 48 h before northern analysis for PTX3. Figure 1A shows that SSc but not normal fibroblasts constitutively express PTX3 mRNA. As shown in Figure 1B SSc fibroblasts release higher levels of PTX3 in vitro than normal fibroblasts in the absence of deliberate stimulation (13.63 ng/ml and 1.06 ng/ml, respectively; p<0.001).

In order to investigate whether PTX3 over-expression was restricted to scleroderma fibroblasts or was a feature of other cell types, using a RT-PCR approach due to the low number of cells obtained by the venous blood of SSc patients, PTX3 was evaluated in the peripheral blood monocytes as well. Figure 1C shows that scleroderma monocytes but not monocytes from normal controls constitutively express the PTX3 gene.

The mean serum level of PTX3 determined in sera from 30 scleroderma patients was not significantly different from the value obtained in sera from normal controls (data not shown).

**Cytokine modulation of PTX3 gene expression in normal and SSc fibroblasts**

To elucidate which cytokines could activate and modulate PTX3 expression in fibroblast cells *in vitro*, normal skin fibroblast were stimulated with recombinant human IL-1β, TNF-α and IL-6, which have been shown to induce PTX3 gene in endothelial cells, and TGF-β, PDGF, IFN-γ, IL-4, and FGF, which have been found to be involved in scleroderma fibroblast activation. As previously demonstrated (14, 16), IL-1β and TNF-α, but not IL-6, were able to induce PTX3 gene expression in normal dermal fibroblasts (Fig. 2A).

These cytokines were also able to increase PTX3 gene expression in SSc fibroblasts (Fig. 2A) that already displayed a constitutive over-expression (Fig. 1A). Actinomycin D, but not cycloheximide, inhibited IL-1β and TNF-α-induced stimulation of PTX3 expression in normal dermal fibroblasts, suggesting a dependency on gene transcription (data not shown). PDGF and FGF did not exert any effect on PTX3 basal expression in normal and SSc fibroblasts (Fig.2A). Interestingly, TGF-β and IFN-γ consistently down-regulated the constitutive PTX3 expression in SSc fibroblasts, acting at the lowest active concentration (0.1 ng/ml and 50 U/ml, respectively), and a similar result was obtained after the addition of IL-4 (Fig. 3A). In contrast, neither TGF-β nor IFN-γ were able to inhibit the PTX3 mRNA induced by IL-1β or TNF-α in normal and SSc fibroblasts (Fig. 2B). In all the experiments, the PTX3 protein concentration determined by the ELISA in the fibroblast supernatants was comparable to its mRNA expression (data not shown).

**PTX3 expression in SSc skin fibroblasts is not maintained by an IL-1β and or TNF-α autocrine pathway**

Since previous studies have reported that an autocrine IL-1β pathway could be responsible for the activation of the SSc fibroblasts *in vitro* (20), in order to rule out that the secretion of very small and undetectable amounts of IL-1β and TNF-α by cultured fibroblasts could stimulate PTX3 gene expression, cells were incubated either with the IL-1 receptor antagonist (IL-1ra), or with a mAb against TNF-α. As shown in Figure 3A, neither IL-1ra nor anti-TNF-α antibody affected the basal PTX3 over-expression, although under the same experimental conditions anti-TNF-α mAb and IL-1ra completely blocked the induction of monocyte chemotactic protein-1 (MCP-1) (data not shown).

To further confirm that this observed PTX3 activation is not consequent to an autocrine cytokine pathway, the same blots which showed the PTX3 message...
were hybridized with IL-1β and TNF-α cDNA probes. Neither SSc nor normal skin fibroblasts expressed an apprecia-
table amount of IL-1β or TNF-α mRNA (Fig. 3B).
Therefore, we can conclude that PTX3 constitutive expression in SSc fibro-
blasts is not maintained by an autocrine production of IL-1β and TNF-α, that previously were been able to induce its
expression.

**Immunohistochemical findings**

Skin biopsies from 5 scleroderma pa-
tients and 5 normal controls were stu-
died by immunohistochemical tech-
niques. In SSc specimens intense stain-
ing was present in dermal fibroblasts
and in vessel walls (Fig.4). Some stain-
ing was also present in the extracellular
matrix as well.

**Discussion**

Pentraxin 3 was the first long pentraxin
to be identified. Long pentraxins con-
sist of a C-terminal domain, which has a sequence similar to that found in C-
reactive protein (CRP) and serum amy-
loid P (SAP) (the short pentraxins),
coupled to an unrelated non-pentraxin
N-terminal portion (12-14). The long
pentraxins already identified include
guinea pig apixin (21), XL-PXN1 from
Xenophus (22), rat neuronal pentraxin
(NP) (23), human neuronal pentraxin
(NPTX2) (24), and Narp (25), which
possibly represents the rat homologue
of apixin.

PTX3 is constitutively expressed at
low levels in endothelial cells and can
be induced by LPS, TNF-α and IL-1β
in several cell types (14, 26-28). These
include endothelial cells, fibroblasts,
mononuclear phagocytes and dendritic
cells. The inducibility of the PTX3
gene by inflammatory signals has
prompted many investigators to hypo-
thesize a link between PTX3 and the
immune system and evidence suggests
that PTX3 plays a role in the regulation
of innate resistance to pathogens, in-
flammatory reactions and the clearance
of self-components (see 15 for a re-
view).

For these reasons it has seemed plausi-
ble to investigate PTX3 regulation, ex-
pression and production in autoimmune
diseases and elevated levels were
found in the sera of patients with small
vessel vasculitis (18) and in the syno-
vial fluid of patients with rheumatoid
arthritis (17).

In the present study, we show that skin
fibroblasts from scleroderma patients,
although quiescent by incubation under
serum-deprived conditions and in the
absence of deliberate stimulation,
strongly express the PTX3 gene in vitro
and release an increased amount of the
protein. Conversely, serum concentra-
tions of the pentraxin did not differ
from normal controls and this finding
is reminiscent of what has been de-
scribed in rheumatoid arthritis and could be ascribed, at least in part, to the
expression of PTX3 in affected tissues
only. In fact the immunohistochemical
staining of SSc skin samples confirmed
the in vitro studies and showed the pre-

cence of the protein in the extracellular
matrix as well.

At variance with data obtained in mo-
nonuclear cell lines (26) and in syno-
vial fibroblasts (17), both TNF-α and
IL-1β were able to induce the gene in
normal skin fibroblasts and, in agree-
ment with previous findings (17,26),
the induction is probably mediated at
the level of transcription, since actino-
mycin D but not cycloheximide abro-
gated it. In scleroderma fibroblasts a
slight further increase in PTX3 expres-
sion could be observed after TNF-α
and IL-1β addition, suggesting that –

albeit activated – they were still capa-
bles of responding to exogenous stimuli.
It is not clear whether IL-β and TNF-α
are the cytokines responsible for PTX3
induction in SSc fibroblasts but, inter-

gestingly, PTX3 expression in SSc fi-
броblasts was still detectable after se-
veral subpassages in vitro and since IL-
1Ra and anti-TNF-α antibodies did not

---

**Fig. 3. Panel A.** Northern blot analysis
showing PTX3 expression in fibroblasts
from one scleroderma (SSc) patient and
one normal control. Fibroblasts were
made quiescent by serum deprivation for
48 h before the addition of IL-1 receptor
antagonist (IRA) at a final concentration
of 300 ng/ml and anti-TNF-α MoAb at a
1:2000 final dilution for 24 h. **Panel B.**
mRNAs of 4 scleroderma patients (SSc)
and 4 normal controls (norm) were run
on agarose gel, blotted and hybridized
with IL-1β or TNF-α cDNA probes. Monocytes
(M) and fibroblasts (F) from normal subjects stimulated with TNF-α
were used as the positive control.

---

**Table 3**

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Norm</th>
<th>SSc</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTX3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>
abrogate PTX3 over-expression, the presence of minimal amounts of IL-β
and TNF-α proteins in the culture medium and a positive autocrine loop
that maintains PTX3 can be ruled out. Thus, PTX3 expression is to be consid-
ered a further feature of scleroderma fi-
broblast activation as collagen genes,
c-myb and c-myc proto-oncogenes, and
substances such as reactive oxygen
species (2-6,11), suggesting that PTX3
could be involved in the metabolic and/
or activated pathways typical of the
scleroderma phenotype and probably to
be traced back to the same, as yet
unclear, mechanisms.
As with rheumatoid arthritis synoviocytes,
IFN-γ, TGF-β and IL-4 were able to
down-regulate the constitutive,
but not stimulated, expression of PTX3
implying that different mechanisms
may be operating in its expression.
PTX3 function has not been complete-
ly defined. It has been demonstrated
that the ability of PTX3 to recognize
several ligands differs considerably
from that of the classical pentraxins
CRP and SAP, and that PTX3 selective-
ly binds C1q (29) and added to human
serum causes consumption of the total
complement hemolytic activity (15).
Moreover, it plays an important role in
the regulation of the response of the
immune system against pathogens (30)
and possibly to some autoantigens.
With regard to the latter, PTX3, inhibit-
ing recognition of apoptotic cells by
dendritic cells, is believed to contribute
to limit autoimmune phenomena (31).
Recent data show that scleroderma fi-
broblasts spontaneously release in-
creased amounts of reactive oxygen
species and are extremely prone to
ROS induced apoptosis. Furthermore,
several of the autoantigens targeted in
diffuse scleroderma are uniquely su-
ceptible to cleavage by reactive oxy-
gen species in a metal dependent man-
ner (32). In this scenario PTX3 over-
expression could be interpreted as an
effort to prevent autoimmune reactions
to autoantigens released by an exagge-
rated oxidative stress.
Studies are in progress to elucidate the
relationship between PTX3 and sclero-
derma fibroblast redox state.

References
1. BLACK, CM, STEPHEN C: Systemic sclerosis
(scleroderma) and related disorders. In MAD-
DISON PJ, ISENBERG DA, WOO P, GLASS
DN (Eds): Oxford Textbook of Rheumatology,
Oxford, Oxford University Press 1993: 771-
89.
2. LEROY EC: Increased collagen synthesis by
scleroderma skin fibroblasts in vitro: a possi-
ble defect in the regulation or activation of
the scleroderma fibroblast. J Clin Invest
3. UITO J, BAUER EA, EISEN AZ: Scleroder-
ma: increased biosynthesis of triplehelical
type I and III procollagens associated with
unaltered expression of collagenase by skin
fibroblasts in culture. J Clin Invest 1979; 64:
921-30.
4. JIMENEZ SA, FELDMAN G, BASHEY RI,
BIENKOWSKI R, ROSENBLoom J: Co-ordi-
nate increase in the expression of type I and
type III collagen genes in progressive sys-
temic sclerosis fibroblasts. Biochem J 1986;
237: 837-43.
5. TROJANOWSKA M, WU LT, LEROY EC: Ele-
vated expression of c-myc proto-oncogene in
scleroderma fibroblasts. Oncogene 1987; 3:
447-81.
6. PICCINNI G, LUCHETTI MM, CANIGLIA
ML et al.: c-myc proto-oncogene is expressed
by quiescent scleroderma fibroblast and,
unlike B-myb, does not correlate with prolif-
7. STEIN CN, TANNER SB, AWAD JA, ROBERTS
LJ, MORROW JD: Evidence of free-radical-

Fig. 4. Skin biopsies from two normal controls (a and b) and two patients with scleroderma (c and d).
A higher magnification of the insert of panel (d) is shown in (e).
Strong immunostaining for PTX3 was detected in dermal fibroblasts and in the vessel walls of SSc skin
biopsies. **Black arrows**: fibroblast cells expressing PTX3 protein. (*) PTX3 protein deposited in the
extracellular matrix. Representative results from 5 experiments with SSc and normal skin samples are
shown. (Immunoperoxidase-haematoxylin, x 250).


25. TSUI CC, COPELAND NG, GILBERT DJ, JENNINGS NA, BARNES C, WORLEYFP: C-reactive protein via the collagen-like region of C1q and inhibition Narp, a novel member of the pentraxin family, promotes neutrophil outgrowth and is dynamically regulated by neuronal activity. J Neurosci 1996; 15: 2463-78.


S-72