

Systemic sclerosis: pathophysiology

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Inserm

Institut national
de la santé et de la recherche médicale



Groupement d'hôpitaux Paris Centre



Conflicts of interest

- Consultant: **Actelion, CSL Behring, Cytheris, GSK, LFB Biotechnologies, Lilly, Pfizer**
 - Financial support to ARMIIC
- Investigator: **Actelion, CSL Behring, Pfizer**
- Financial support (grants): **Actelion, CSL Behring, GSK, LFB Biotechnologies, Pfizer**

Systemic sclerosis

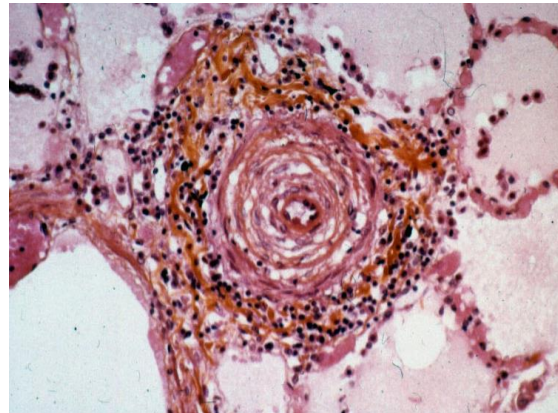
Fibrosis

Skin
Lung
Gastrointestinal
Heart



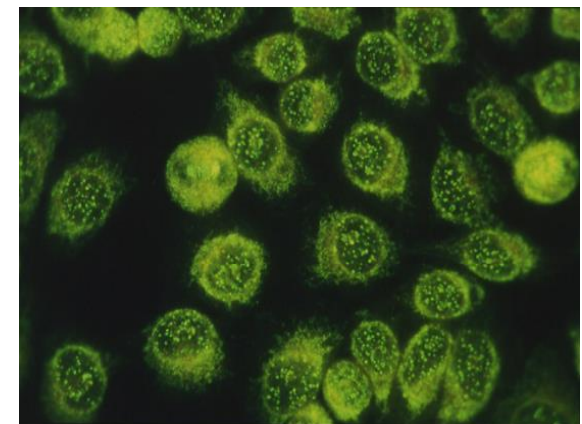
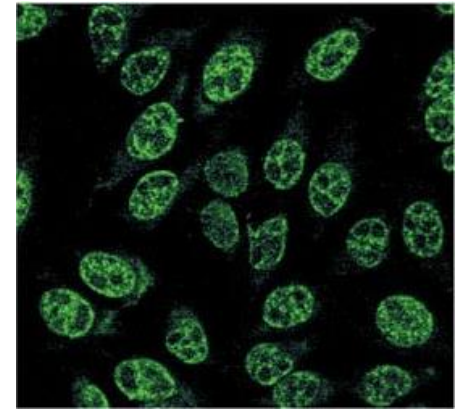
Vascular involvement

Raynaud's phenomenon
Renal crisis
Pulmonary arterial hypertension

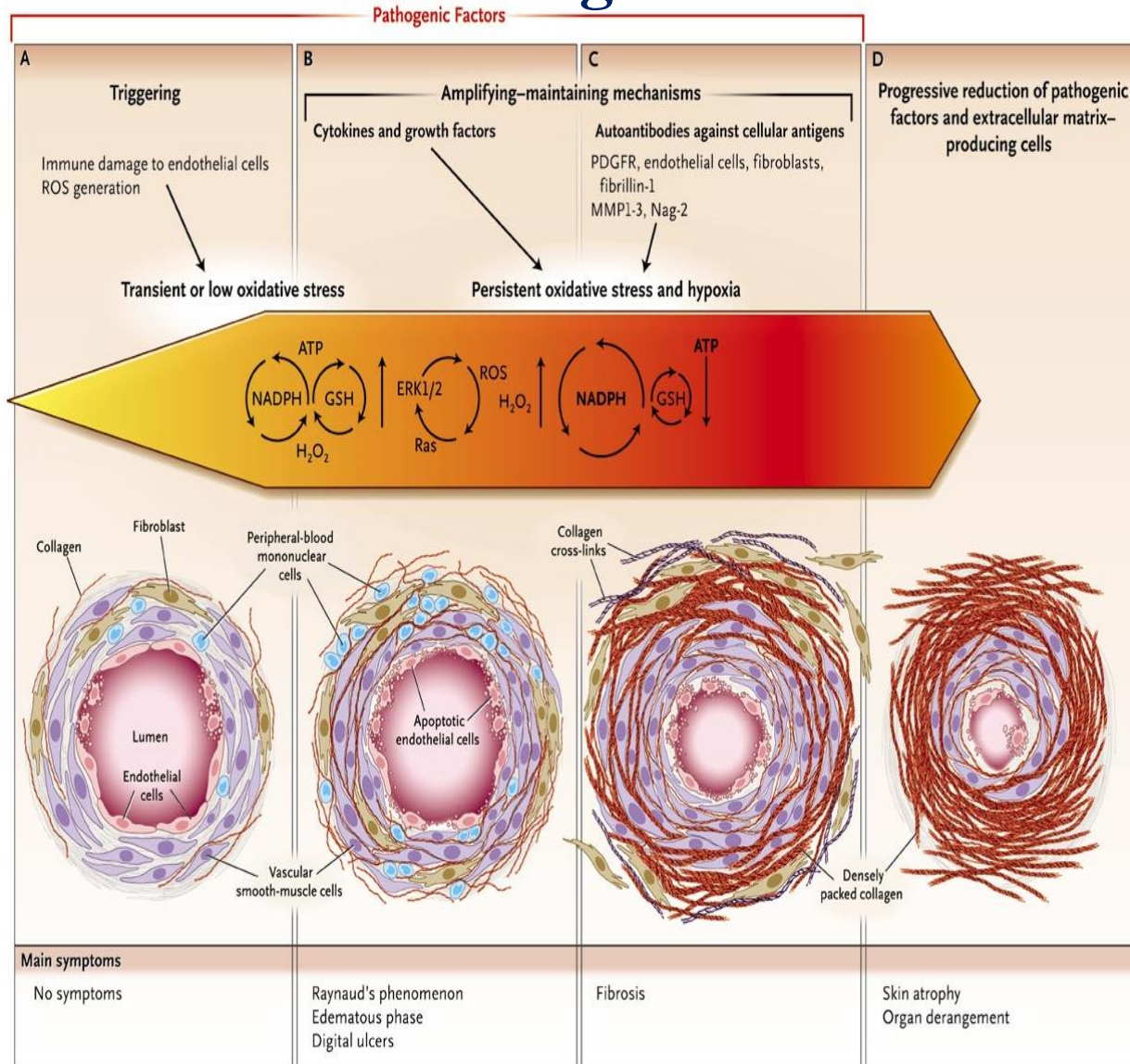


Autoimmunity

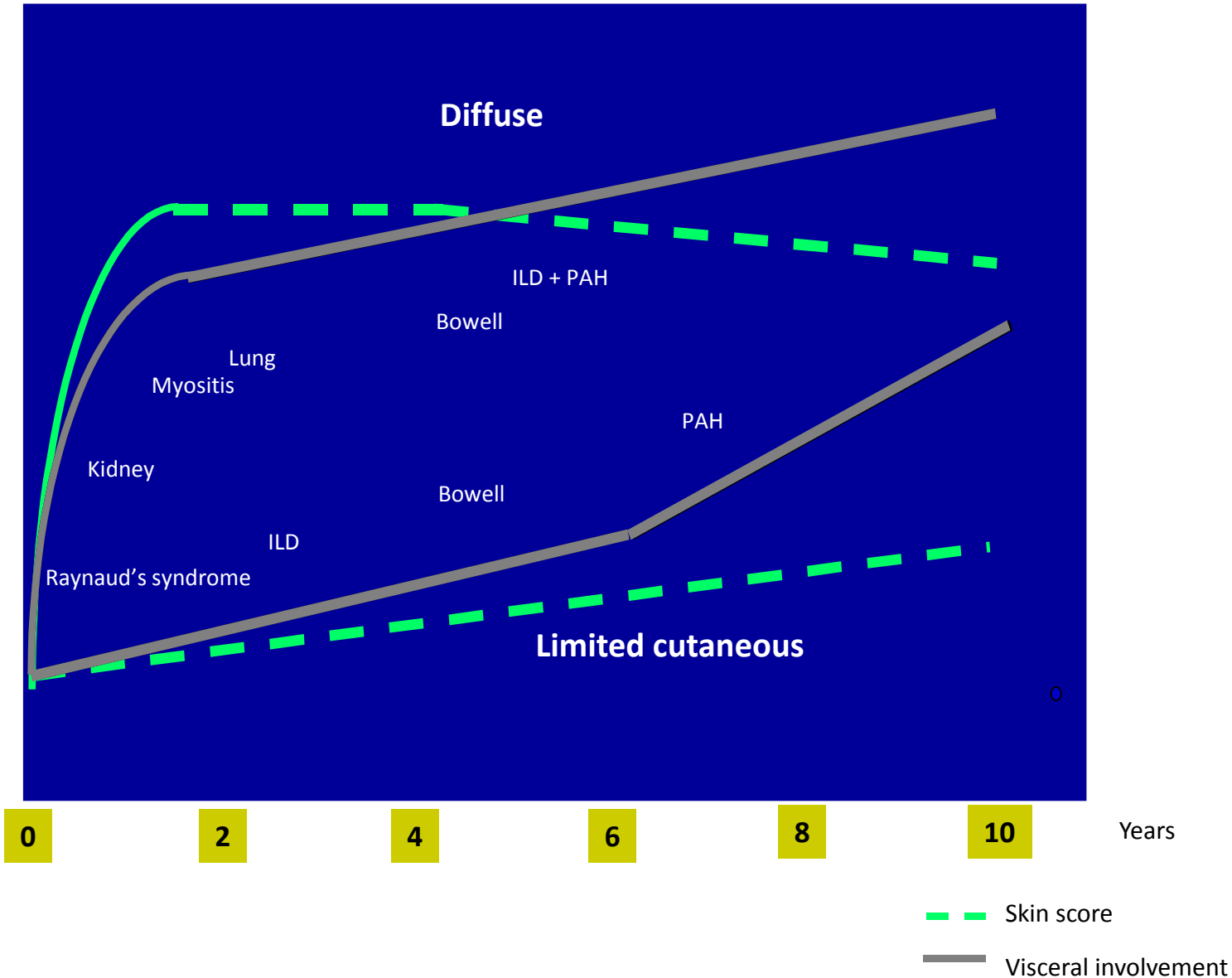
Specific autoantibodies
Anti-Scl70
Anti-centromere
Anti-ARNP0133
Non specific autoantibodies



Systemic sclerosis: lesions at different stages



SYSTEMIC SCLEROSIS : EVOLUTION



2013 classification criteria for SSc: an ACR/EULAR collaborative initiative (I)

- Skin thickening of the fingers extending proximal to the metacarpophalangeal joints: SSc;
- If that is not present, 7 additive items apply:
 - skin thickening of the fingers,
 - fingertip lesions,
 - telangiectasia,
 - abnormal nailfold capillaries,
 - interstitial lung disease or pulmonary arterial hypertension,
 - Raynaud's phenomenon,
 - SSc-related autoantibodies.

Skin thickening of the fingers (I)



Score = 2

Puffy fingers

Only count higher score

Skin thickening of the fingers (II)



Sclerodactily

Score = 4

Only count higher score



fingertip lesions

Digital ulcers

Score = 2



Fingertip pitting scars

Score = 3



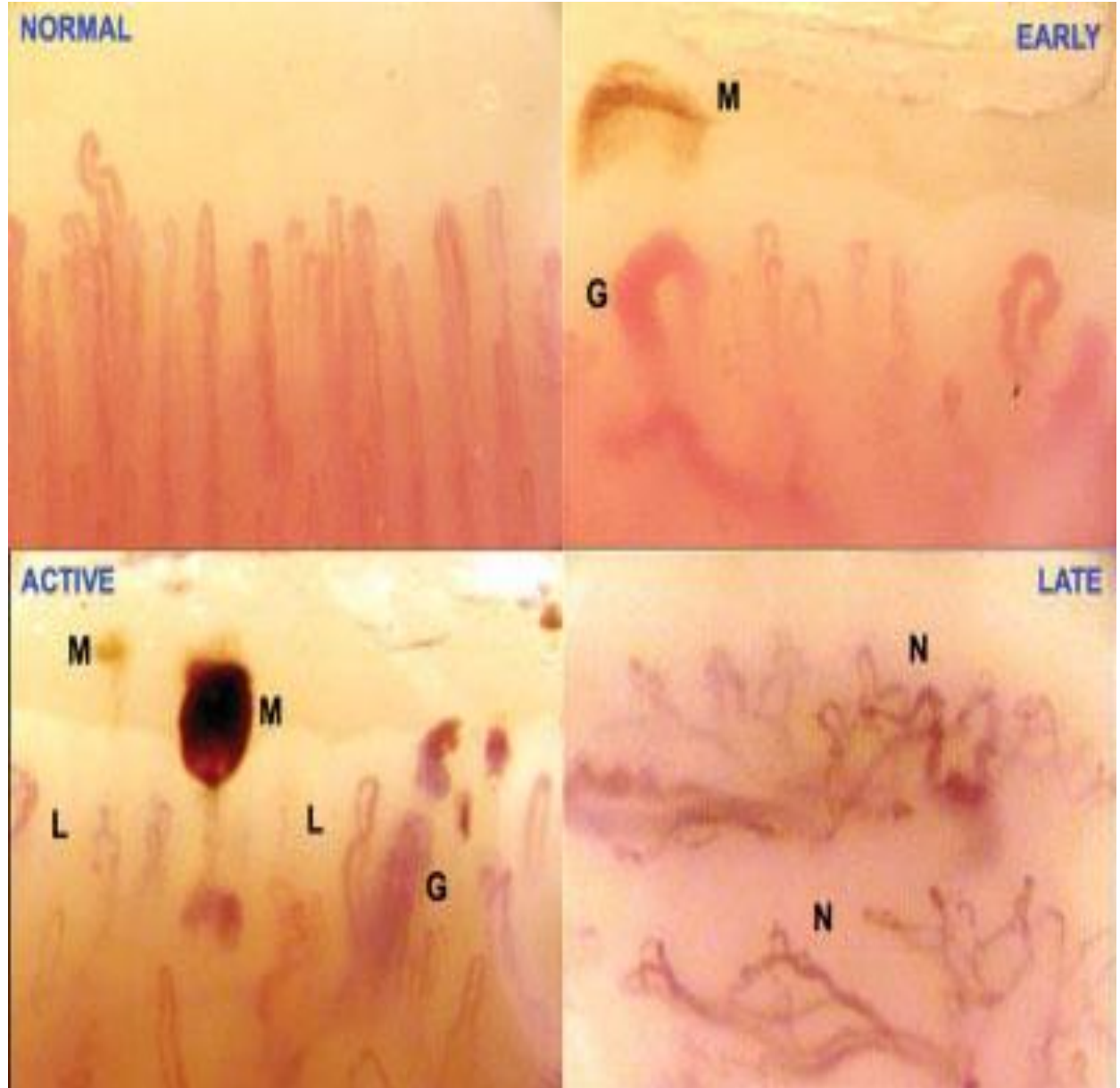
Only count higher score

telangiectasia



Score = 2

Abnormal nailfold capillaries

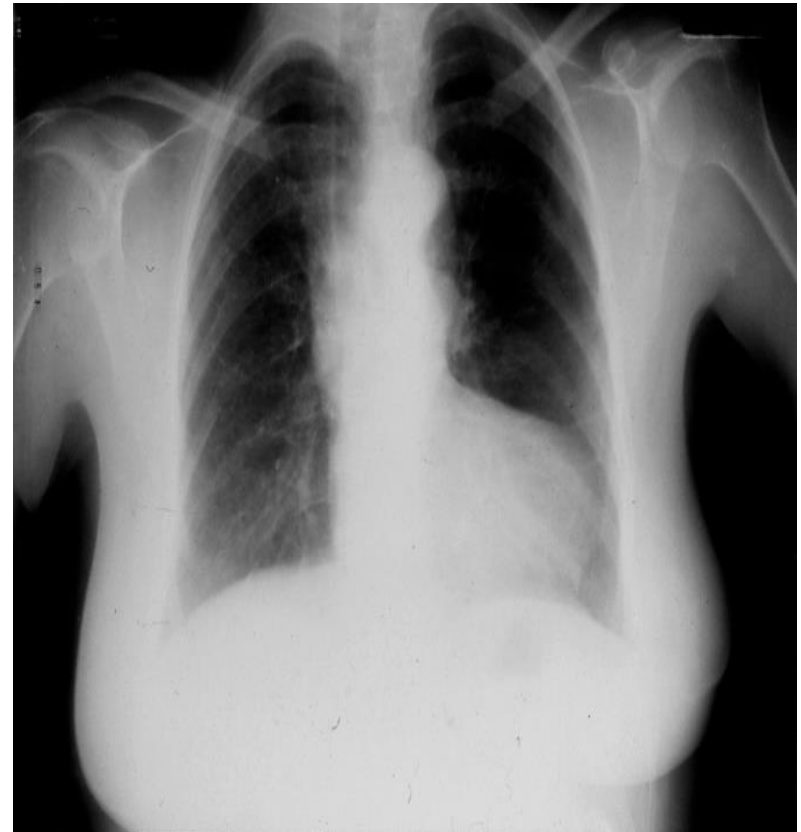
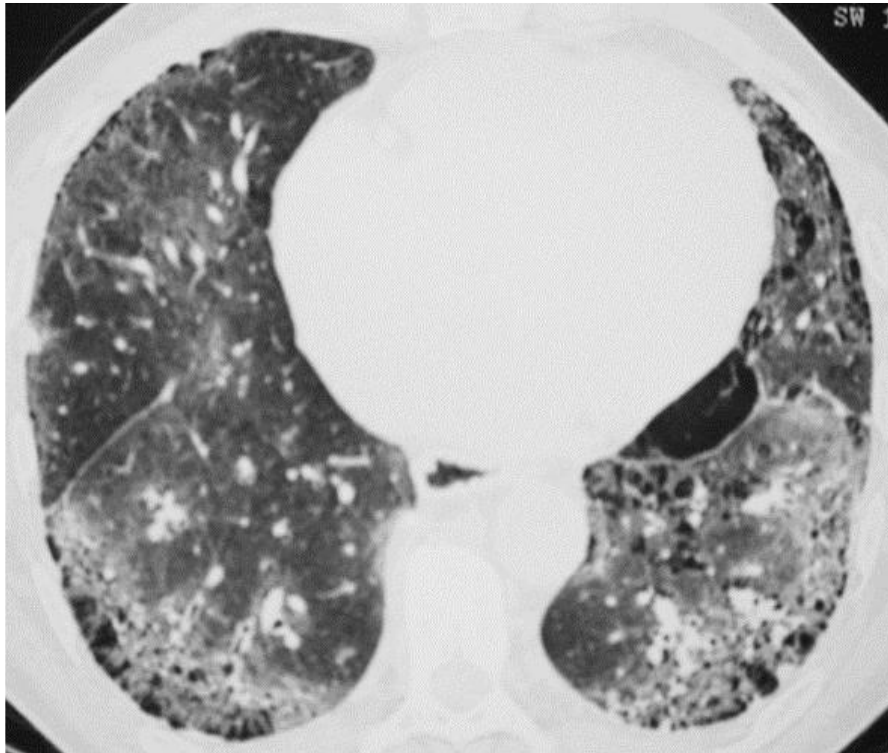
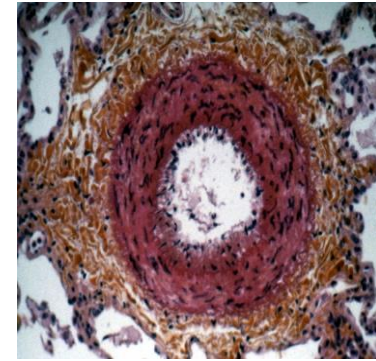


Score = 2

Interstitial lung disease/pulmonary arterial hypertension

Score = 2

Score = 2



Maximum score = 2

Raynaud's phenomenon

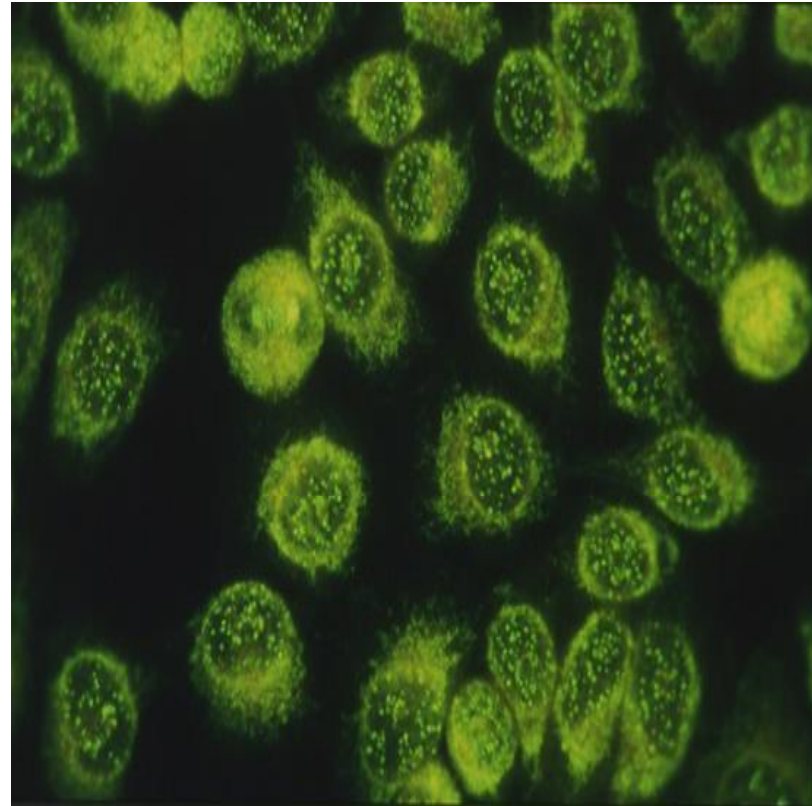


Score = 3

SSc-related autoantibodies

Anti-centromere
Anti-topoisomerase I
Anti-RNA polymerase III

Score = 3



Maximum score = 3

Prevalence

Authors

Regions

technique

**Prevalence
/million**

USA

Michet	Rochester	Hospital	138
Mayes	Detroit	Multiple sources	242
Maricq	Caroline du sud	Population	190-750

Oceania

Chandran	Australie du sud		147-208
Roberts-Thomson	Australie du sud	Multiple sources	233

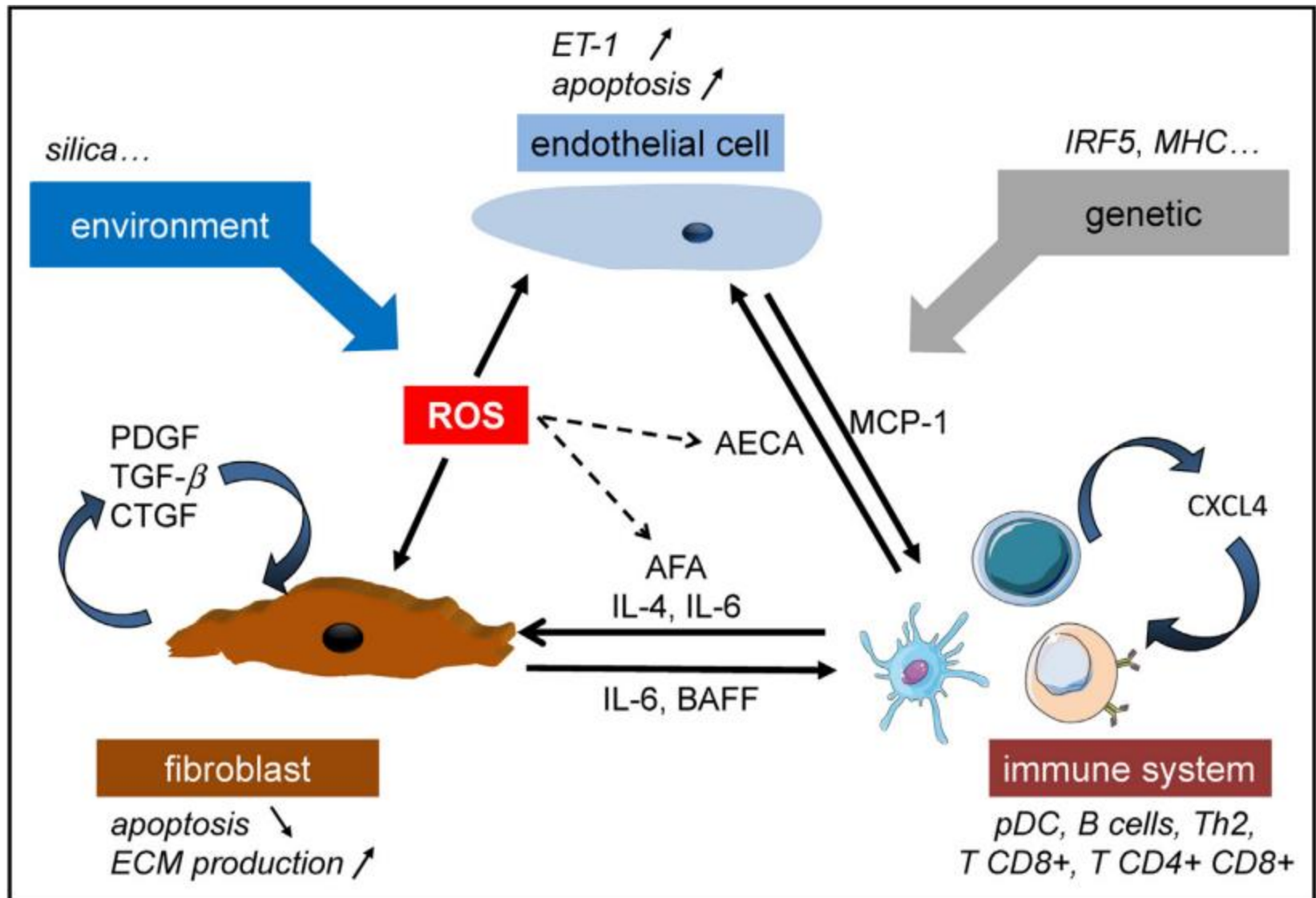
Asia

Shinkai	Japon	Public health	7
Tamaki	Tokyo	Public health	21-53

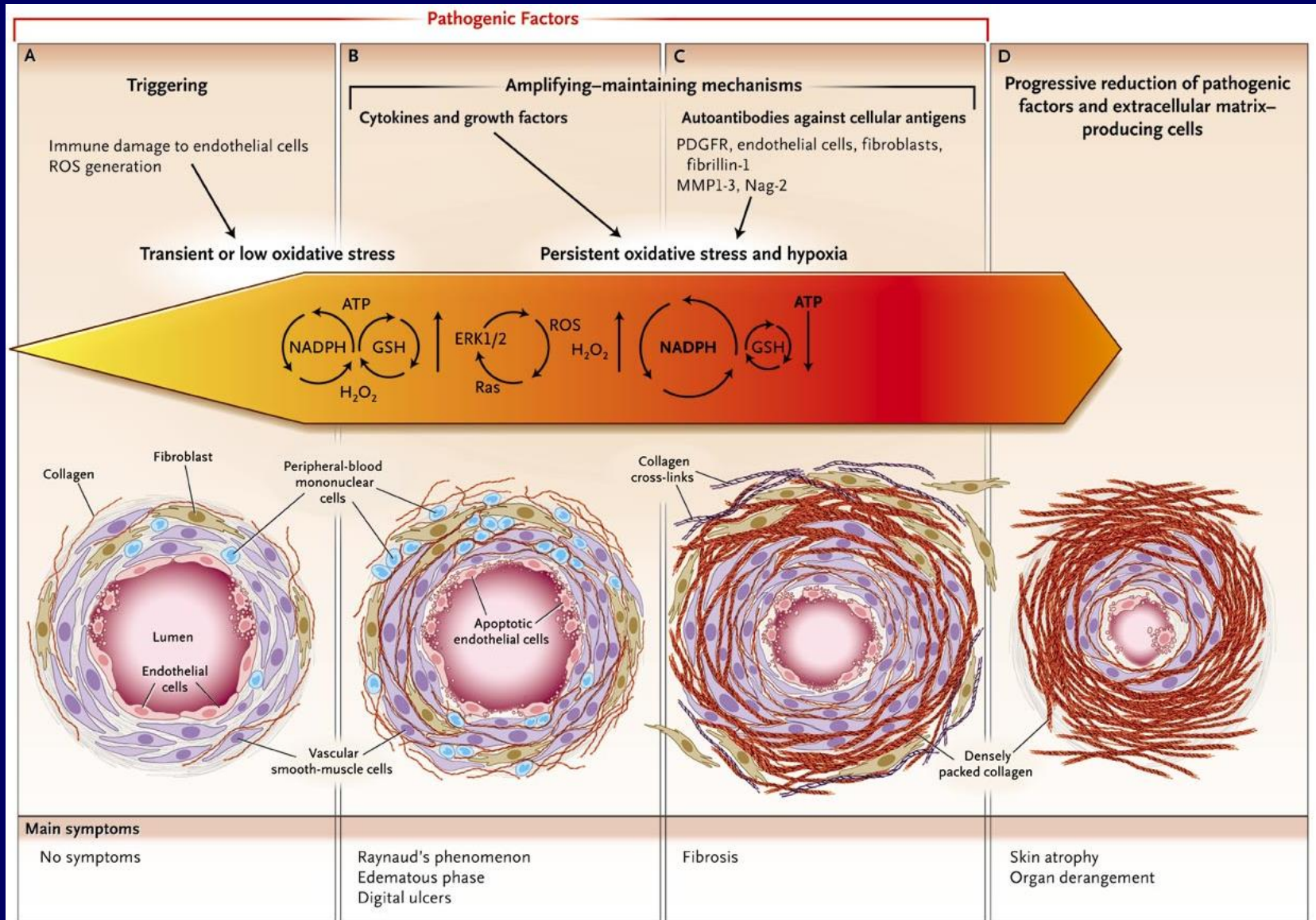
Europe

Silman	West midland	Multiple sources	31
Asboe-Hansen	Danemark	Hospital	126
Le Guern	Seine Saint Denis	Multiple sources	158
El Adssi	Lorraine	Multiple sources	132

Systemic sclerosis: pathophysiology



Systemic sclerosis: lesions at different stages



Environmental exposures associated with SSc or SSc-like illnesses

Exposure	Disease	Evidence (reference)
Crystalline silica/silica dust	SSc	Meta-analysis [72, 73]
Solvents	SSc	Meta-analysis [75]
Vinyl chloride monomer	Vinyl chloride disease	Investigation of outbreak [76]
Adulterated cooking oil	Toxic oil syndrome	Investigation of outbreak [77]
Tryptophan	Eosinophilic myalgia syndrome	Investigation of outbreak [79]
Gadolinium	Nephrogenic systemic fibrosis	Multiple case series (review [81, 82])
Drugs		
Bleomycin	Pulmonary fibrosis	Multiple observations (review [83, 84,])
Pentazocine	Localized dermal fibrosis at injection site	Multiple observations (review [85])

Familial risk

- Only 4 studies that have investigated heritability in a large case cohort.
- Frech et al. [65] studied 1,037 unique SSc cases and, reported a RR of SSc among first-degree relatives as 3.07 (95% CI 1.25–7.57, $p = 0.0148$).
- An Australian study (18) of 353 SSc cases reported a RR for SSc among first-degree family members of 14.3 (95% CI 5.9–34.5)
- US study by Arnett et al. [66] of 703 families that found a RR of 13 (95% CI 2.9–48.6, $p < 0.001$) for SSc among first-degree family members.
- A study using cases from Canada and Columbia [67] found increased frequency of multiple autoimmune disease in family members but did not find an increased RR for SSc.
- Assassi et al. [68] compared disease type, organ involvement, and autoantibody status among 18 familial SSc cases and 692 sporadic cases. SSc families tended to be concordant for SSc-specific autoantibodies and HLA haplotypes, but otherwise familial SSc did not appear to be a unique disease subset.

Systemic sclerosis: susceptibility genes

Fibrosis

Vascular involvement

Autoimmunity

Fibrillin-1 (FBN1)

VEGF

*CMH-HLA: HLA II and autoantibodies
(HLA-DRB1*01-DBQ1*0501
associated to ACA)*

Fibronectin (FN))

*Endothelin and its
receptors*

*Lymphocytic activation : STAT4,TBX21
regulators of TH1-TH2 balance;*

*Secreted Protein Acid and
Rich Cystein (SPARC) or
osteonectin*

*Hypoxia-inducible factor
1A*

*Protein tyrosine phosphatase
nonreceptor type 22 (PTPN22),*

*Connective tissue
growth factor
(CTGF)*

*Endothelial nitric oxide
synthase
(eNOS/NOS3) and
inducible NOS
(iNOS/NOS2)*

*B cell scaffold protein with ankyrin
repeats 1 (BANK1)*

TGF-β

B lymphocyte kinase (BLK);

*Serotonin 5-HT2A
receptor*

*Tumour necrosis factor alpha-
induced protein 3 (TNFAIP3);*

*Interleukine-1α
et 1β*

Fibrinogen

Interleukin-23 receptor

*Matrix metalloproteinase
(MMP)*

*Stromal cell-derived
factor 1 (SDF-
1/CXCL12):)*

*Innate immunity: IRF5, control of IFN
production*

Animal models of SSc

		Experimental model	Vasculopathy	Fibrosis	Inflammation	Autoantibodies	Limits
Genetic models	Spontaneous	Tak-1 [46]					Unclear various organs defects
		Tak-2 [52]					Unclear various organs defects
		UCD-200 UCD-206 [54]					Chicken model with poor background
		TβRIIΔk and TβRI ^{lox} [56, 57]					Absence of autoimmunity
	Genetic modification	Caveolin 1 ⁺ [61]					Not well characterized
		Fra-2 ⁺ [61]					Not well characterized
		Flt1 ^{ΔCAF/ACTA} [58]					Not well characterized/ absence of autoimmunity
		Flt1 endothelial cell KO [58]					Not well characterized/ absence of autoimmunity

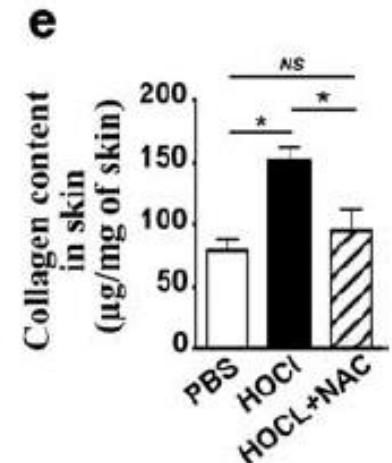
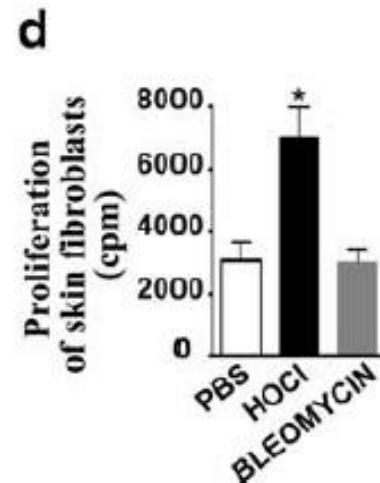
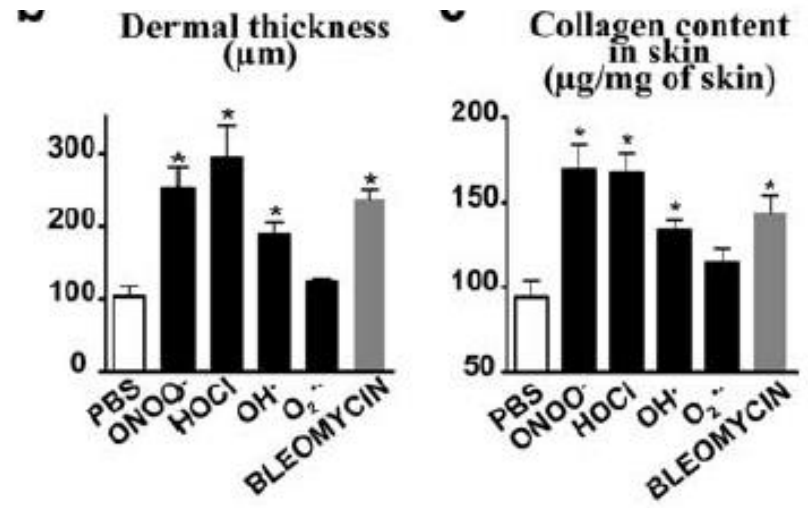
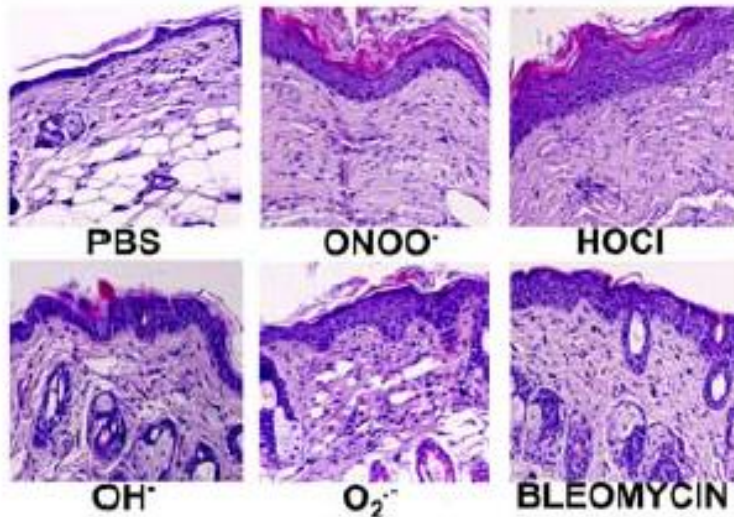
Animal models of SSc

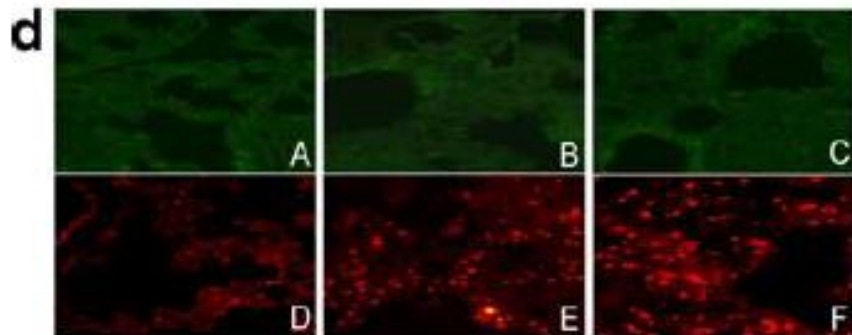
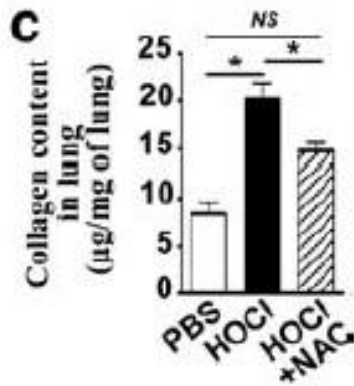
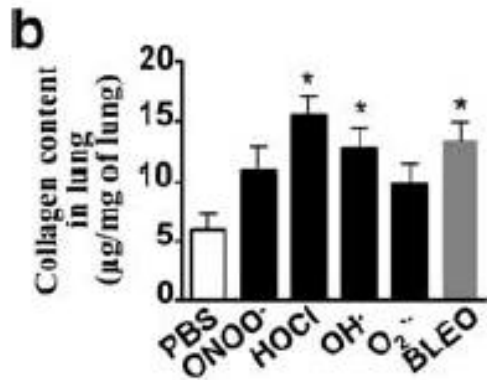
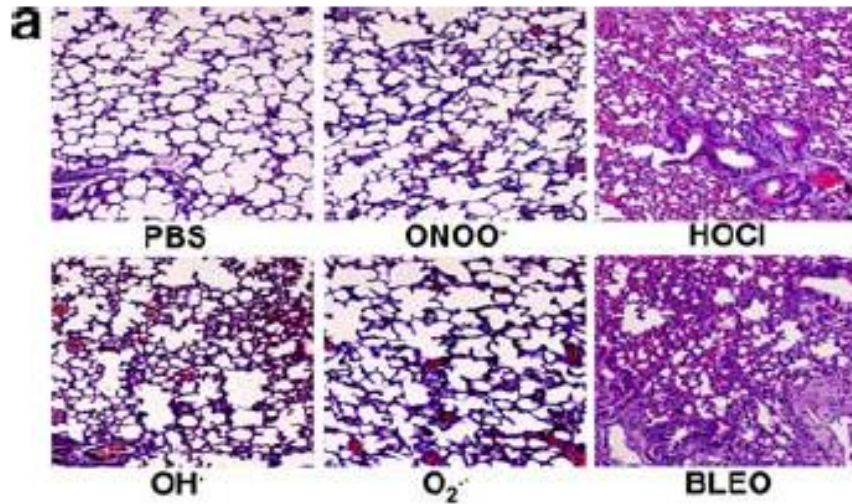
Inducible models	Bleomycin induced model [55]					Overestimation of drug effects
	ROS-induced model [52]					TGF- β independent model
	Topo I/CFA's adjuvant induced SSc [61]					Unclear function of anti-topoisomerase I antibody
	Angiotensin II induced SSc [51]					Poor links with human SSc
	Sclerodermatous GVHD [61]					Not well characterized

Selective Oxidation of DNA Topoisomerase 1 Induces Systemic Sclerosis in the Mouse¹

Amélie Servettaz,^{2*‡} Claire Goulvestre,^{2*} Niloufar Kavian,^{*} Carole Nicco,^{*} Philippe Guilpain,^{*†} Christiane Chéreau,^{*} Vincent Vuiblet,[§] Loïc Guillevin,[†] Luc Mouthon,^{*†} Bernard Weill,^{*} and Frédéric Batteux^{3*}

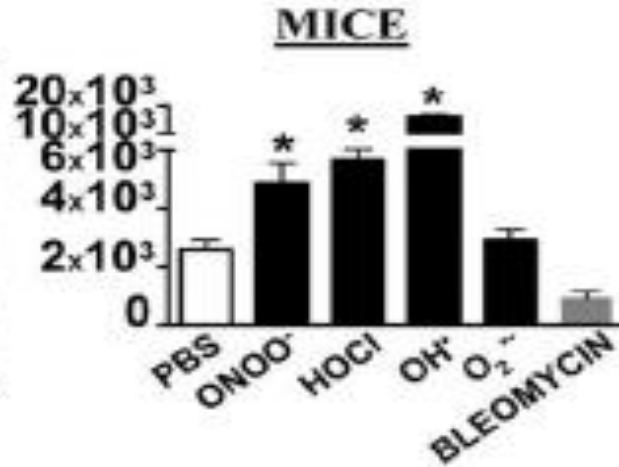
a



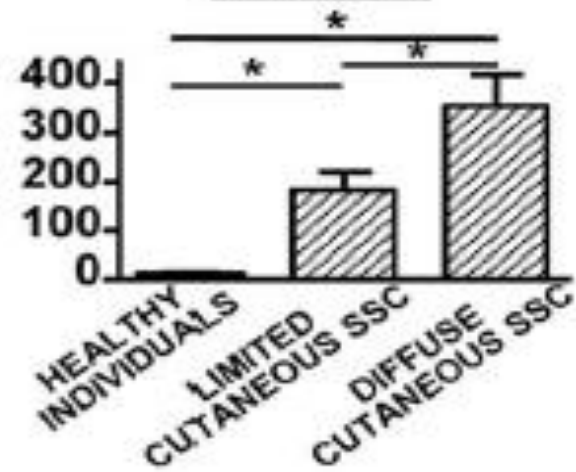


a

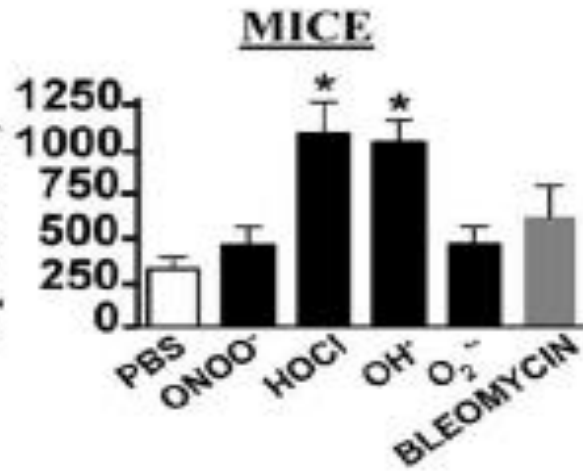
H₂O₂ production
(A.U./mm²/10⁶ cells)



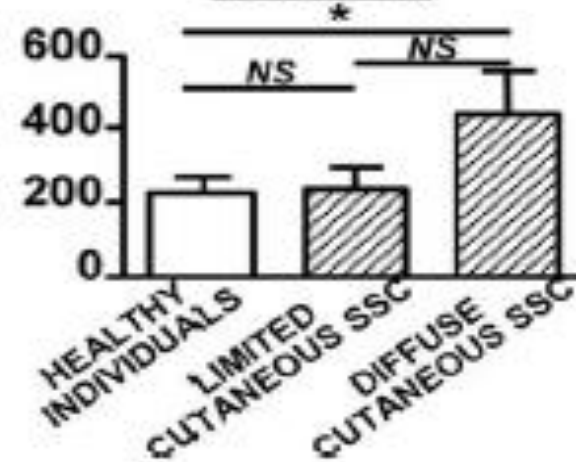
HUMANS

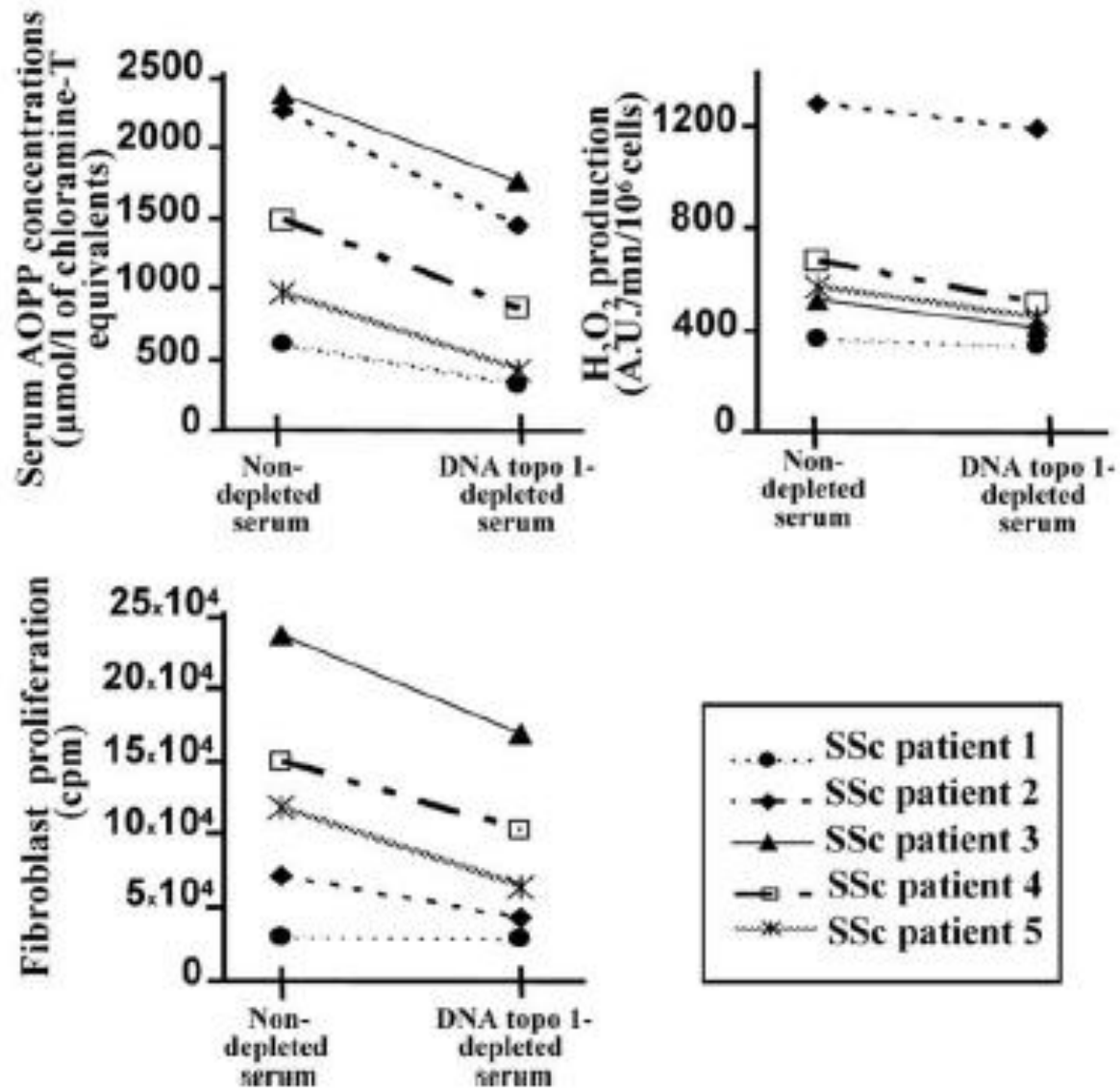
**b**

Serum AOPP concentrations
(μmol/l of chloramine-T
equivalents)



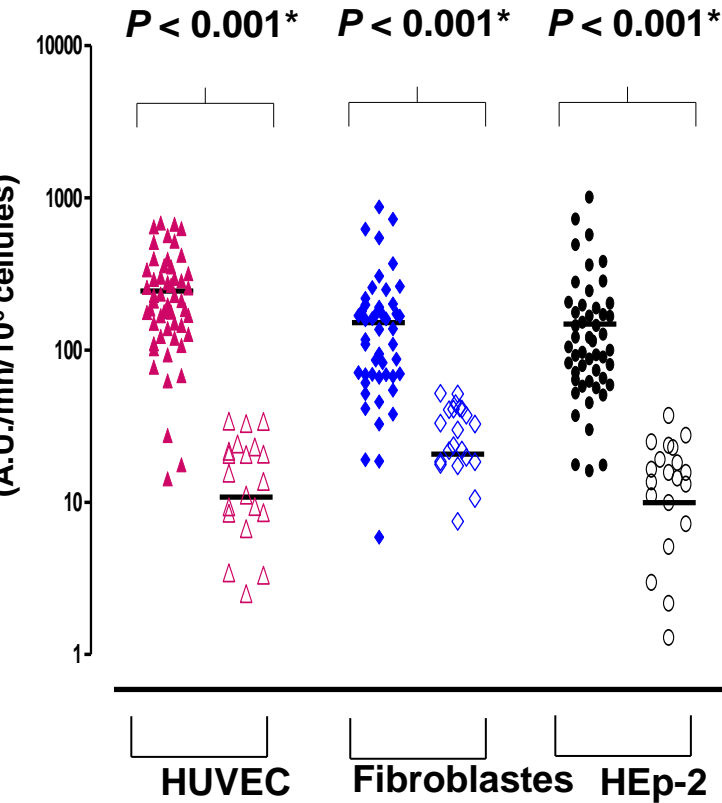
HUMANS



d

Generation of H₂O₂ in the presence of SSc serum

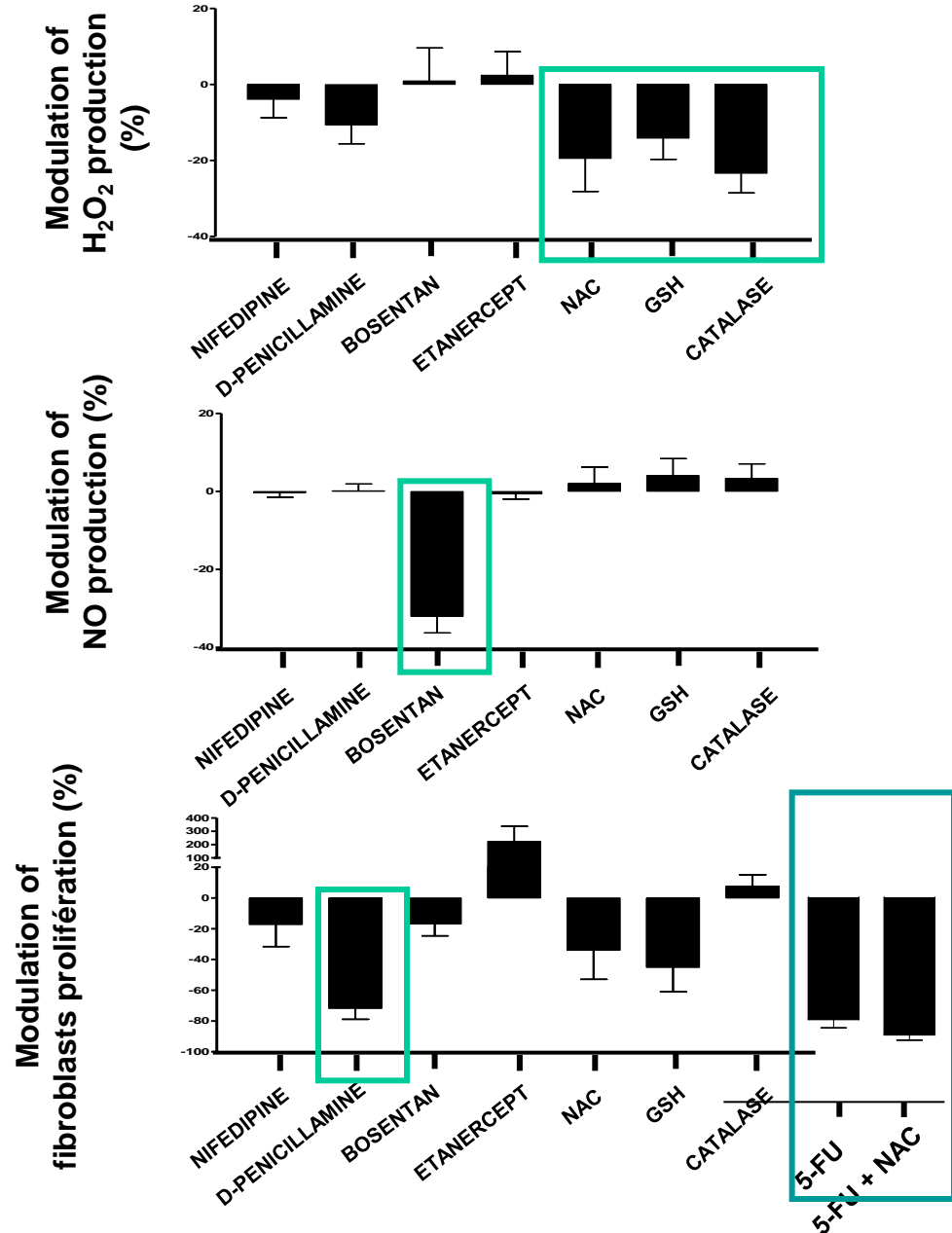
A



B

C

Effect of drugs and anti-oxydizing molecules

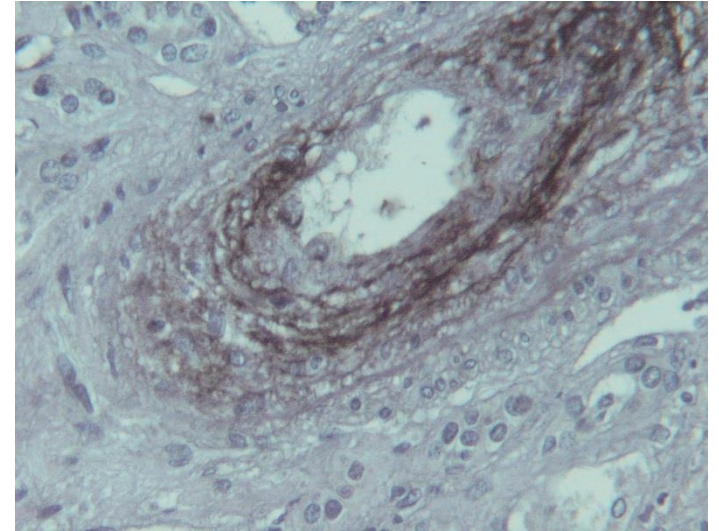


Vascular involvement

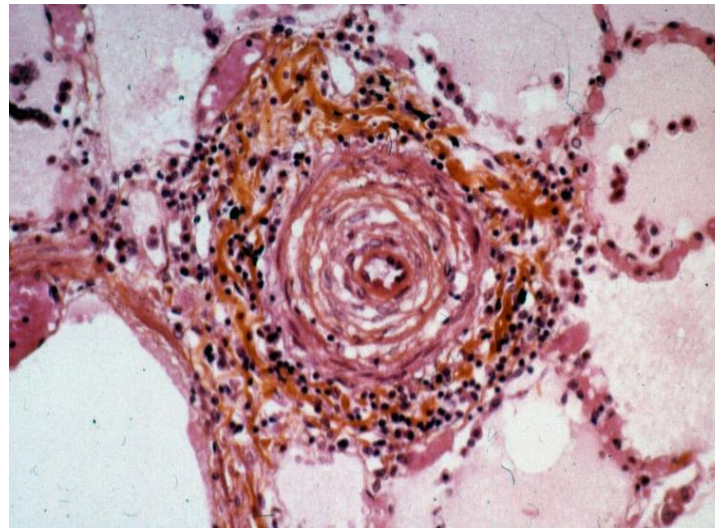
Digital ulcers



Renal crisis



Pulmonary arterial hypertension



A disease of the endothelium

Major dysfunction of endothelial cells *(Matucci-Cerinic, Semin Arthritis Rheum. 2003)*

Apoptosis at early stages (AECA ?) *(Sgonc, J Clin Invest. 1996)*

Loss of physiological barrier: permeabilisation of vessels

Abnormal vascular tone regulation

Increased endothelin-1 synthesis *(Mayes, Arthritis Rheum, 2003)*

Defective prostacyclin synthesis

Perturbed NO synthesis *(Cotton, J Pathol. 1999; Herrick, Clin Exp Rheumatol. 2001)*

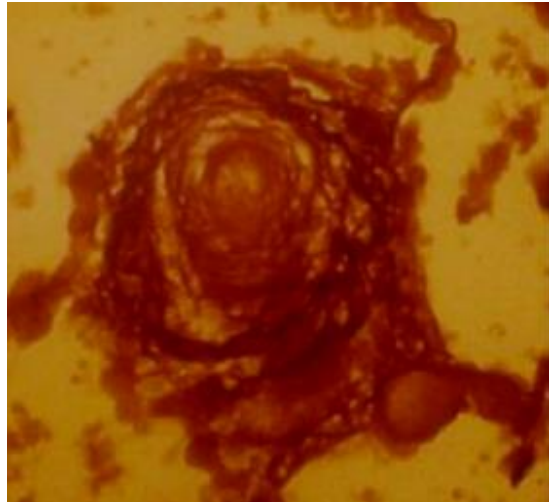
Perturbed angiogenesis: VEGF decreased or not detectable *(Distler O., Circ Res, 2004)*

Synthesis of MCP-1 and VCAM-1: recruitment of lymphocytes *(Andereg, Arch Dermatol Res. 2000)*

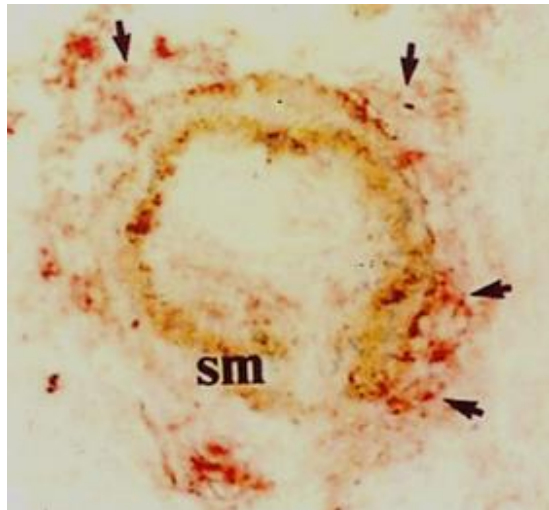
Synthesis of TGF β and PDGF: activation of fibroblasts *(Cotton, J Pathol, 1998)*

Endothelin-1 expression in pulmonary and renal vasculature

PAH

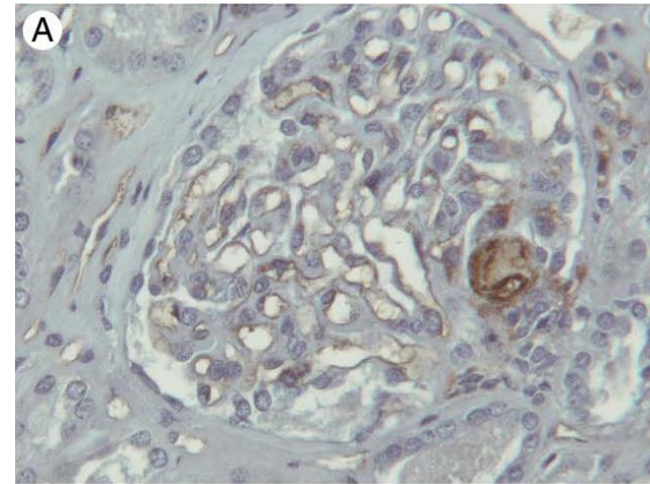


Sirius red stain - collagen

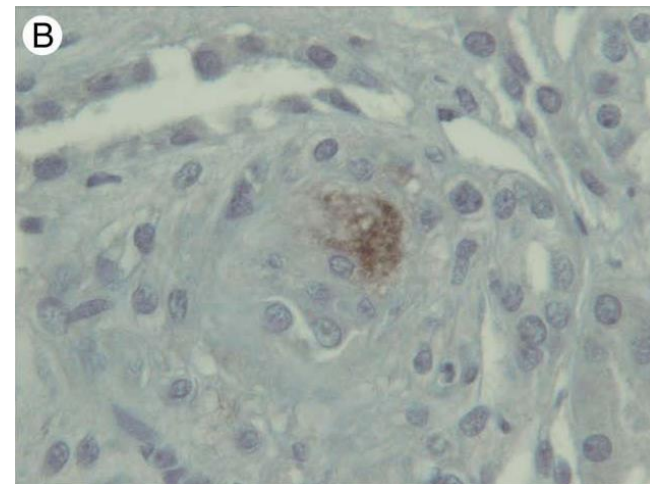


Immunolocalisation of ET-1 ligand

Scleroderma renal Crisis



ET-1 in glomerular thrombosis and along glomerular basement membranes

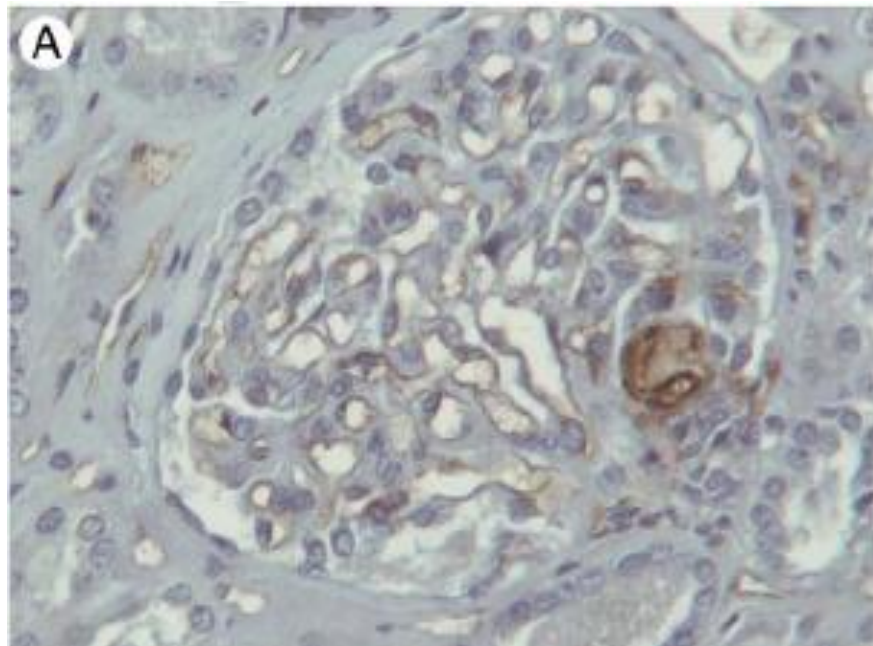


ET-1 in arteriolar thrombosis

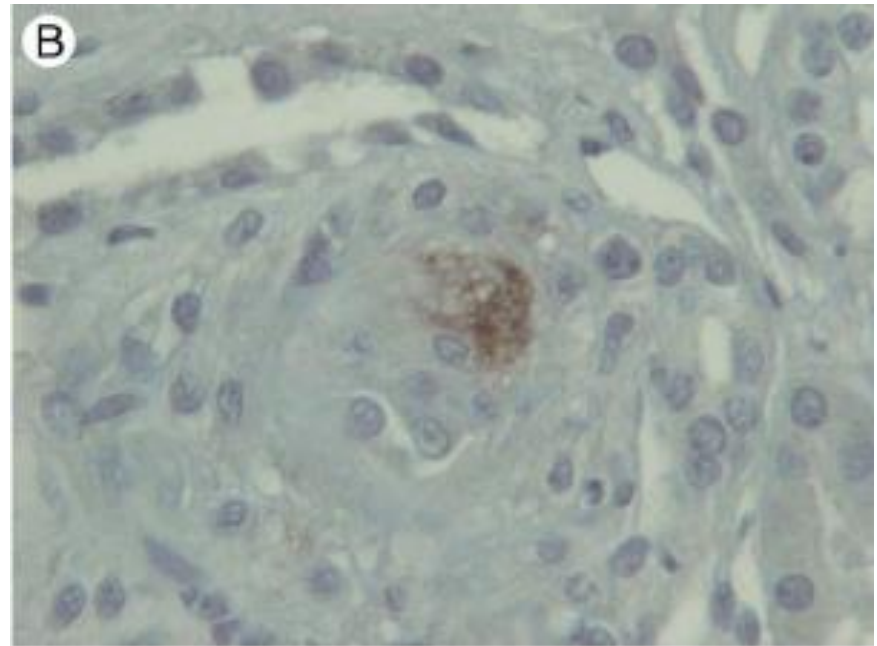
Endothelin 1 expression in scleroderma renal crisis

Table 3 Immunoperoxidase staining of ET-1 and vWF in kidney biopsies of selected groups with nephropathic abnormalities

Condition	No. of patients	Glomeruli		Arterioles		Interlobular arteries	
		ET-1	vWF	ET-1	vWF	ET-1	vWF
Negative controls	5	-	+/- (3)	+/- (1)	+/- (2)	+/- (3)	+/- (5)
SRC	14	6+/8-	++ (12)	++ (12)	++ (14)	++ (10)	++ (13)
HUS	5	+ (4)	+++ (5)	-	++ (5)	-	++ (5)
APLN	6	-	++ (4)	+/- (3)	++ (6)	+/- (2)	++ (5)
Cyclosporine A toxicity	5	-	+/- (1)	+(1)	++(4)	-	++(4)
Nephroangiosclerosis	5	-	+ (4)	+/- (1)	++(3)	+/- (3)	++(5)
Diabetic nephropathy	5	-	++ (3)	+(2)	++(4)	+(3)	++(4)



ET-1 in glomerular thrombosis and along glomerular basement membranes



ET-1 in arteriolar thrombosis

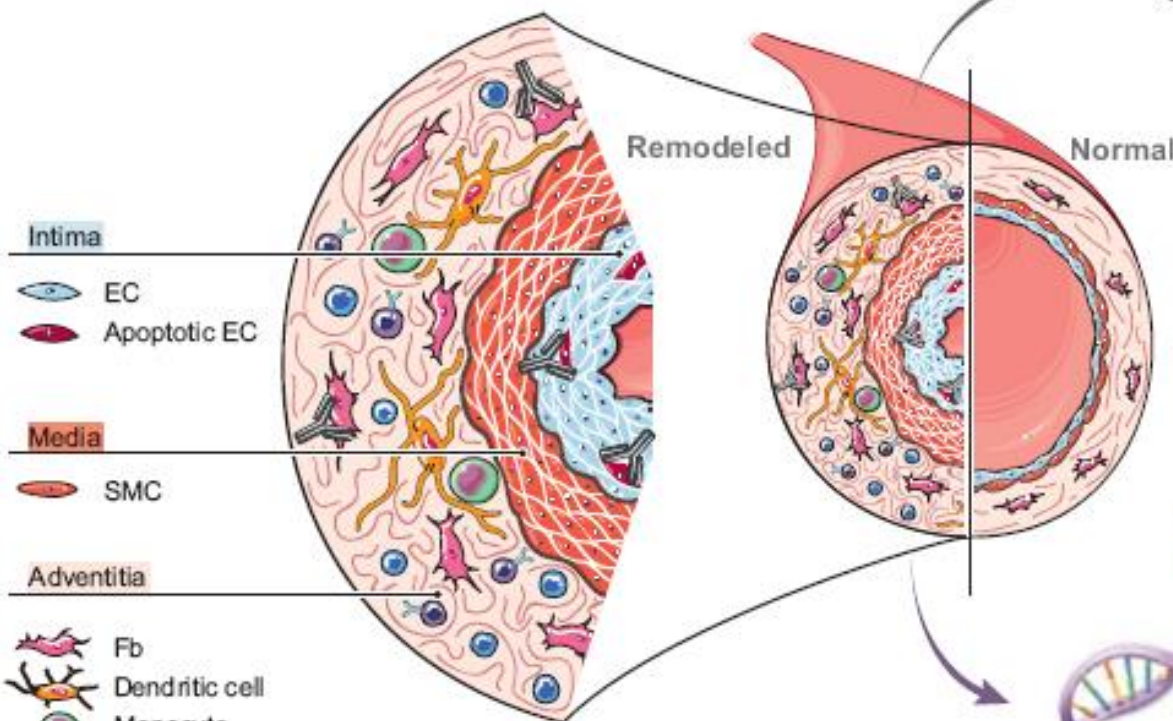
Pulmonary vascular remodeling in SSc-PAH

Vascular remodeling

Intima : EC apoptosis, activation and/or proliferation
 Media: SMC hyperplasia/hypertrophy
 Adventitia: inflammatory cell recruitment, cell proliferation, and fibrosis

Circulating autoantibodies

- Anti-EC
- Anti-Fb
- Anti-PDGF receptor
- Anti-Centromere
- Anti-Topoisomerase 1
- Anti-RNA-polymerase III
- Anti-Fibrillarin (U3 small nucleolar RNP)
- Anti-Th/To
- Anti-PM/Scl
- Anti-Fibrillarin 1
- Anti-Matrix Metallo Proteinase 1-3
- Anti-Nag-2



Intima

- EC
- Apoptotic EC

Media

- SMC

Adventitia

- Fb
- Dendritic cell
- Monocyte
- B Lymphocyte
- T Lymphocyte
- IgG
- Collagen

Candidate genes

- CCL2 (MCP-1)
- CD 19
- TNF alpha
- IL1 alpha
- IL10 (3-SNP haplotype)
- CTGF
- IRF5
- STAT4
- Endoglin

Pulmonary Lymphoid Neogenesis in Idiopathic Pulmonary Arterial Hypertension

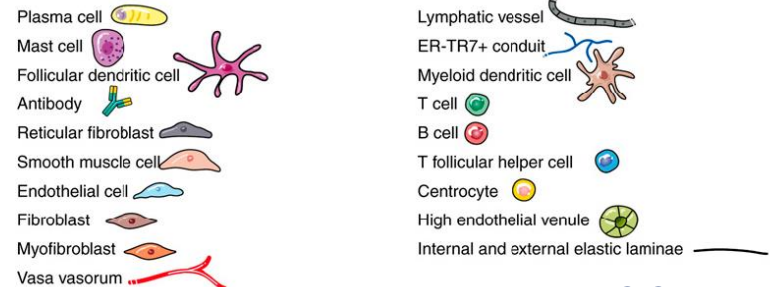
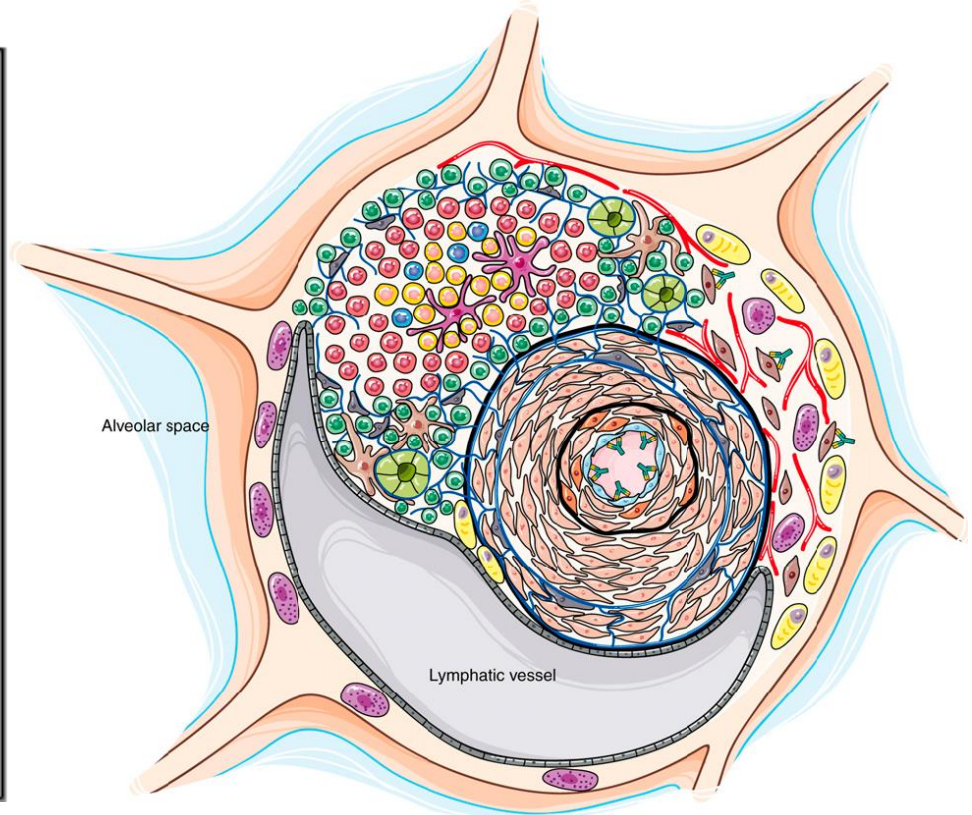
AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

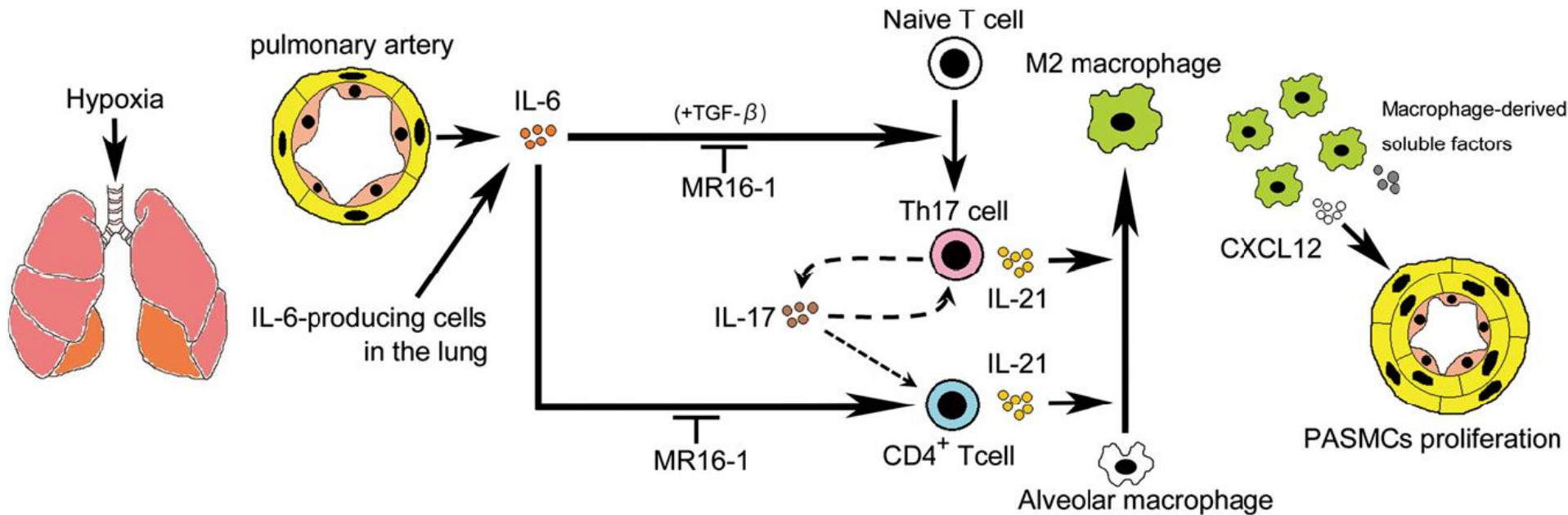
Work on chronic inflammatory disorders and autoimmune diseases suggests that pathogenic antibodies and T cells may be generated locally, in the targeted organ, in highly organized ectopic lymphoid follicles commonly called *tertiary lymphoid tissues*. Despite the importance of inflammatory influx in idiopathic pulmonary arterial hypertension (IPAH) lesions, lymphoid neogenesis has not been studied.

What This Study Adds to the Field

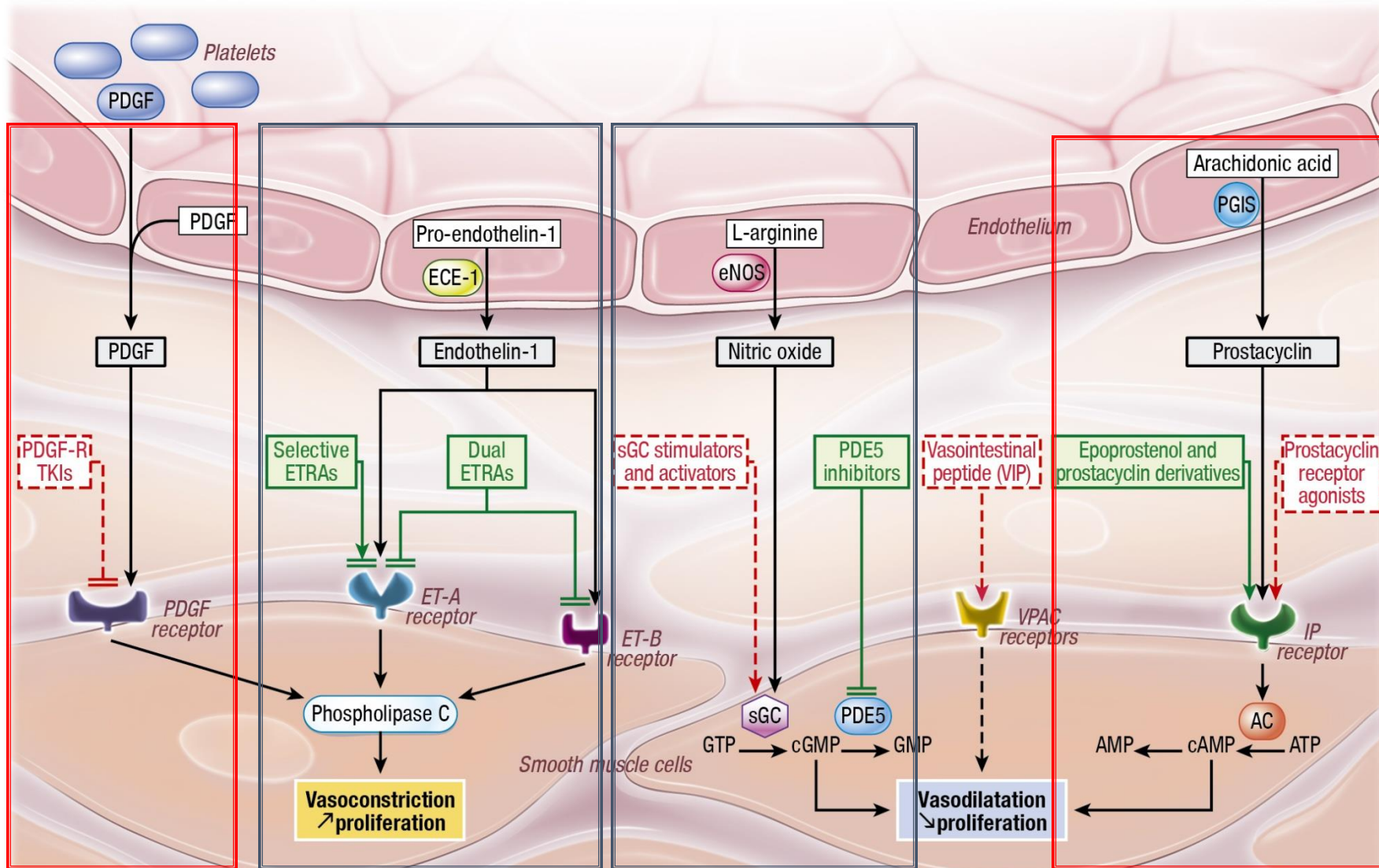
The presence of highly organized perivascular follicles in IPAH lungs argues for specific immune-adaptive mechanisms in the pathophysiology of the disease. It is highly important to understand how modulating factors that drive and maintain lymphoid neogenesis in IPAH lungs can contribute to disease progression.



Interleukin-6/interleukin-21 signaling axis is critical in the pathogenesis of pulmonary arterial hypertension



Current and Emerging Targets and Therapies in PAH



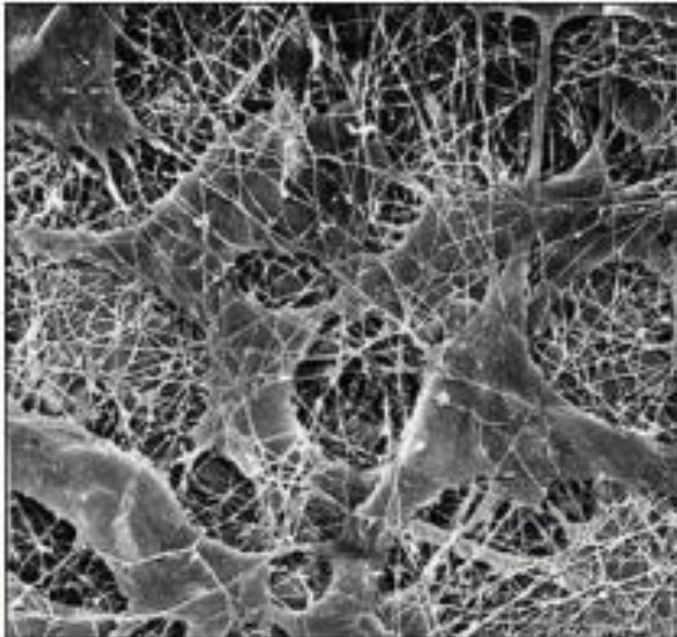
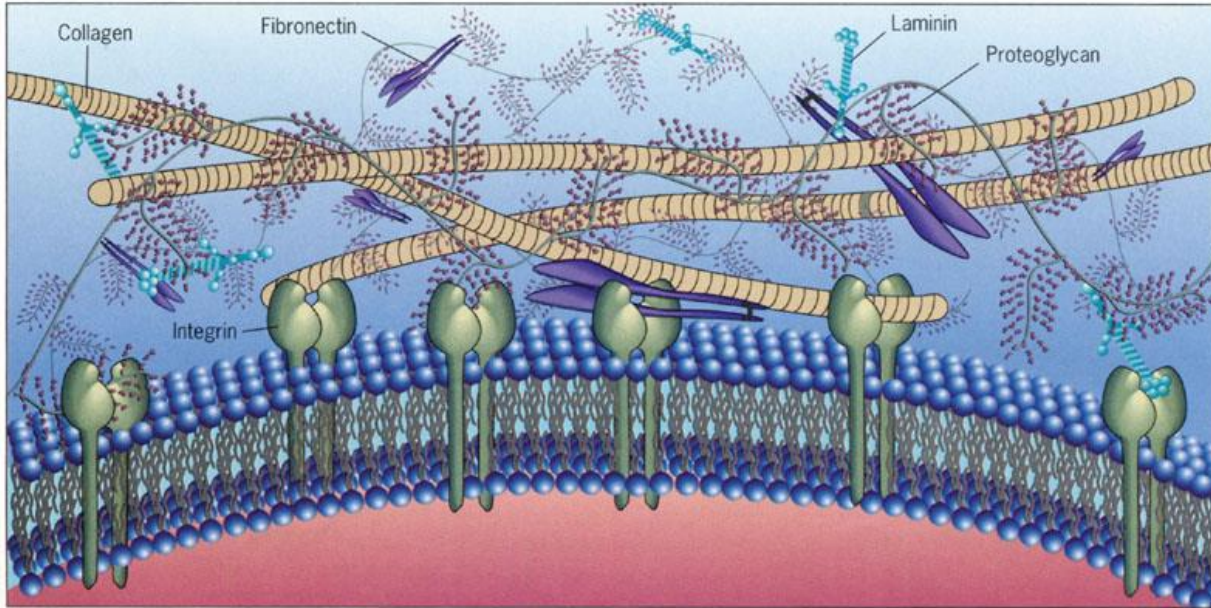
EXTRA-CELLULAR MATRIX

Collagens:
I, II, III, V, XI,...

Fibers: Elastin, Fibrillin

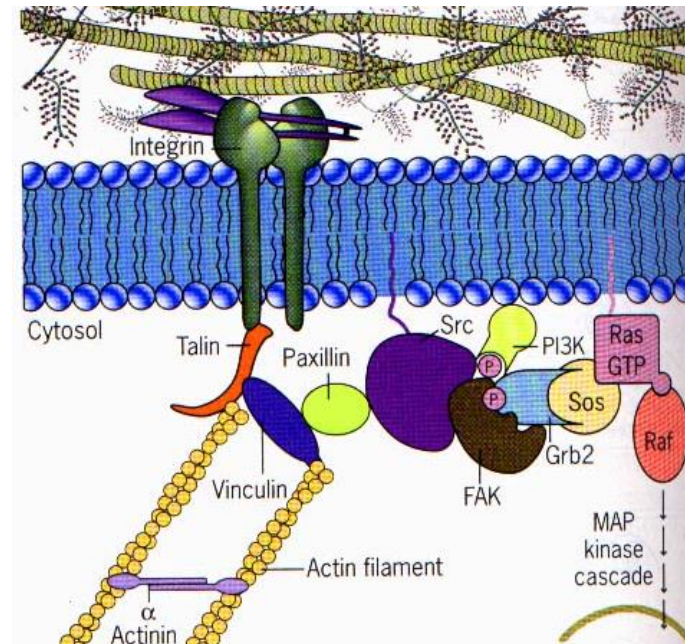
Glycosaminoglycans:
Hyaluronan, Heparan sulfate,...

Growth factors:
TGF- β , CTGF, PDGF,...



Fibroblasts
Pericytes
Vascular
smooth muscle
cells

Intégrins:
 $\alpha 1\beta 2$
 $\alpha v\beta 3$
 $\alpha v\beta 5$



SCLERODERMA FIBROBLASTS

Activation phenotype: myofibroblasts *(LeRoy, E.C.. J.Clin Invest, 1974; KIRK, J Biol Chem, 1995)*

α -smooth actin *(Abraham, D.J. Curr. Rheumatol. Rep. 2007)*

Focal Adhesion Kinase *(Mimura, Y. J. Invest. Dermatol, 2005)*

Defective apoptosis through Fas/Fas-ligand *(Santiago B., Arthritis Rheum 2001)*

Defective synthesis of ECM regulators (metalloproteinases) *(VAN DER SLOT, J Biol Chem. 2003)*

Activation and increased collagen synthesis influenced by

IL-4: proliferation *(POSTLETHWAITE, J Clin Invest, 1992)*

Connective Tissue Growth Factor (CTGF) *(Leask, A., J. Cell Sci. 2006)*

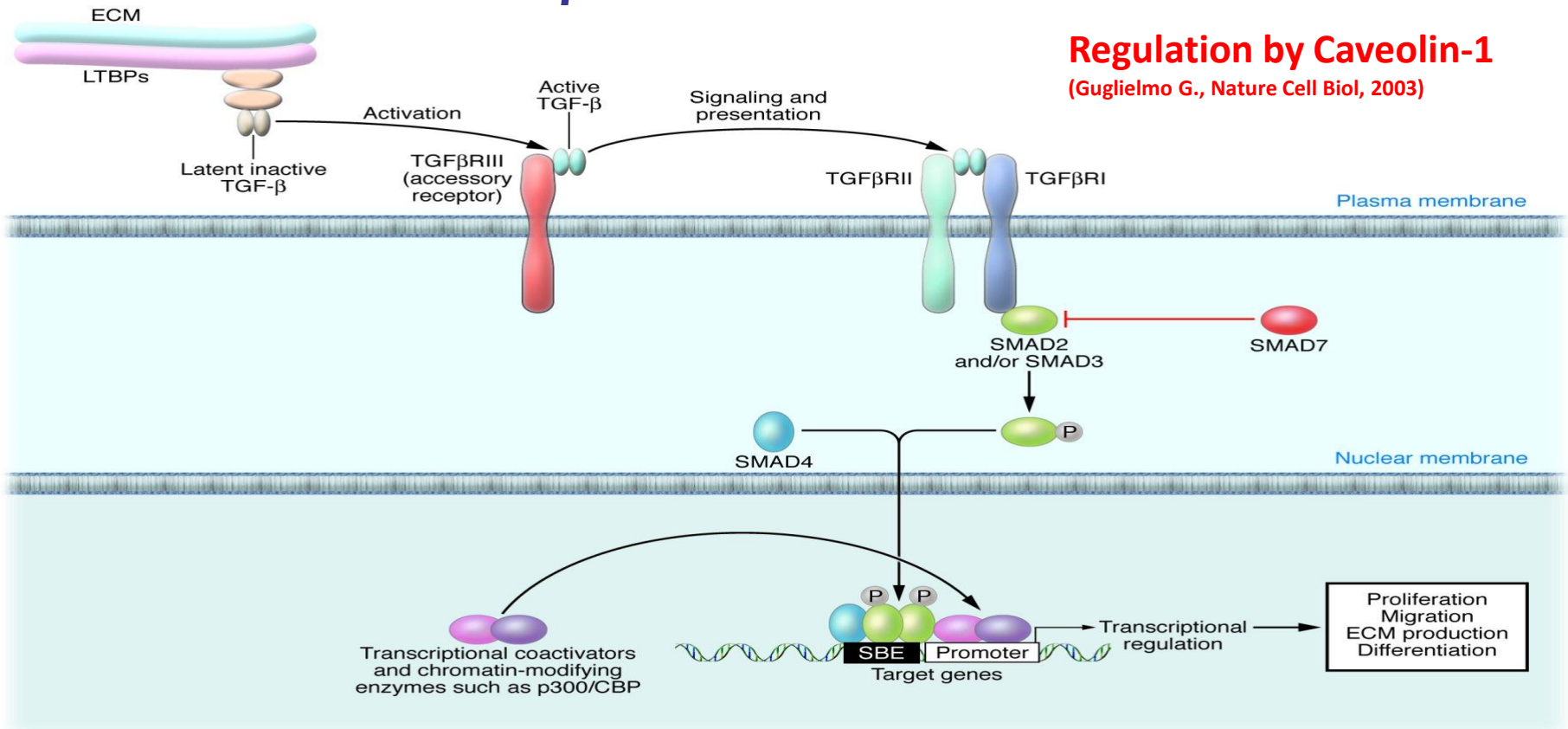
Platelet Derived Growth Factor (PDGF) *(Ludwicka, A., J. Rheum. 1995)*

Reactive Oxygen Species (ROS) *(Sambo P., Arthritis Rheum.,2001)*

Anti-fibroblasts and anti-PDGFR antibodies *(Chizzolini C., Arthritis Rheum 2001, Sevgliati Baroni S., NEJM, 2006)*

Transforming Growth Factor- β (TGF- β) *(Pannu, J., Curr. Opin. Rheumatol. 2004)*

TGF- β and fibroblasts in SSc



produced by EC, dermal perivascular macrophages

Activation of Smads

Activation of non-smads pathways: p21 activated kinase 2, Rho associated Kinase, ...

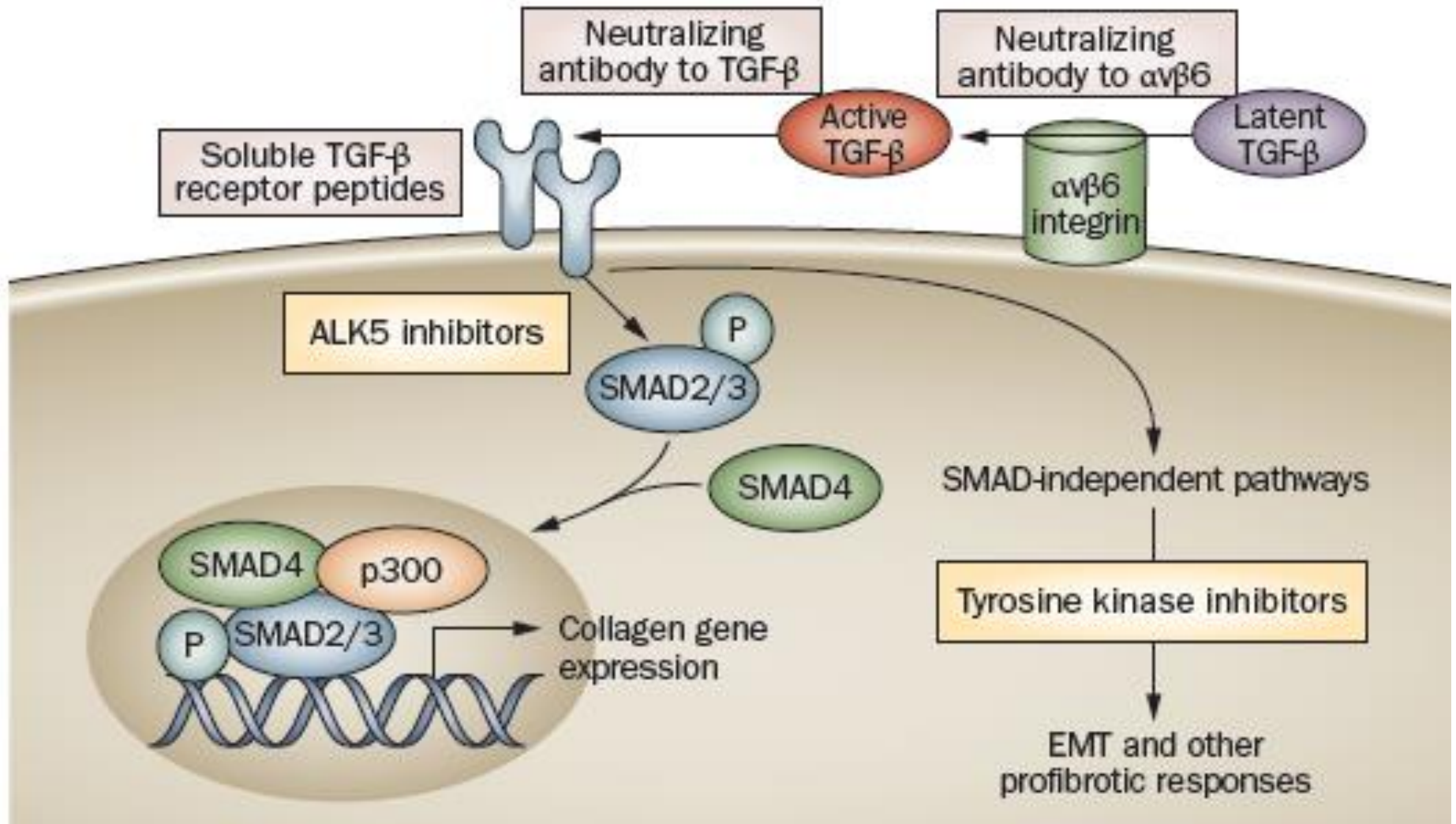
Transcription of genes encoding for:

Type I collagen

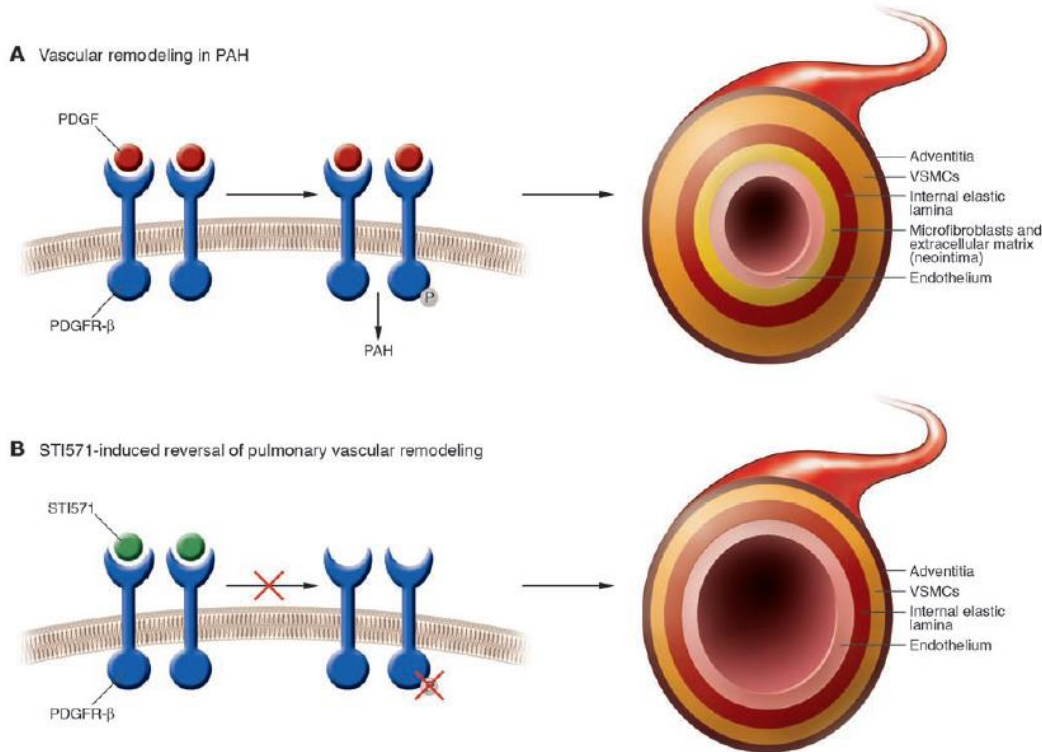
PDGF

CTGF

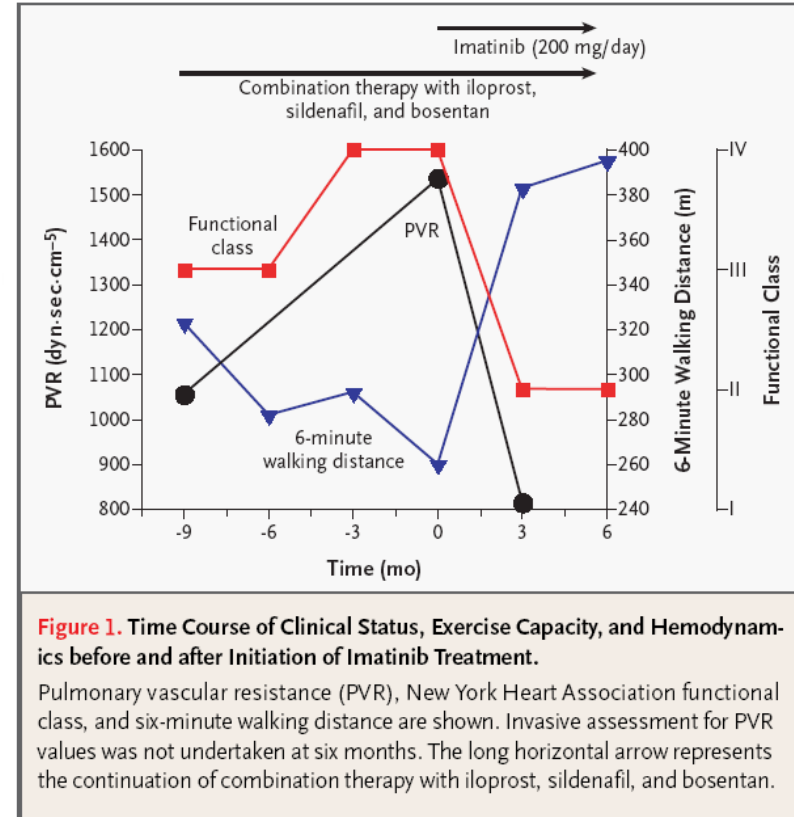
Proposed therapeutic strategies to block TGF- β



Imatinib inhibits PDGF signaling in PAH



Barst, J Clin Invest, 2005

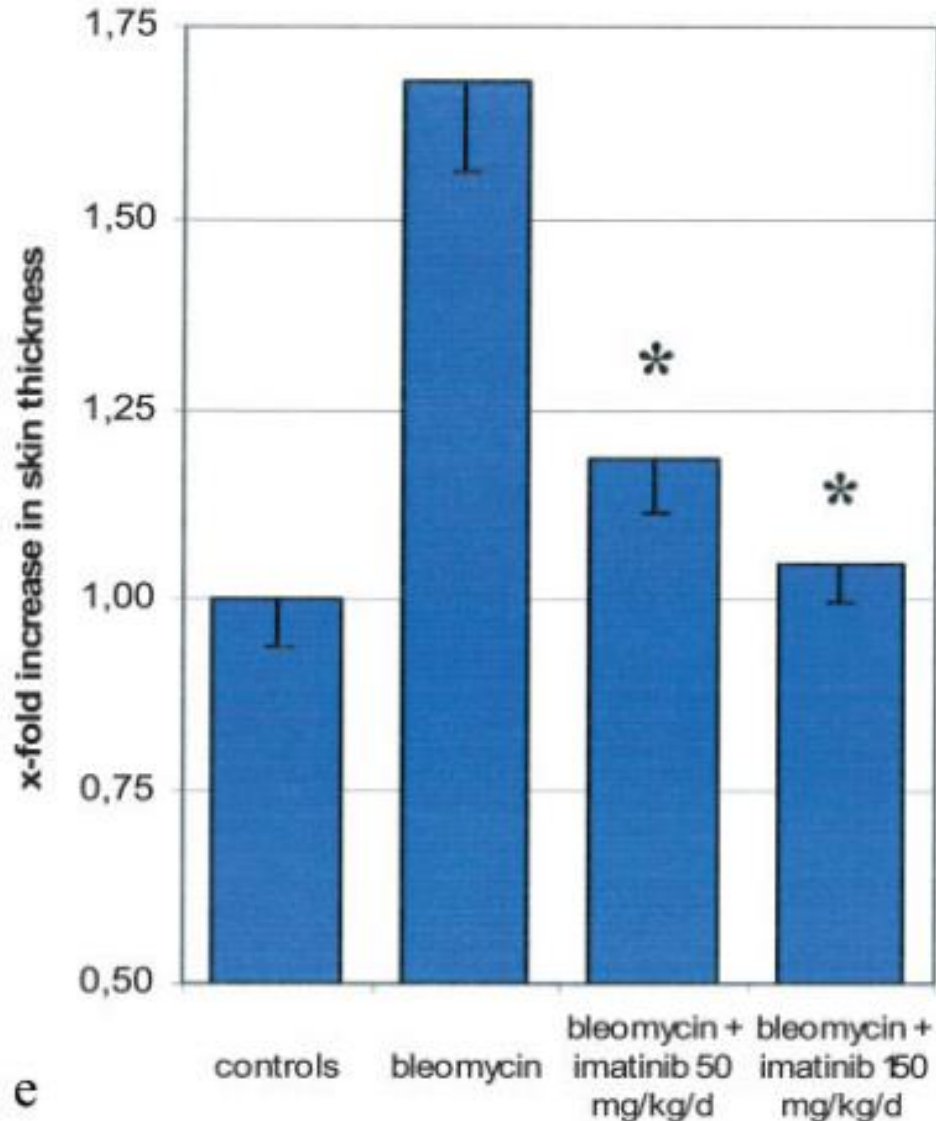


Ghofrani et al, N Engl J Med 2005
 Farber et al, Ann Int Med 2006
 Souza et al, Thorax 2006

Prospective randomized trial in PAH patients: negative on the primary end point

Ghofrani et al, ERS 2008

Reduction of the skin thickness in bleomycin induced dermal fibrosis by imatinib



e

Distler et al, A & R 2007

Novel therapies

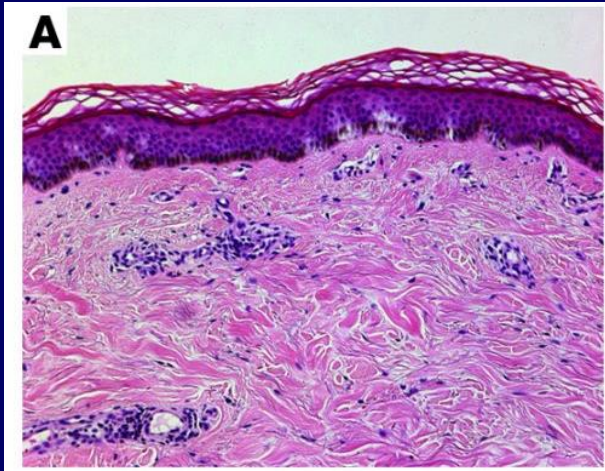
Inhibition of:

- SRC kinases
- Rho associated kinases (ROCK)
- Fos related antigen-2 (Fra2)
- Histone deacetylases
- DNA methyl transferases

Distler et al, A & R 2008

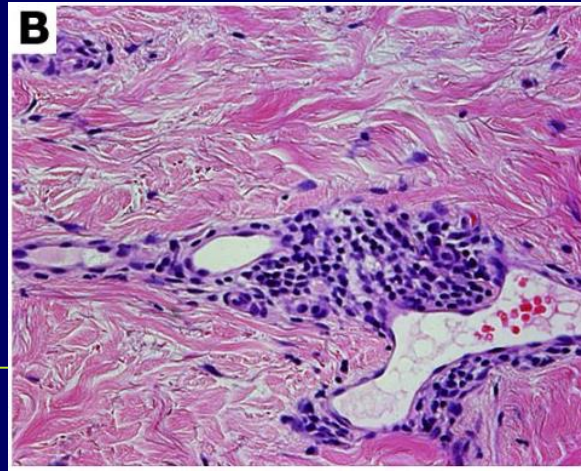
Distler et al, ACR meeting 2008

Skin inflammation and fibrosis in SSc



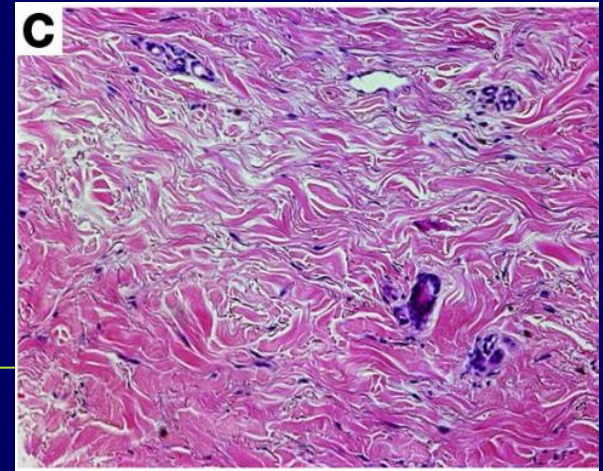
(A) Early diffuse cutaneous SSc

- Moderate fibrosis
- Inflammatory infiltrates in the dermis and near the dermal-epidermal junction, predominantly around small blood vessels



(B) Early-stage diffuse disease

- Profound dermal inflammation perivascular mononuclear cellular infiltrate
- Perivascular fibrosis and loss of pericytes and vessel integrity



(C) Established fibrosis

- Dermal thickening
- Loss of the microvasculature and dermal structures and the dermis-subcutaneous adipose tissue interface

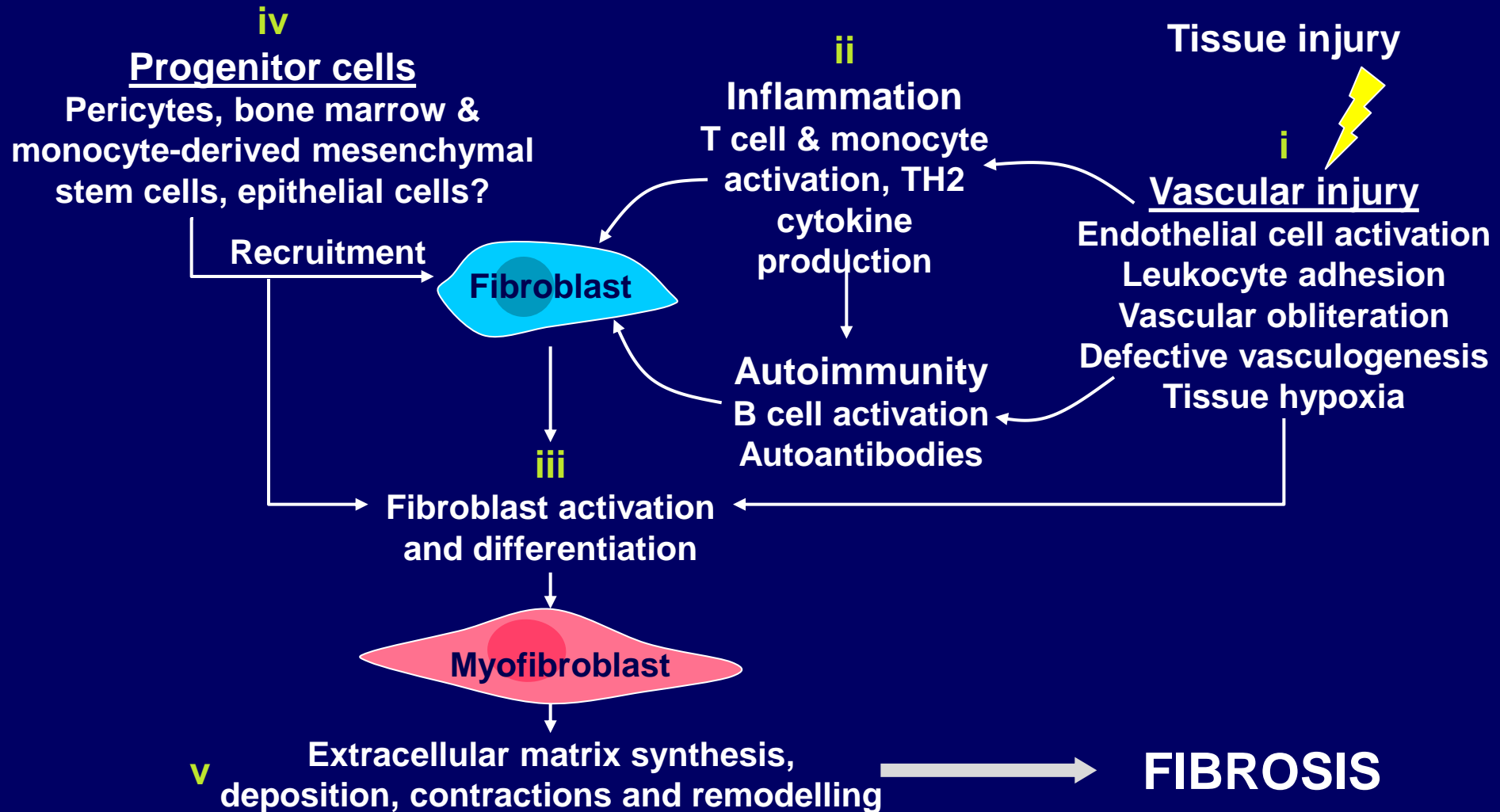
Elevated levels of cytokines in SSc

- ◆ **Growth factors**
 - **TGF- β , CTGF, VEGF**, FGF, etc
- ◆ **Interleukins**
 - IL-2, **IL-4**, IL-6, IL-10, **IL-13**, etc
- ◆ **Chemokines**
 - MCP-1, IL-8 (CXCL8), TARC, fractalkine, etc
- ◆ **Other cytokines**
 - TNF- α , etc

CTGF = connective tissue growth factor; FGF = fibroblast growth factor; IL = interleukin; MCP = monocyte chemoattractant protein; TARC = thymus and activation-regulated chemokine; TGF = tumour growth factor; TNF = tumour necrosis factor; VEGF = vascular endothelial growth factor

Slide courtesy of Kazuhiko Takehara.

Integration of vasculopathic and immunological processes leading to fibrosis



CYTOKINES I

TGF- β

TGF- β , chef d'orchestre de la régulation de la fibrogénèse, l'angiogénèse, la régulation immunitaire, prolifération et différenciation cellulaire *(Blobe GC, NEJM, 2000)*

TGF- β , produits par CE, les monocytes, les lymphocytes T *(Blobe GC, NEJM, 2000)*

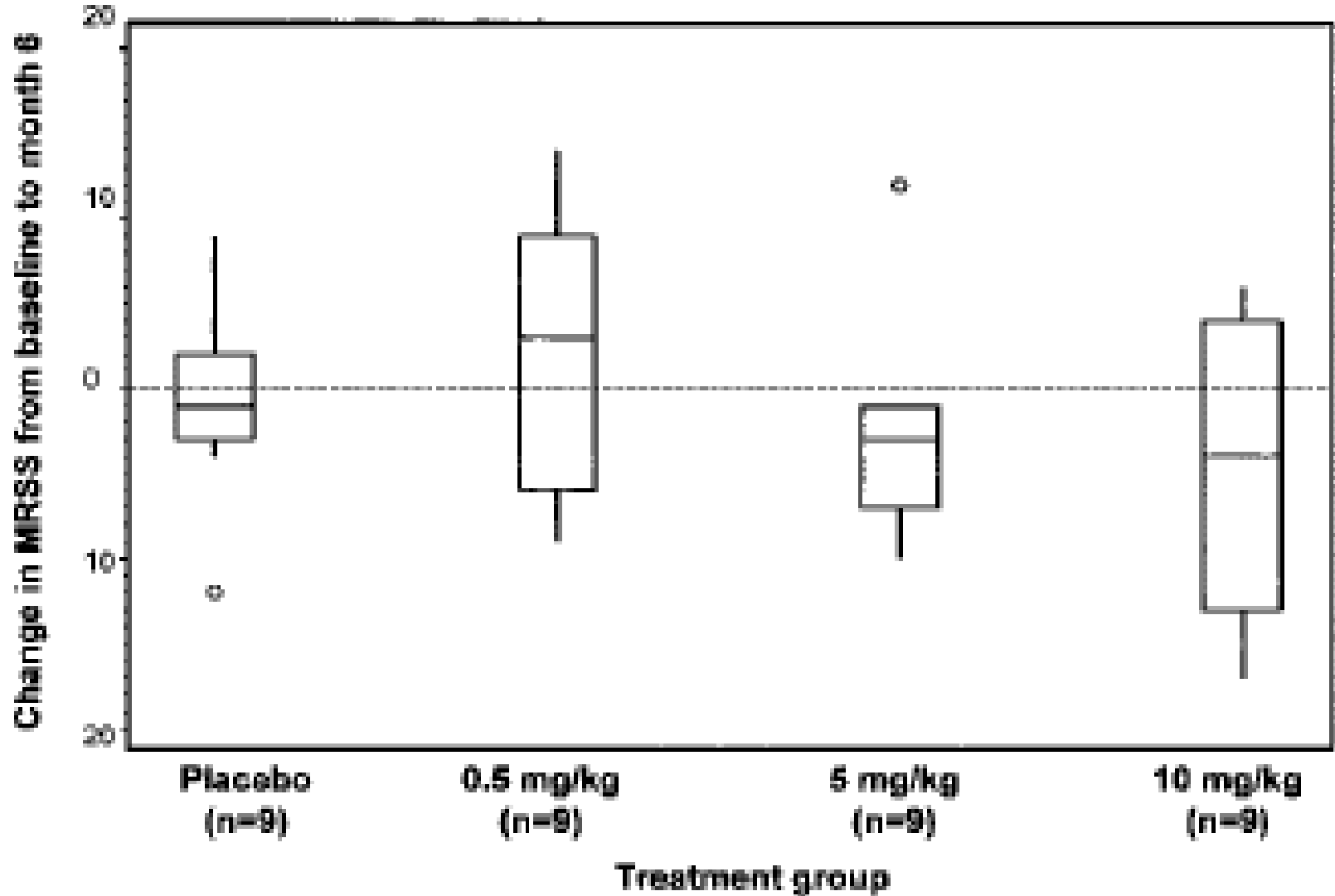
TGF- β induits la différenciation des fibroblastes en myofibroblastes *(Kawakami T, J Invest Dermatol 1998)*

PDGF

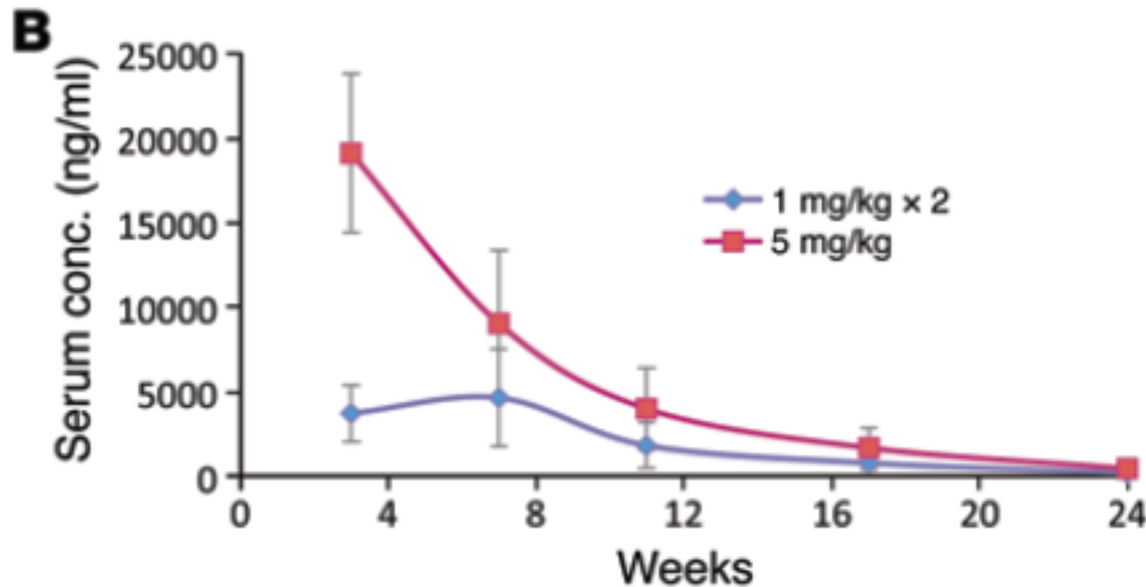
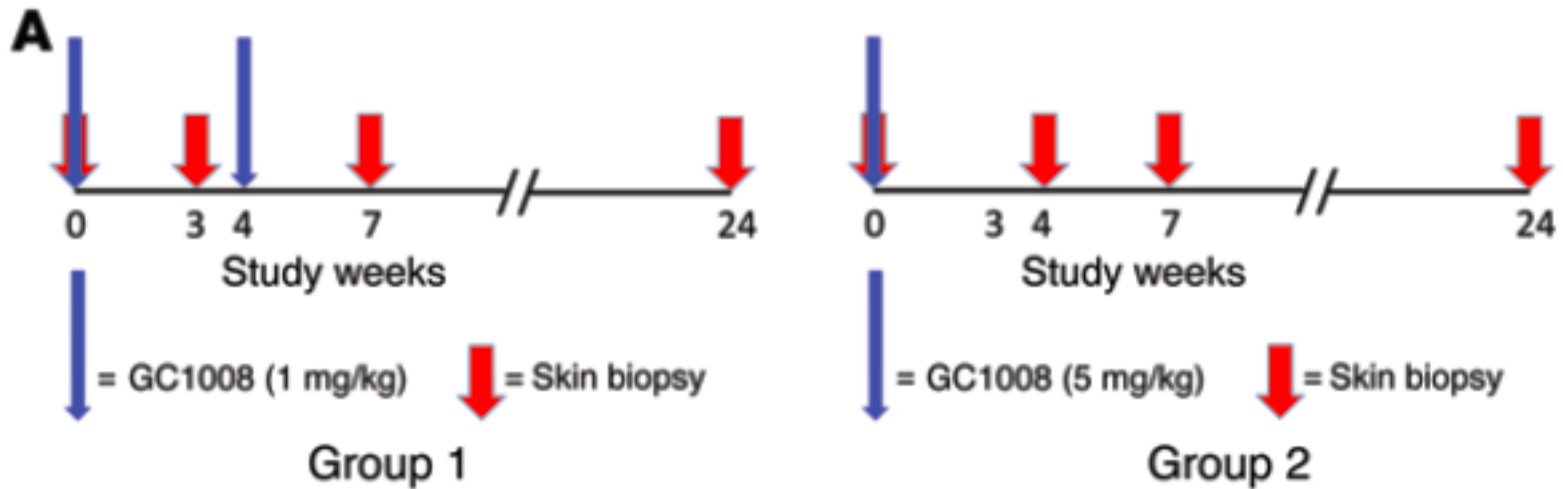
PDGF produits par plaquettes, macrophages, CE, fibroblastes

PDGF induit prolifération activation des fibroblastes: synthèse de collagène, fibronectine, MCP1, IL-6 *(Gay S, J Invest Dermatol 1989)*

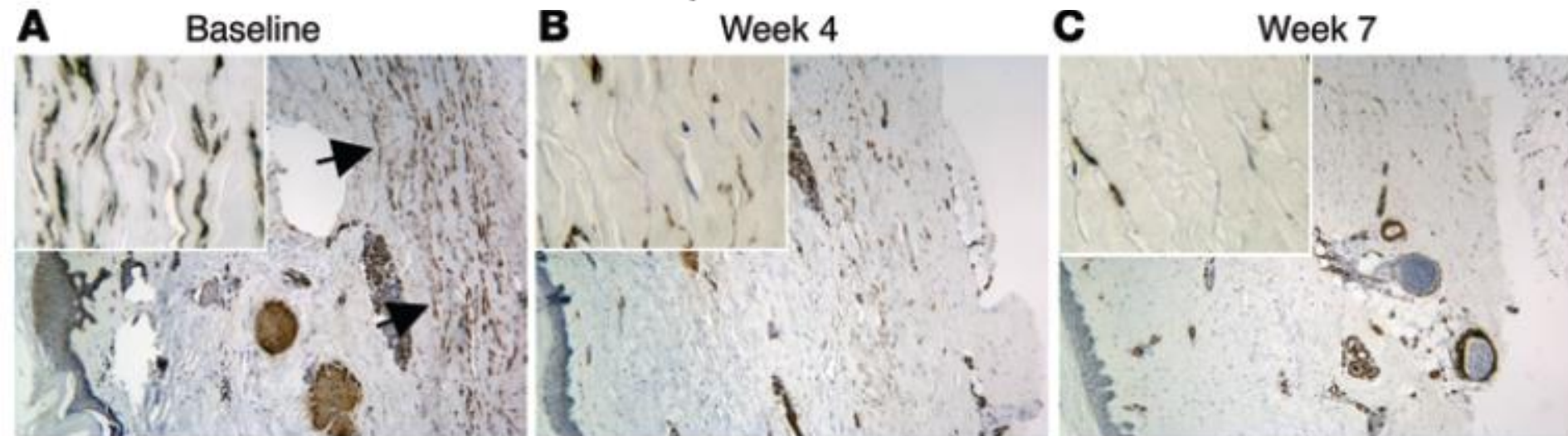
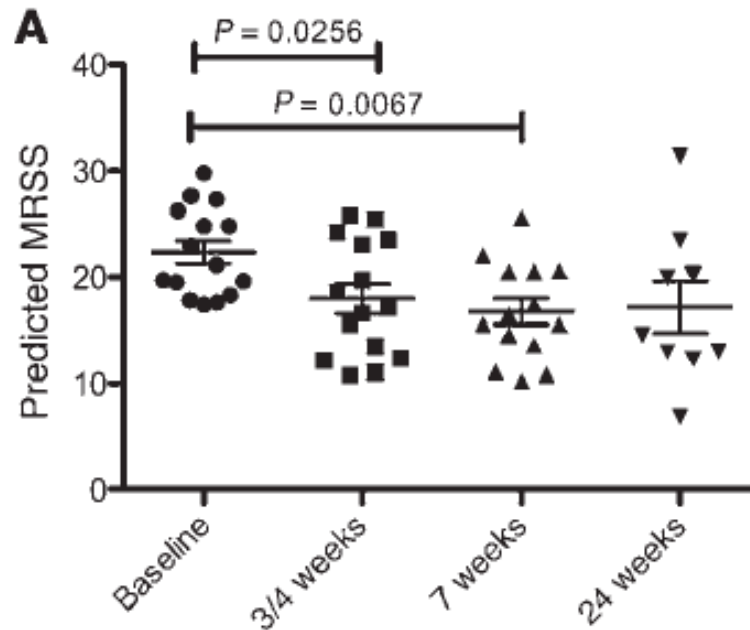
Change in the modified Rodnan skin score from baseline to month 6 in patients with diffuse cutaneous SSc treated with placebo or with 3 different doses (0.5, 5, or 10 mg/kg) of CAT-192.



Fresolimumab treatment decreases biomarkers and improves clinical symptoms in SSc patients



Fresolimumab treatment decreases biomarkers and improves clinical symptoms in SSc patients



Fresolimumab treatment decreases biomarkers and improves clinical symptoms in SSc patients

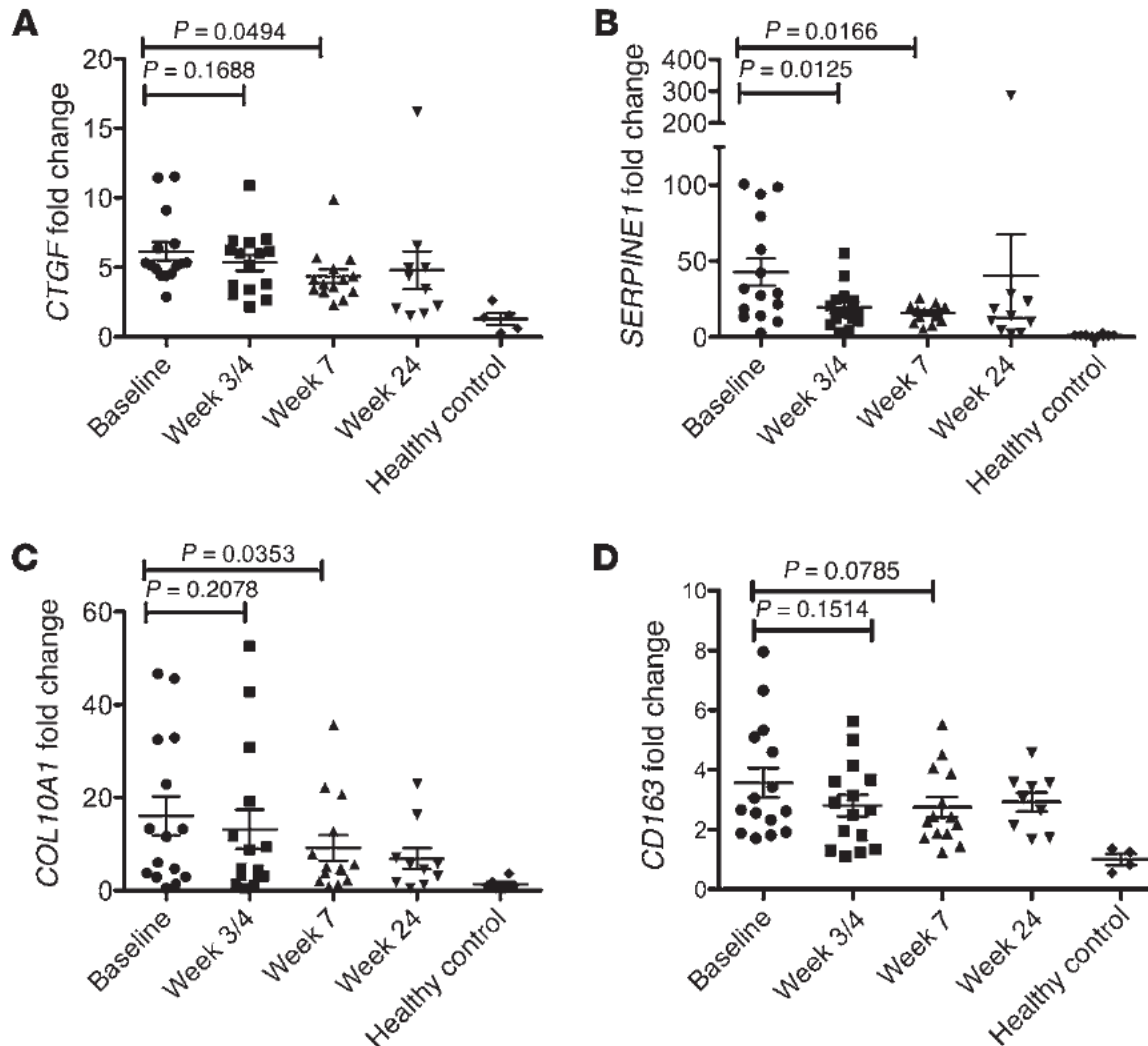
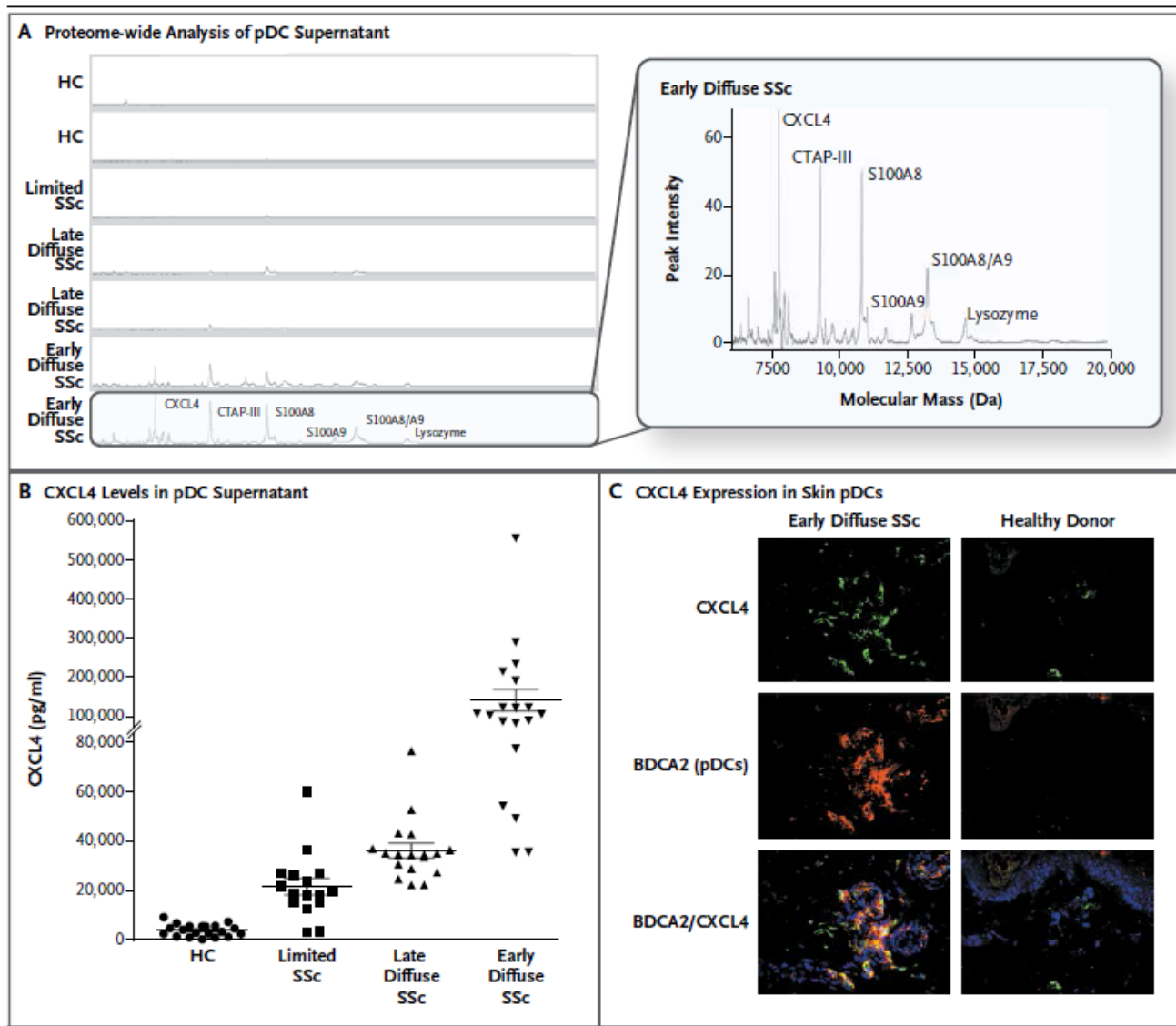


Figure 5. Changes in gene expression before and after fresolimumab treatment. Skin biopsy RNAs were analyzed for expression of TGF- β -regulated genes, (A) *CTGF*, (B) *SERPINE1*, (C) and *COL10A1*, and a macrophage marker, (D) *CD163*, at baseline and after fresolimumab treatment. Levels from 5 healthy controls skin samples are also shown. *CTGF*, *SERPINE1*, and *COL10A1* mRNA expression was assayed by RT-PCR; *CD163* expression was assayed by NanoString. Statistical significance was assessed by Wilcoxon signed-rank. Error bars indicate SEM.

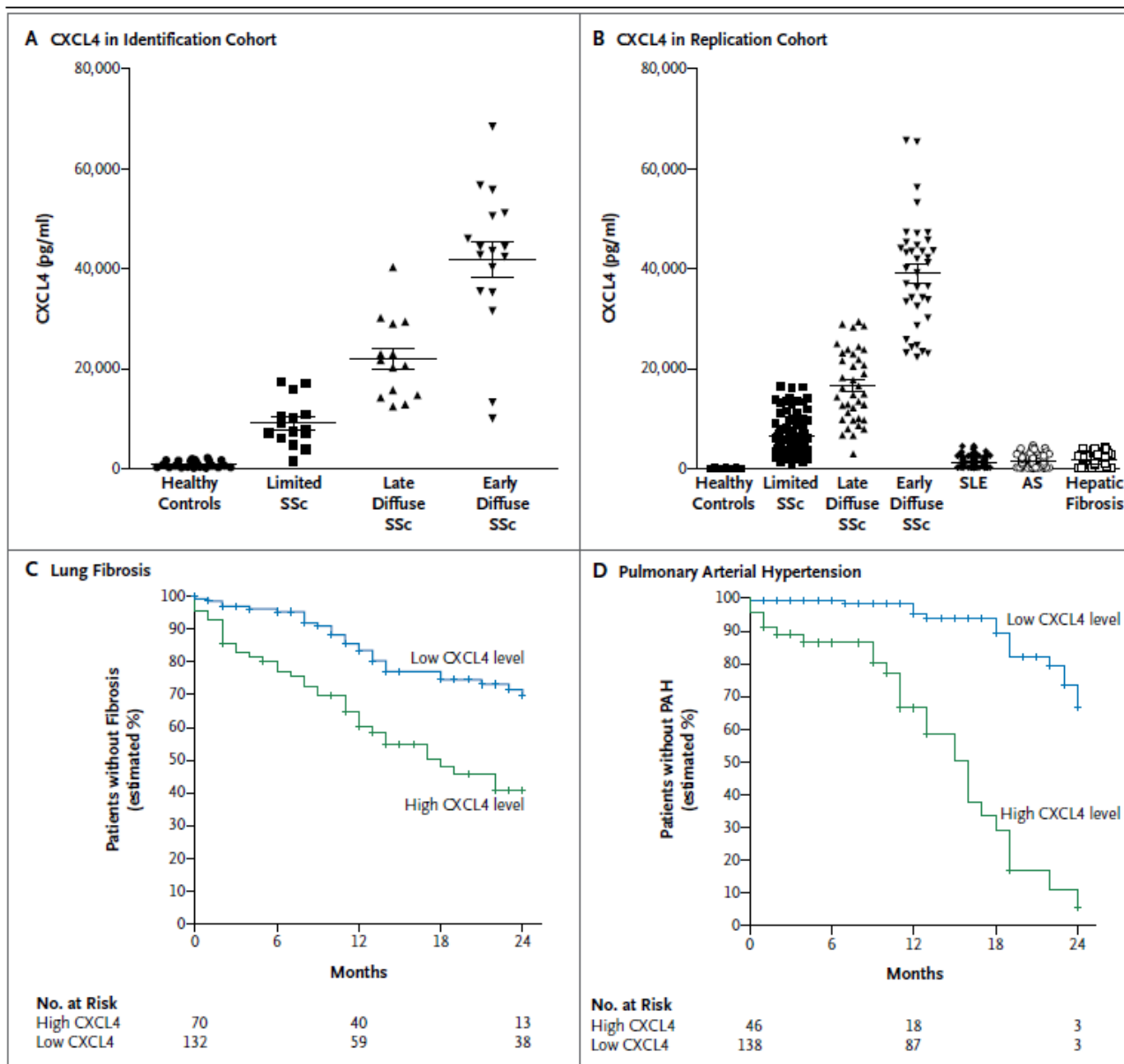
Proteome-wide Analysis and CXCL4 as a Biomarker in Systemic Sclerosis

L. van Bon, A.J. Affandi, J. Broen, R.B. Christmann, R.J. Marijnissen, L. Stawski, G.A. Farina, G. Stifano, A.L. Mathes, M. Cossu, M. York, C. Collins, M. Wenink, R. Huijbens, R. Hesselstrand, T. Saxne, M. DiMarzio, D. Wuttge, S.K. Agarwal, J.D. Reveille, S. Assassi, M. Mayes, Y. Deng, J.P.H. Drenth, J. de Graaf, M. den Heijer, C.G.M. Kallenberg, M. Bijl, A. Loof, W.B. van den Berg, L.A.B. Joosten, V. Smith, F. de Keyser, R. Scorza, C. Lunardi, P.L.C.M. van Riel, M. Vonk, W. van Heerde, S. Meller, B. Homey, L. Beretta, M. Roest, M. Trojanowska, R. Lafyatis, and T.R.D.J. Radstake

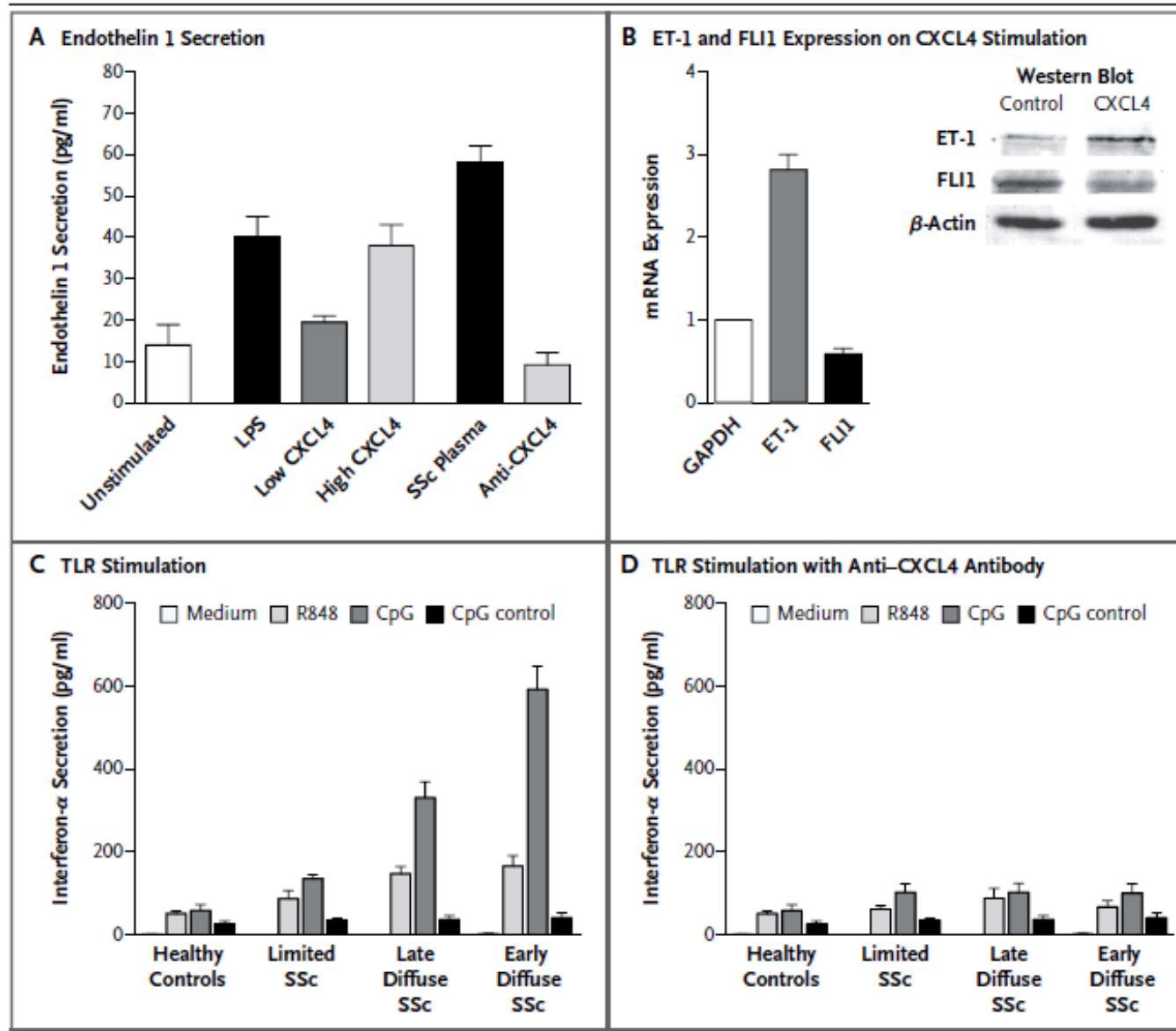
Identification of CXCL4 as the Major Protein Product of Plasmacytoid Dendritic Cells in Systemic Sclerosis.



Increased Levels of Circulating CXCL4 in Systemic Sclerosis and the Association with Lung Fibrosis and PAH

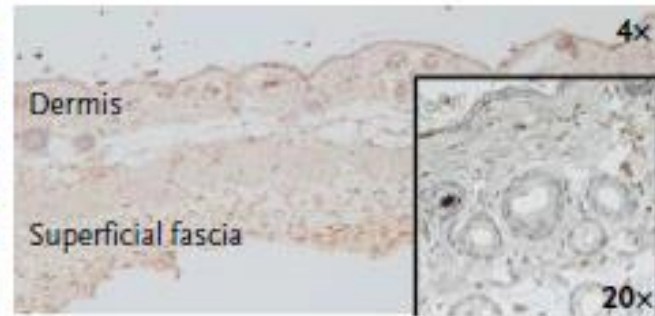
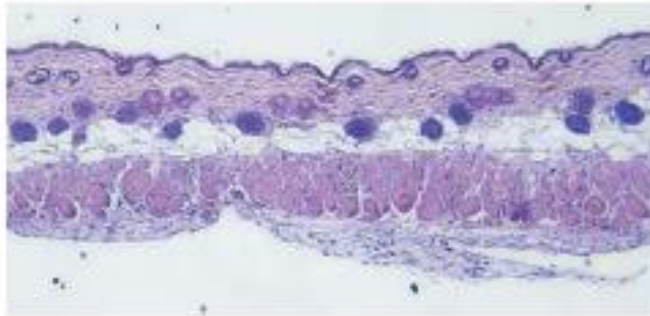


Changes in Endothelial Cells and Augmented Responses in Toll-Like Receptors Induced by CXCL4.

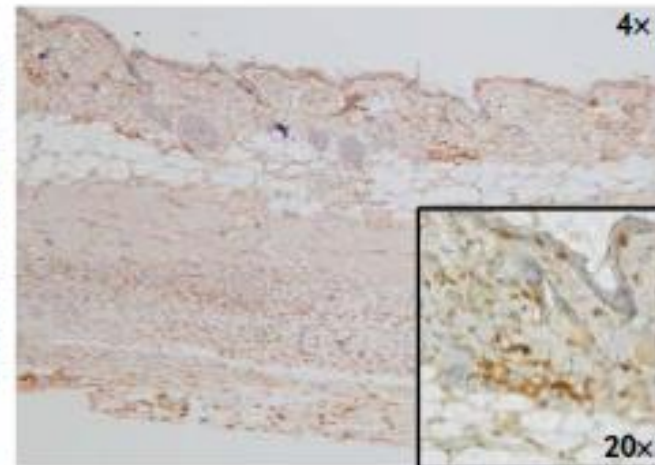
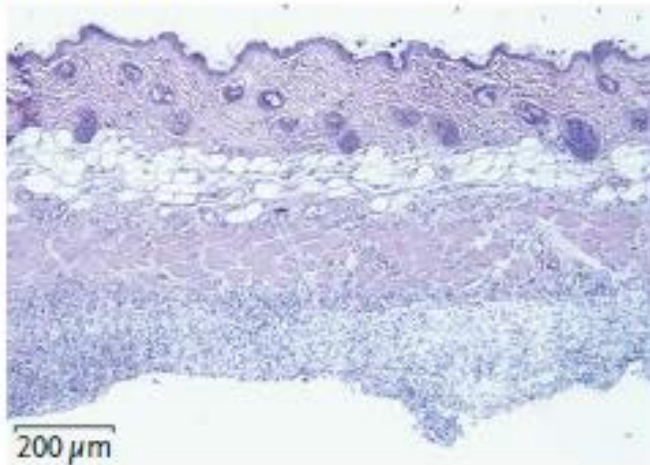


Inflammatory Skin Changes Mimicking Those in Systemic Sclerosis Induced by CXCL4 In Vivo in Mice.

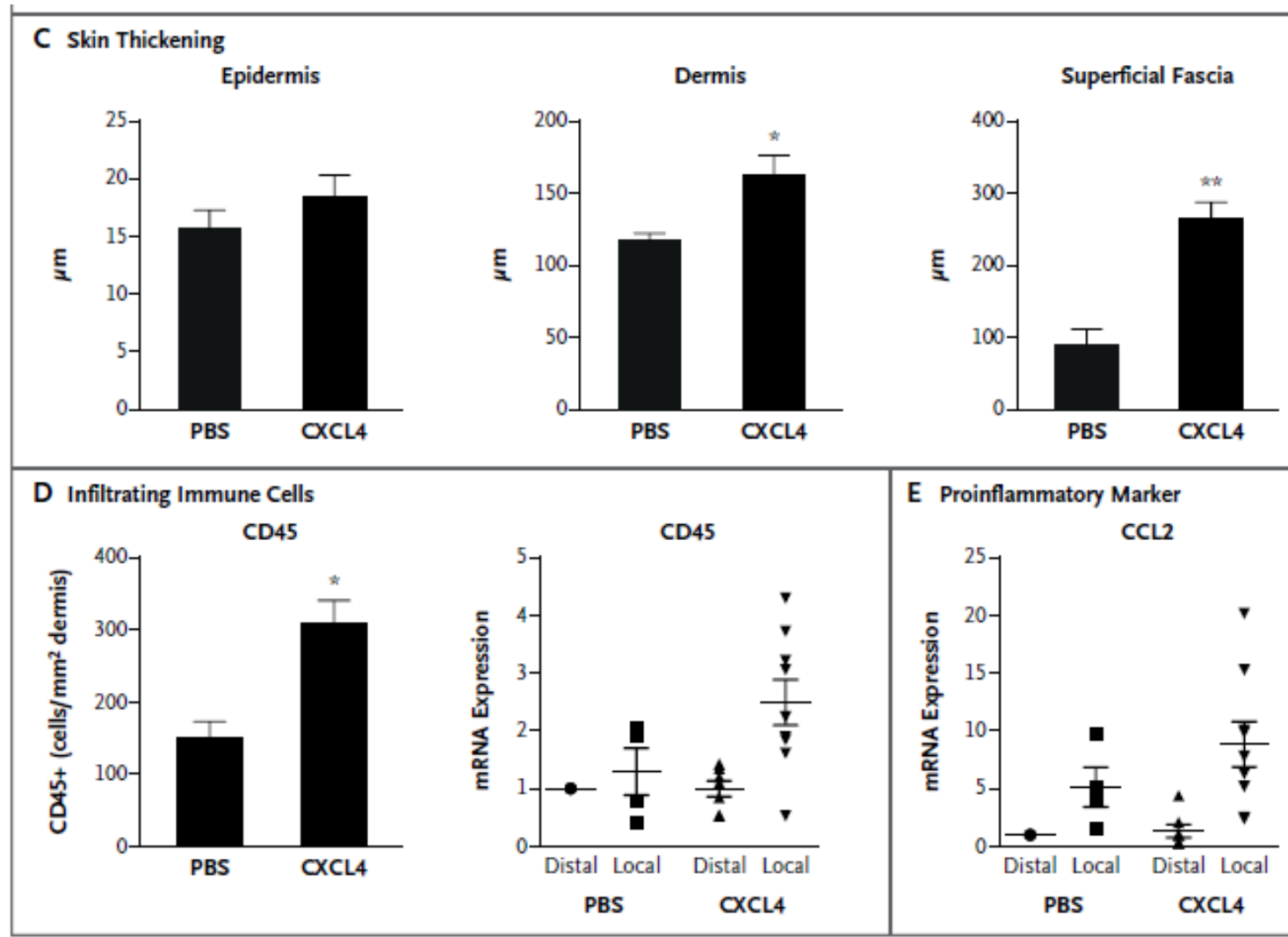
A Murine Exposure to PBS



B Murine Exposure to CXCL4



Inflammatory Skin Changes Mimicking Those in Systemic Sclerosis Induced by CXCL4 In Vivo in Mice.



T cell activation in SSc

- ◆ T cell activation in blood
 - Soluble IL-2R level correlated with the extent of skin fibrosis¹
 - Clonal expansion of blood T cells²
- ◆ T cell activation in skin
 - Oligoclonal T cell expansion in the skin³
 - Enhanced transendothelial migration of CD4⁺ T cells⁴
- ◆ Pronounced Th17 profile in SSc; intracellular expression of TGFβ and IFNγ distinguishes SSc phenotypes

1. Steen VD, et al. *J Rheumatol* 1996; 23:646-9

2. French LE, et al. *Arch Dermatol* 2001; 137:1309-13

3. Sakkas LI, et al. *J Immunol* 2002; 168:3649-59

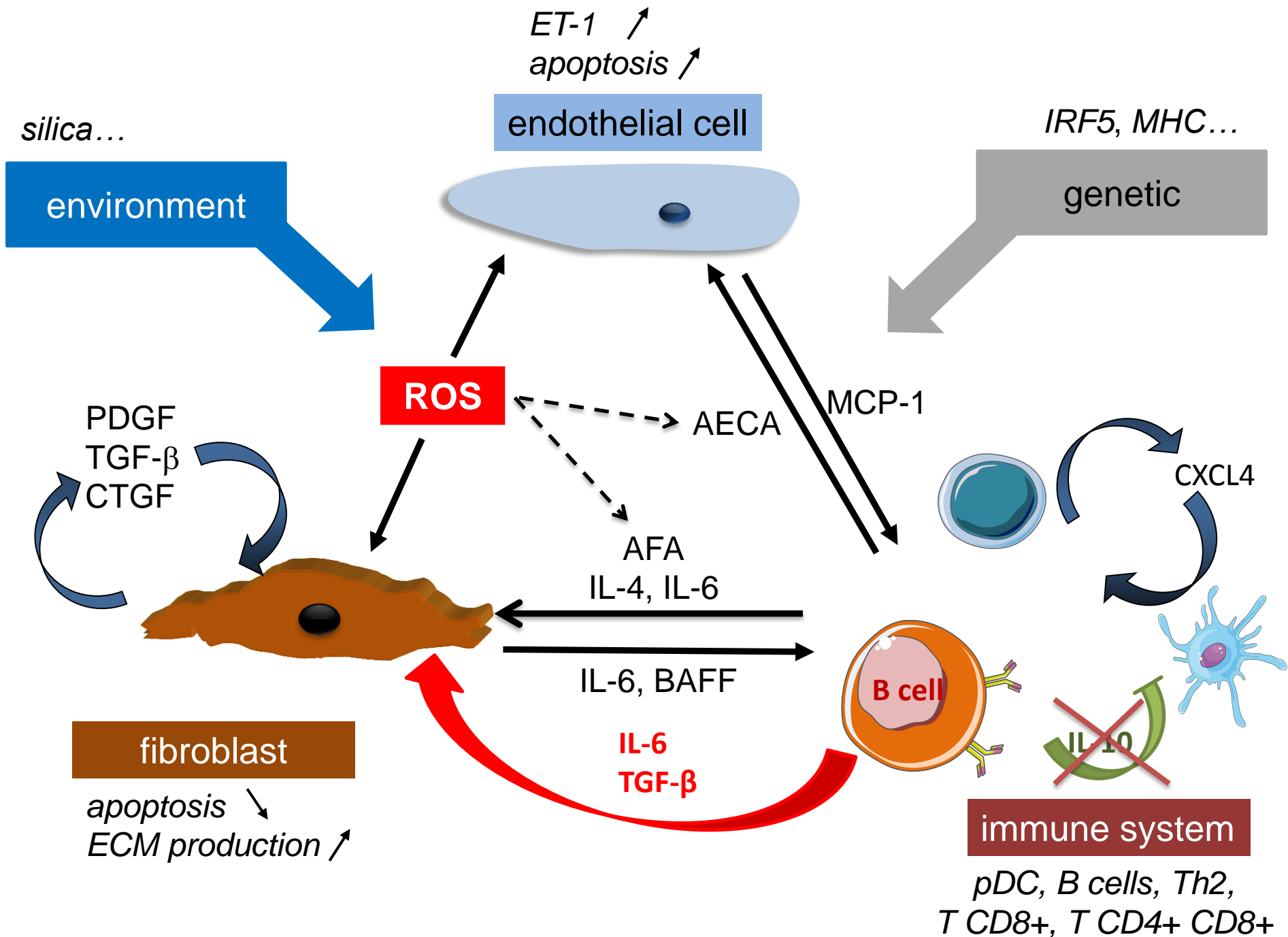
4. Stummvoll GH, et al. *Ann Rheum Dis* 2004; 63:569-74

Radstake, et al. *Plos One* 2009

SSc: involvement of B lymphocytes

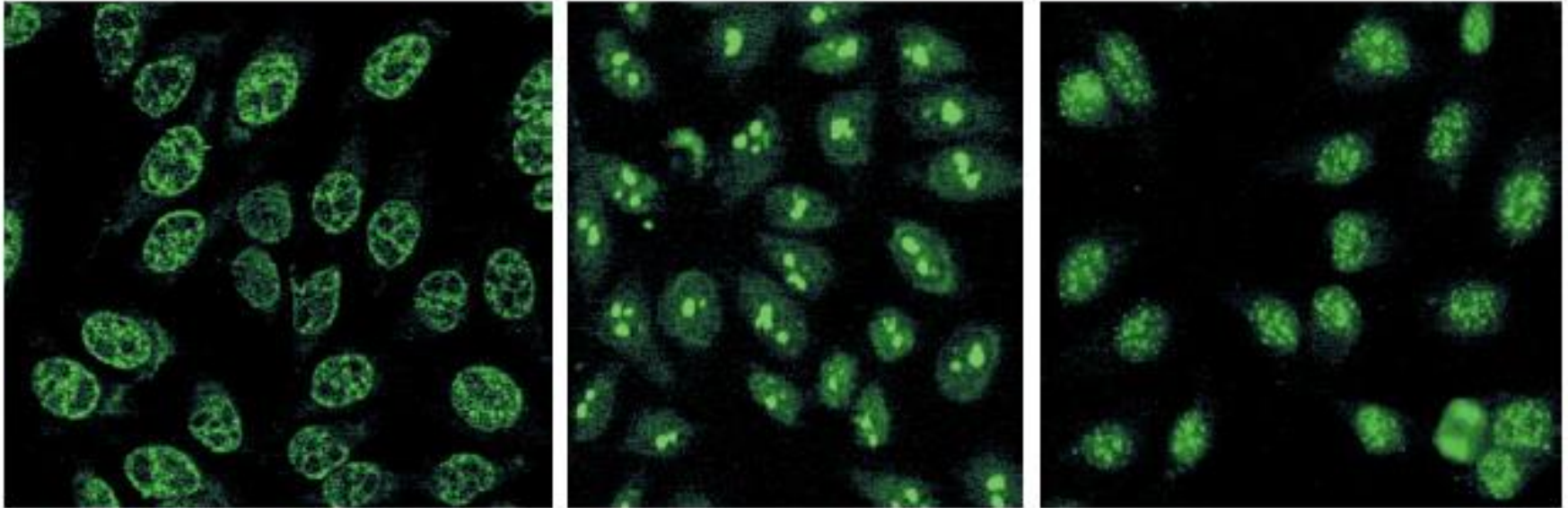
- ◆ Abnormal B cell signalling in TSK/+ mice¹
- ◆ **Presence of B cells in skin² and in lungs from SSc patients³**
- ◆ Expanded naive B cells and diminished but activated memory B cells⁴
- ◆ Presence of serum autoantibodies and elevated serum levels of cytokines such as **IL-6** which correlate with skin fibrosis
- ◆ **Elevated serum BAFF levels correlate with disease severity⁵**
- ◆ Preliminary results from pilot studies in SSc patients with rituximab^{2,6}

1. Saito E, et al. *J Clin Invest* 2002; 109:1453–62.
2. Bosello, et al. *Arthritis Res Ther* 2010; 12:R54.
3. Lafyatis R, et al. *Arthritis Rheum* 2007; 56:3167–8.
4. Sato S, et al. *Arthritis Rheum* 2004; 50:1918–27.
5. Matsushita T, et al. *Arthritis Rheum* 2006; 54:192–201.
6. Lafyatis R, et al. *Arthritis Rheum* 2009, 60:578-83.



Autoantibodies in scleroderma

A



B

Classic Autoantibodies	Clinical Features	New Autoantibodies	Role
Anti-topoisomerase I	Diffuse cutaneous scleroderma	Anti-endothelial cell	Induce apoptosis of endothelial cells
Anticentromere proteins	Limited cutaneous scleroderma, pulmonary hypertension	Anti-FBN 1	Activate normal human fibroblasts
Anti-RNA polymerase I/II	Diffuse cutaneous scleroderma, renal involvement	Anti-MMP 1 and 3	Prevent degradation of ECM proteins
Antipolymyositis, sclerosis	Polymyositis, calcinosis	Anti-PDGFR	Stimulate normal human fibroblasts through Ha-Ras-ERK1/2-ROS
Antifibrillar (U3RNP)	Diffuse cutaneous scleroderma, internal-organ involvement	Anti-Nag-2	Induce endothelial-cell apoptosis
Anti-Th/To	Limited cutaneous scleroderma, pulmonary fibrosis		

SSc: origin of autoantibodies

- ◆ Molecular mimicry (topo I and CMV)¹
- ◆ Polyclonal B cell activation with excess of **IL-4**
- ◆ **Fragmentation of autoantigens** by metalloproteinases, favoured by hypoxia² and by mercury chloride³
- ◆ Selective **oxidation** of DNA topoisomerase 1 induces SSc in the mouse⁴
- ◆ A subset of SSc patients shows a “lupus-like” high **IFN- α** inducible gene expression pattern⁵

1. Lunardi C, et al. *Nat Med* 2000; 6:1183-6.

2. Casciola-Rosen L, et al. *J Exp Med*. 1997; 185:71-9.

3. Arnet F. 1990.

4. Servettaz, et al. *J Immunol* 2009; 182:5855-64..

5. Assassi S, et al. *Arthritis Rheum* 2010; 62:589–98.

Anti-RNA polymerase III

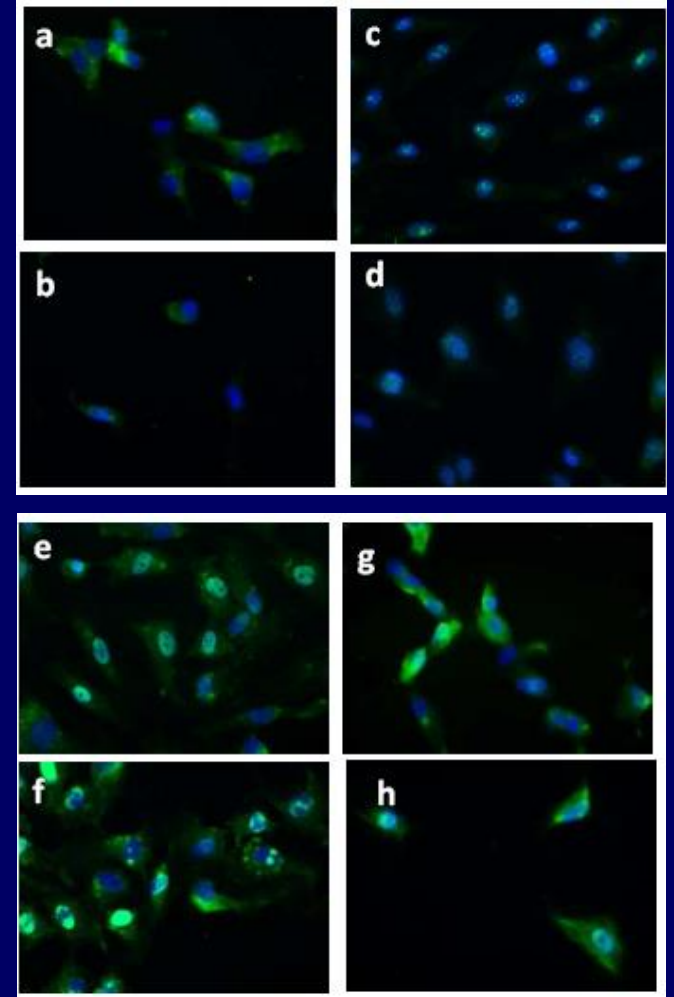
Key Points

- The importance of SSc-associated antibodies in the diagnosis and classification of SSc is now recognized. Autoantibodies are incorporated into the 2013 ACR/EULAR clinical classification criteria for SSc.
- The prevalence of SSc-associated antibodies varies by geographic region, although the internal organ associations are similar across all populations, consistent with earlier studies. New associations include digital ulcers with ACA positivity, and GAVE with RNAP.
- Anti-RNA polymerase III antibodies have now been linked to malignancy in three separate SSc cohort studies, with the cancers occurring during the period close to the diagnosis of SSc.

Anti-endothelial cell antibodies (AECA) in SSc

- **Not disease specific**
- **Absence of standardization**
- **Activate EC and induce the expression of adhesion molecules (IL-1 dependent)¹**
- **Induce apoptosis in the presence of NK cells²**
- **Cross-reactivity of AECA with a CMV protein³**
- **Target antigens unknown except "scleroderma specific" autoantigens^{4,5}**

6. Ab: controls; cd: ssc w/o PAH; ef: SSc-PAH; gh: IPAH



1. Carvalho D. *Arthr Rheum* 1999. 2. Bordron A. *J Clin Invest* 1998.
3. Lunardi C, et al. *Nat Med* 2000. 4. Garcia de la Pena et al. *Clin Immunol* 2004.
5. Servettaz et al. *Clin Immunol* 2006. 6. Dib H, et al. *Eur Resp J* 2011

Anti-fibroblast Abs in SSc

- Anti-fibroblast antibodies (AFA) are present in the serum of 20 to 80% of SSc patients¹
- AFA can activate fibroblasts and induce extracellular matrix proteins synthesis²
- Induce a proadhesion fibroblast phenotype by up-regulating ICAM-1 and increase fibroblast synthesis of pro-inflammatory cytokines
- AFA induce fibroblasts to produce profibrotic chemokines, with partial exploitation of TLR4³
- Target antigens
 - DNA topoisomerase 1⁴
 - PDGF receptor⁵

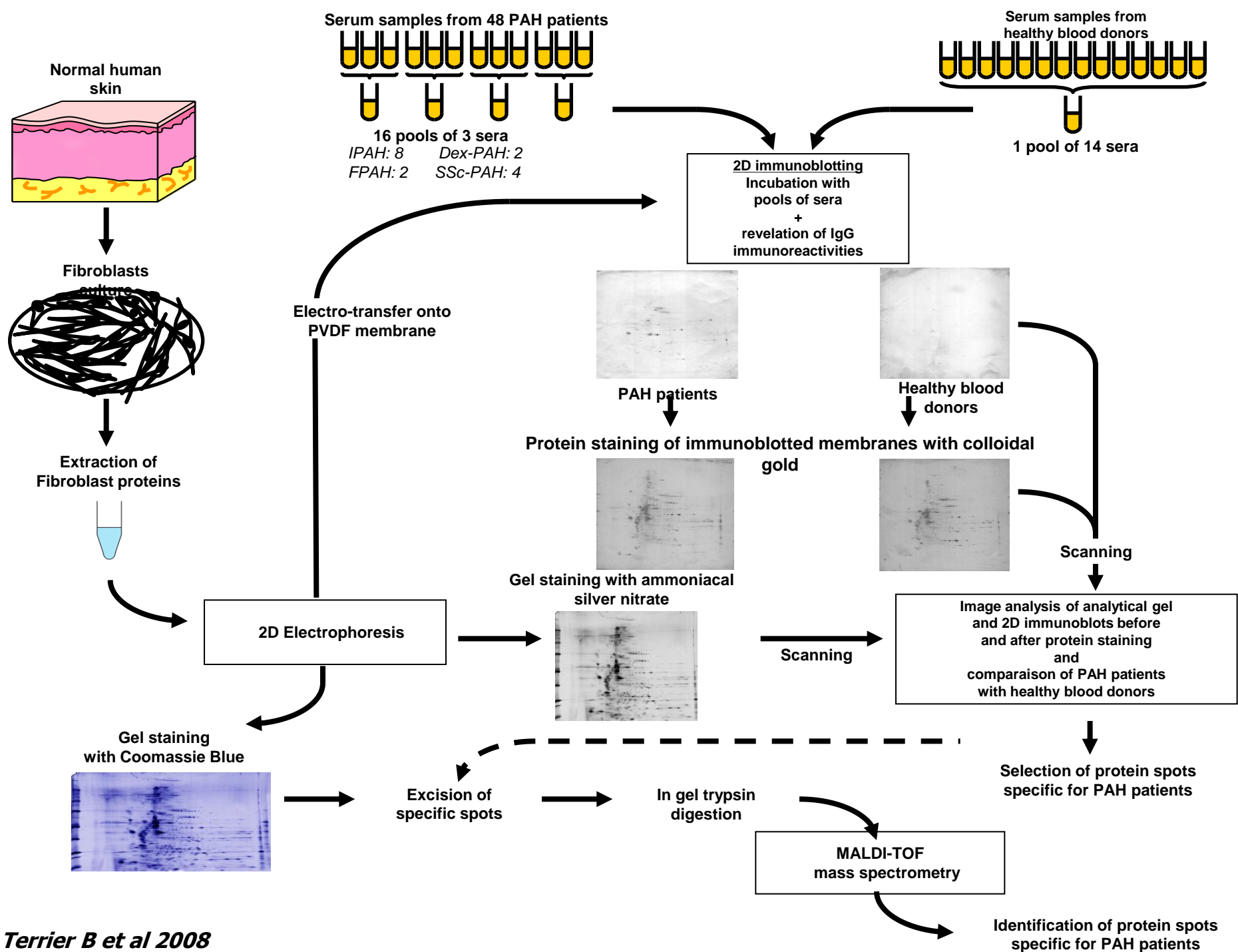
1. Brentnall, 1982; Chizzolini, 2002; Alderuccio, 1989; Ronda, 2002.

2. Chizzolini C. *Arthritis Rheum* 2002.

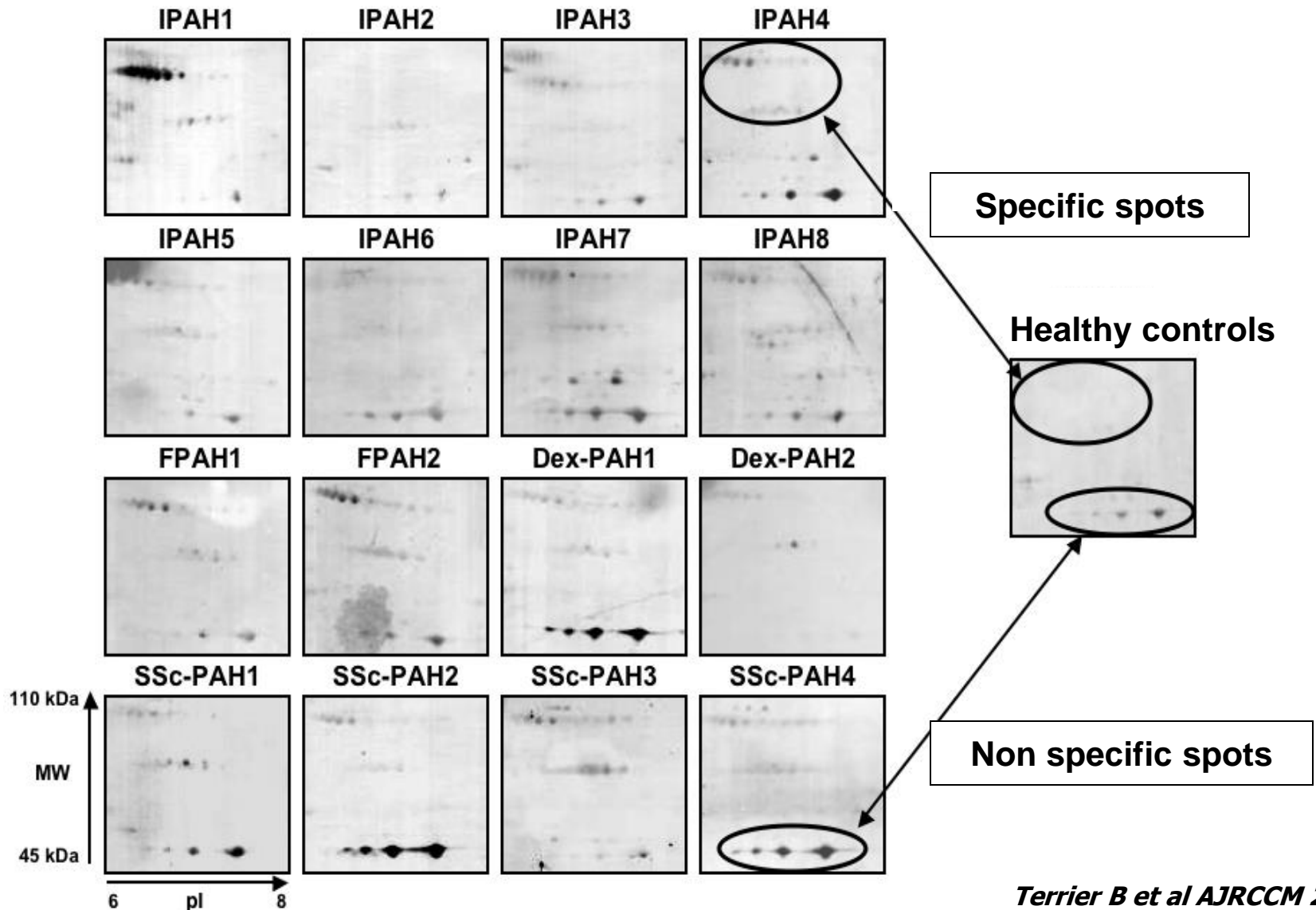
3. Fineschi S. *Arthritis Rheum* 2008.

4. Henault G. *Arthritis Rheum* 2004; Henault G. *Arthritis Rheum* 2006; Tamby MC *et al.* 2008.

5. Baroni S, *et al.* *NEJM* 2006; Classen, *et al.* 2009; Loizos, *et al.* 2009.



Fibroblasts: selection of protein spots



Identification of target antigens of anti-fibroblast Abs in idiopathic and systemic sclerosis associated pulmonary arterial hypertension

➤ Organization of cytoskeleton and cell contraction

- ✓ Phosphatidyl inositol 3-kinase
- ✓ Vimentin
- ✓ Calumenin
- ✓ Tropomyosine 1

➤ Oxydative stress

- ✓ G6PD
- ✓ HSP27
- ✓ HSP70

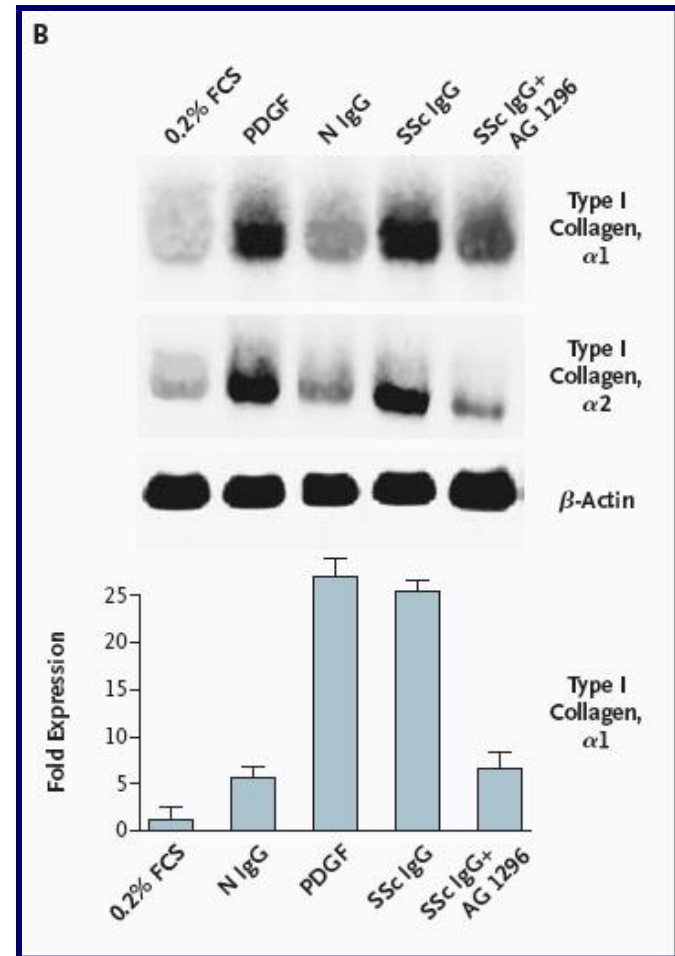
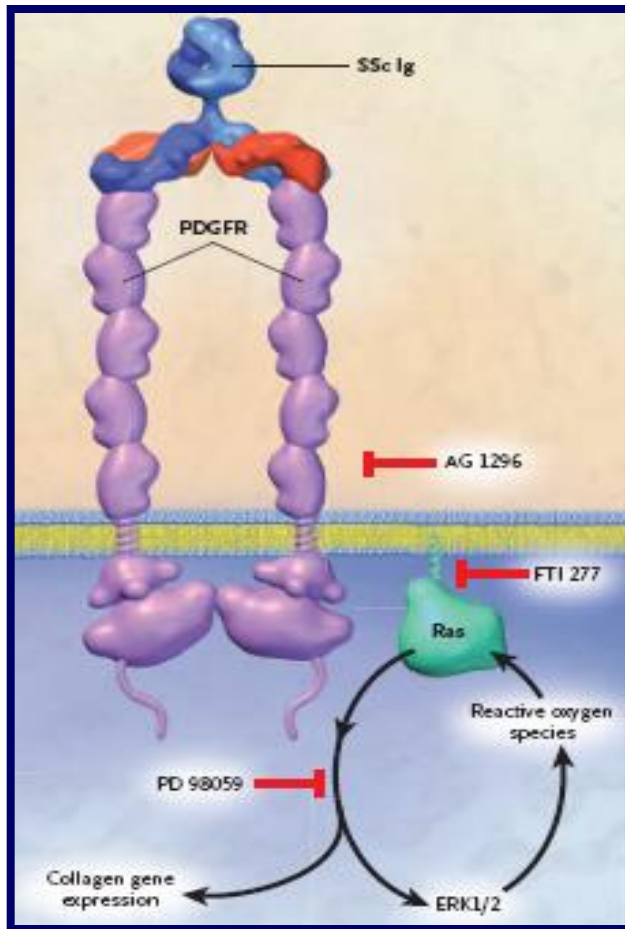
➤ Protein metabolism

- ✓ Glutaminase
- ✓ alanine-glyoxylate amino-transferase2
- ✓ glutamate carboxy-peptidase

➤ Others

- ✓ death-associated protein kinase
- ✓ P61-YES
- ✓ protein Jade-2
- ✓ Kelch-like ECH
- ✓ zinc finger protein 51
- ✓ bromodomain testis-specific protein

ANTICORPS ANTI-PDGFR

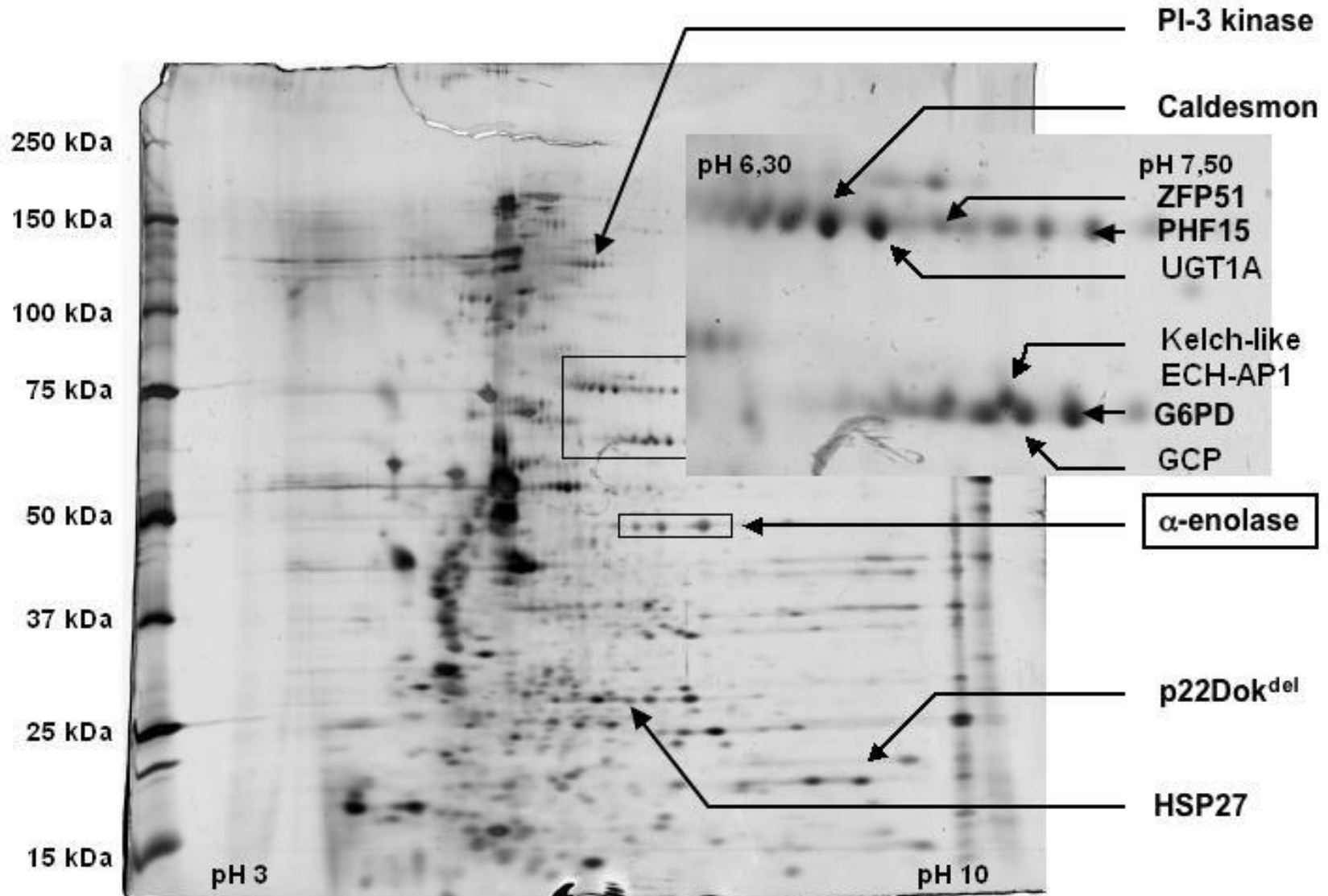


Les IgG sériques stimulent le récepteur de PDGF, qui stabilise RAS et induit ERK1/2

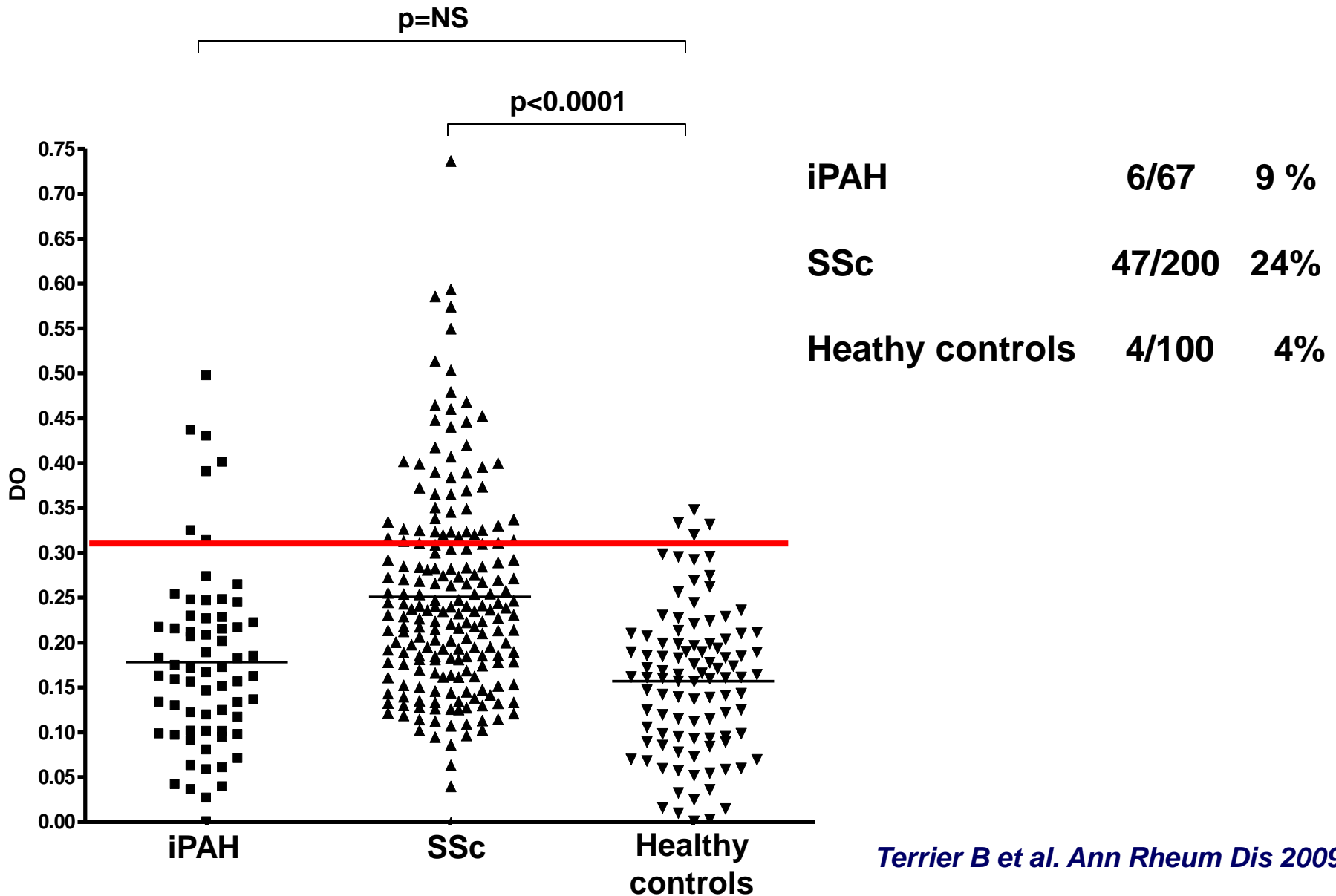
L'induction de ERK1/2 entraîne la production de FRO (ROS)

La persistance à long terme de ROS et ERK1/2 entraîne une augmentation de l'expression du gène du collagène

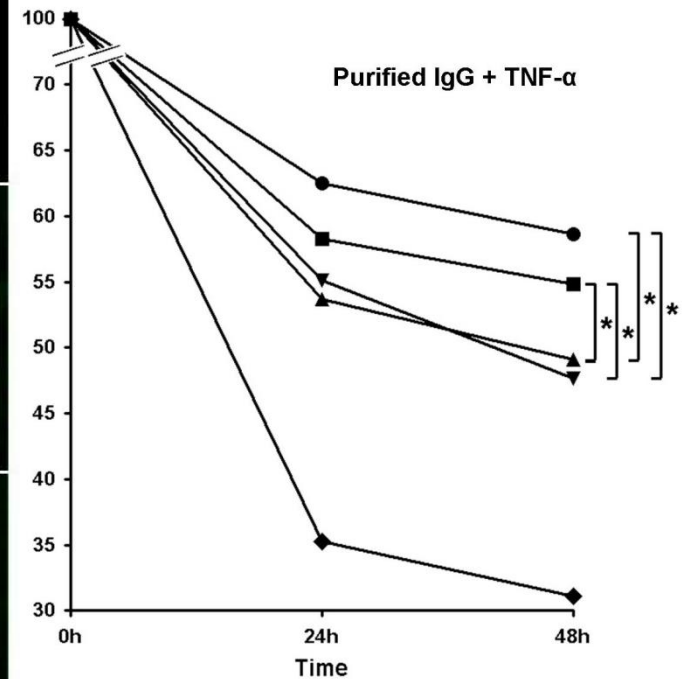
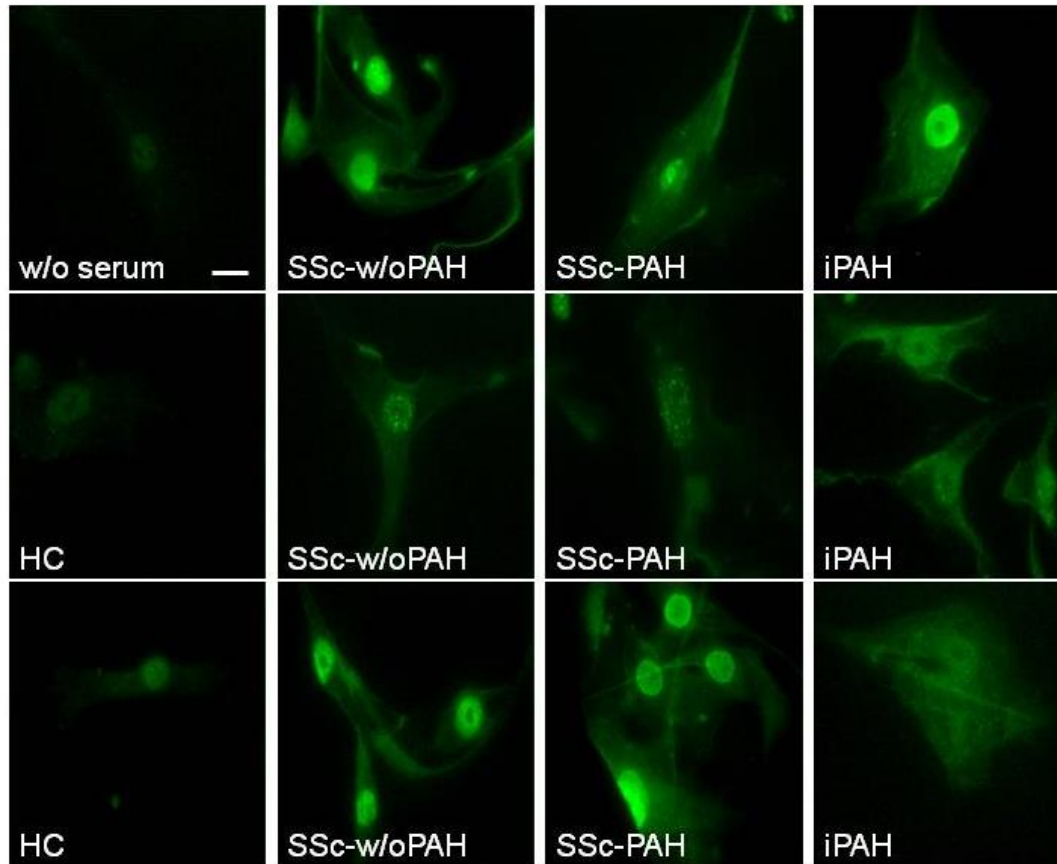
Anti-fibroblast antibodies from systemic sclerosis patients bind to α -enolase



Anti-fibroblast antibodies from systemic sclerosis patients bind to α -enolase



Indirect immunofluorescence on permeabilized human aortic vascular smooth muscle cells, with sera from HC or with sera from SSc-w/oPAH, SSc-PAH and iPAH.



Inhibition of contraction



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Rare Systemic and
Autoimmune Diseases

