Sclérodermie systémique: physiopathologie

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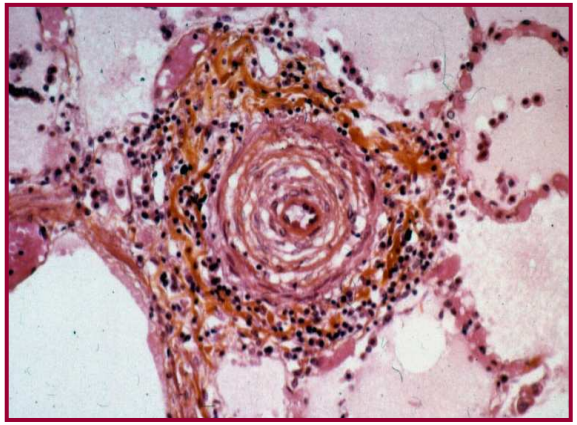
Conflicts of interest

- **Consultant**: Actelion, CSL Behring, LFB Biotechnologies, Lilly, Pfizer, Octapharma
  - Financial support to ARMIIC
- **Investigator**: Actelion, CSL Behring, Pfizer
- **Financial support (grants to ARMIIC)**: Actelion, CSL Behring, GSK, LFB Biotechnologies, Pfizer
- **Invited conference**: SOBI, Roche, Actelion, CSL Behring, Octapharma, GSK, LFB Biotechnologies, Pfizer, Lilly, UCB pharma

SCLÉRODERMIE SYSTÉMIQUE

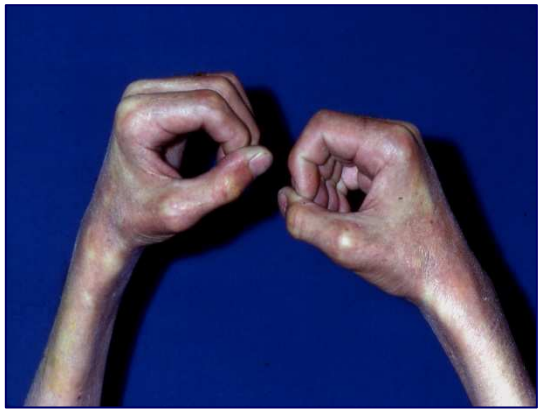
➢Hyperréactivité vasculaire

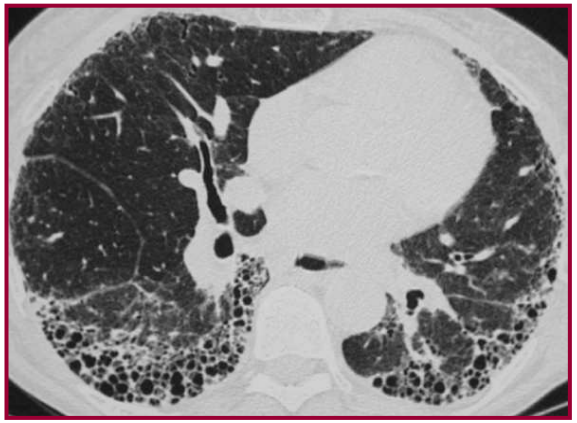
Phénomène de Raynaud
Crise Rénale
Hypertension artérielle pulmonaire



➢Fibrose

Peau
Poumon
Appareil digestif
Coeur

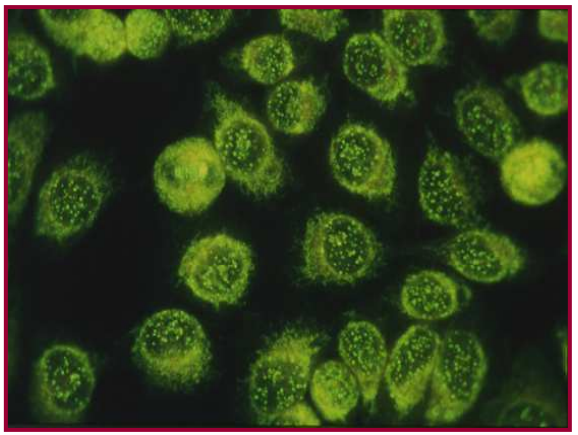


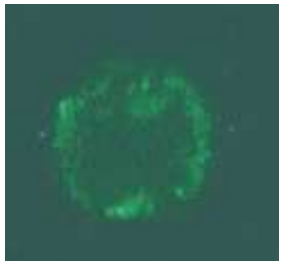


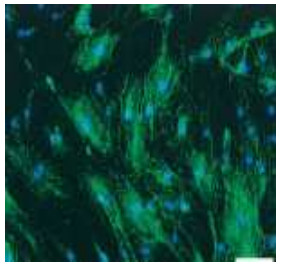
➢Autoimmunité

Autoanticorps
- Anti-Scl70
- Anti-centromère
- Anti-ARNPolIII

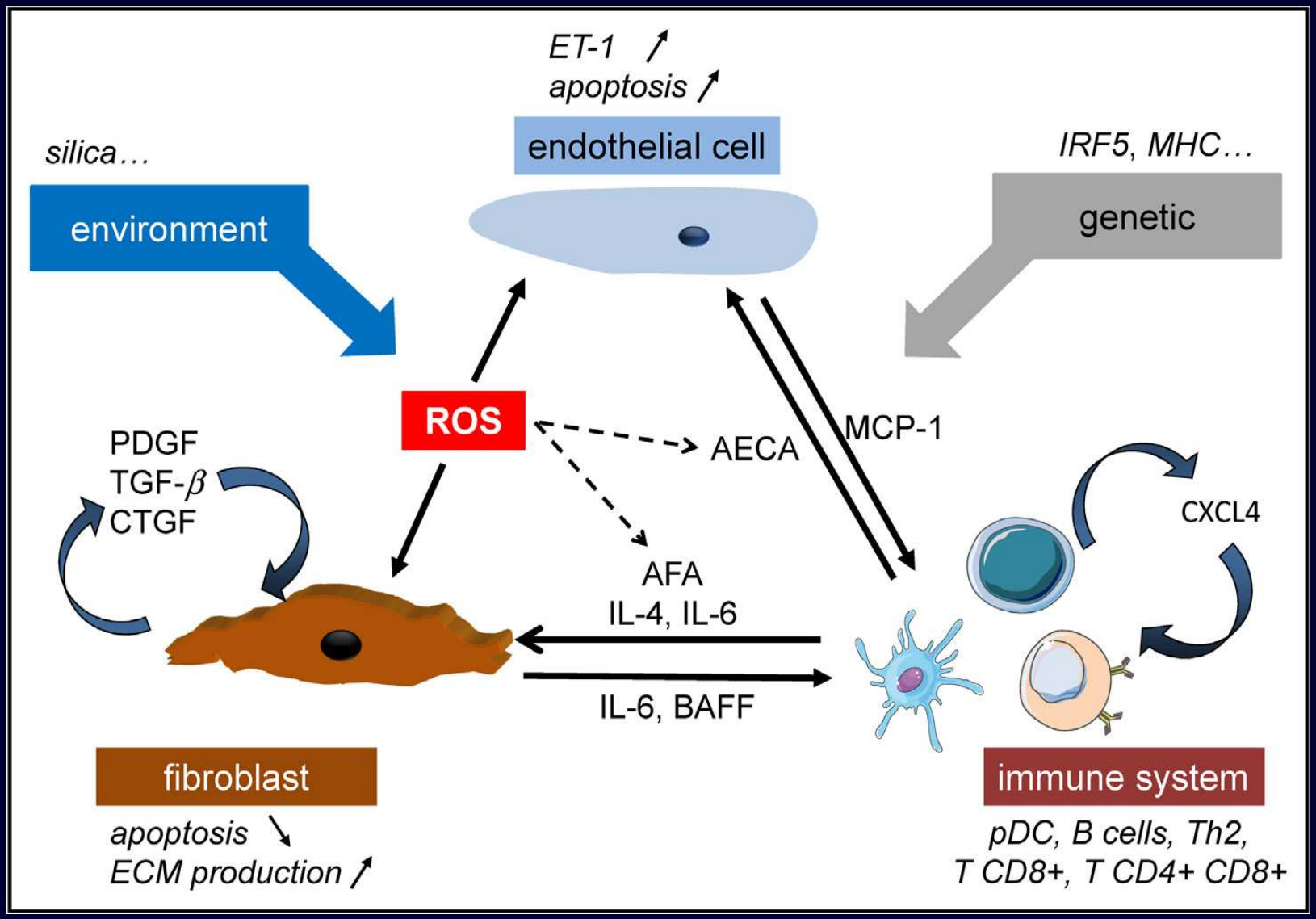
Ac anti-fibroblastes







Systemic sclerosis: pathophysiology



Dumoitier N et al. Presse Med 2014

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	***Fibrinogen***	*Tumour necrosis factor alpha-induced protein 3 (TNFAIP3);*
Interleukine-1α et 1β	***Stromal cell-derived factor 1 (SDF-1/CXCL12):)***	*Interleukin-23 receptor*
Matrix metalloproteinase (MMP)		**Innate immunity: IRF5, control of IFN production**

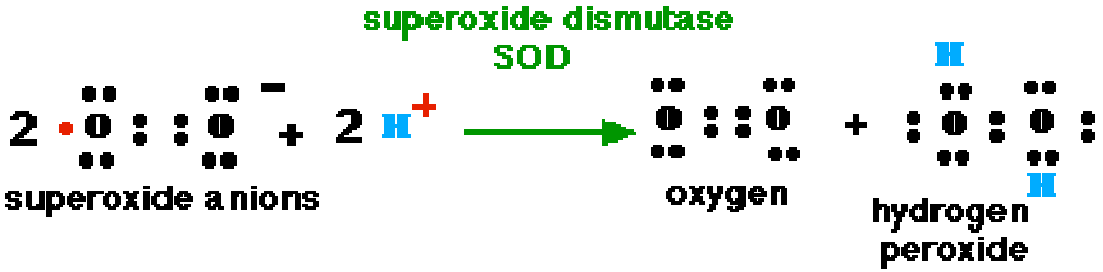
Romano E, Clin Exp Rheumatol, 2011

Animal models of SSc

Model	Fibrosis	Inflammation	Vasculopathy	Autoimmunity
Tsk/1		-	-	
Tsk/2			-	
Mutant fibrillin transgene		-	-	
Bleomycin induced			-	-
TGF-βRII DN			-	-
Fli1 -/-		-		-
Conditional TGF-βRI				-
Fra2 (Fos related Ag-2)				-
Oxydation of DNA topoisomerase 1				
UCD-200 chicken				

RÔLE ÉMERGENT DU STRESS OXYDATIF

NADPH oxidase
myeloperoxidase



Arguments directs:

synthèse d'O2 •– par les monocytes et des fibroblastes de sujets atteints de ScS *(SAMBO, J Invest Dermatol. 1999)*

Prolifération des fibroblastes et production de collagène dépendante de FRO dans la ScS
(SAMBO, Arthritis Rheum, 2001)

Arguments indirects:

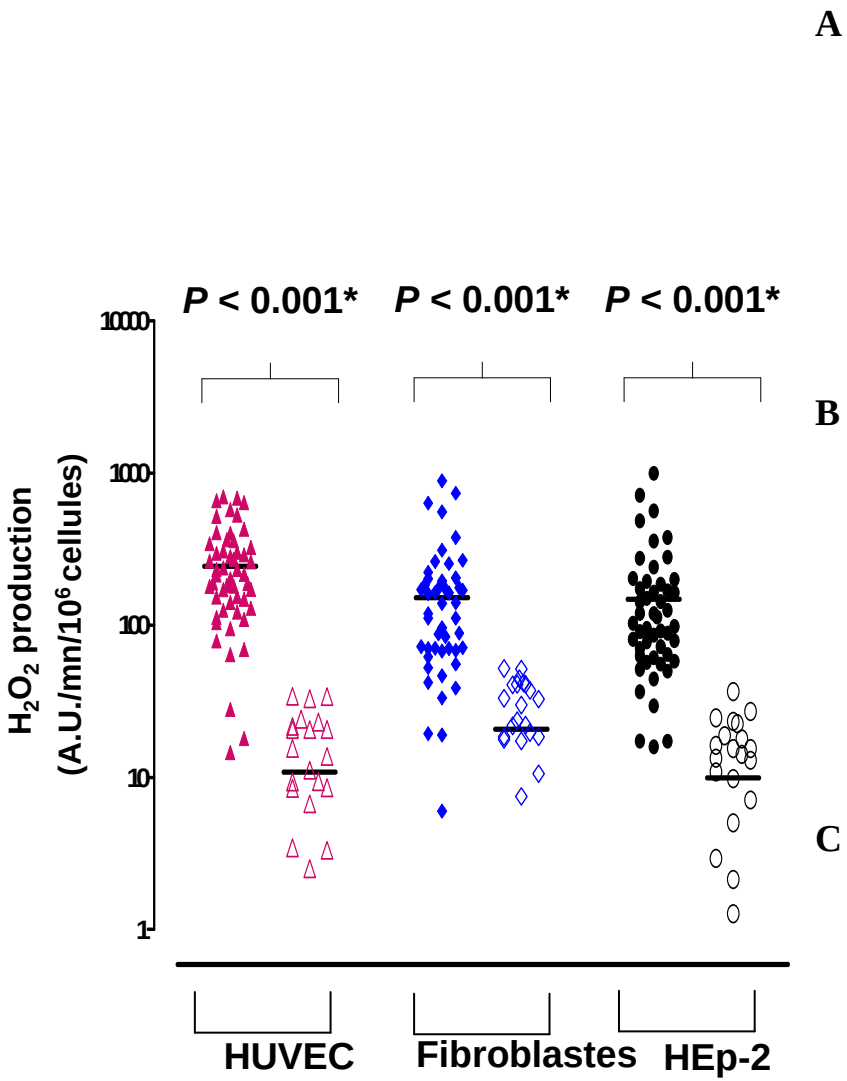
Phénomènes d'ischémie –reperfusion: production d'anions superoxydes (O2 •–) (HERRICK, Clin Exp Rheumatol. 2001)

Toxicité de la silice et de la bléomycine médiée par le stress oxydatif (FUBINI, Free Radic Biol Med. 2003)

protéines oxydées sériques (carbonyls et advanced oxidation protein products, AOPP)(ALLANORE, Am J Med. 2004)

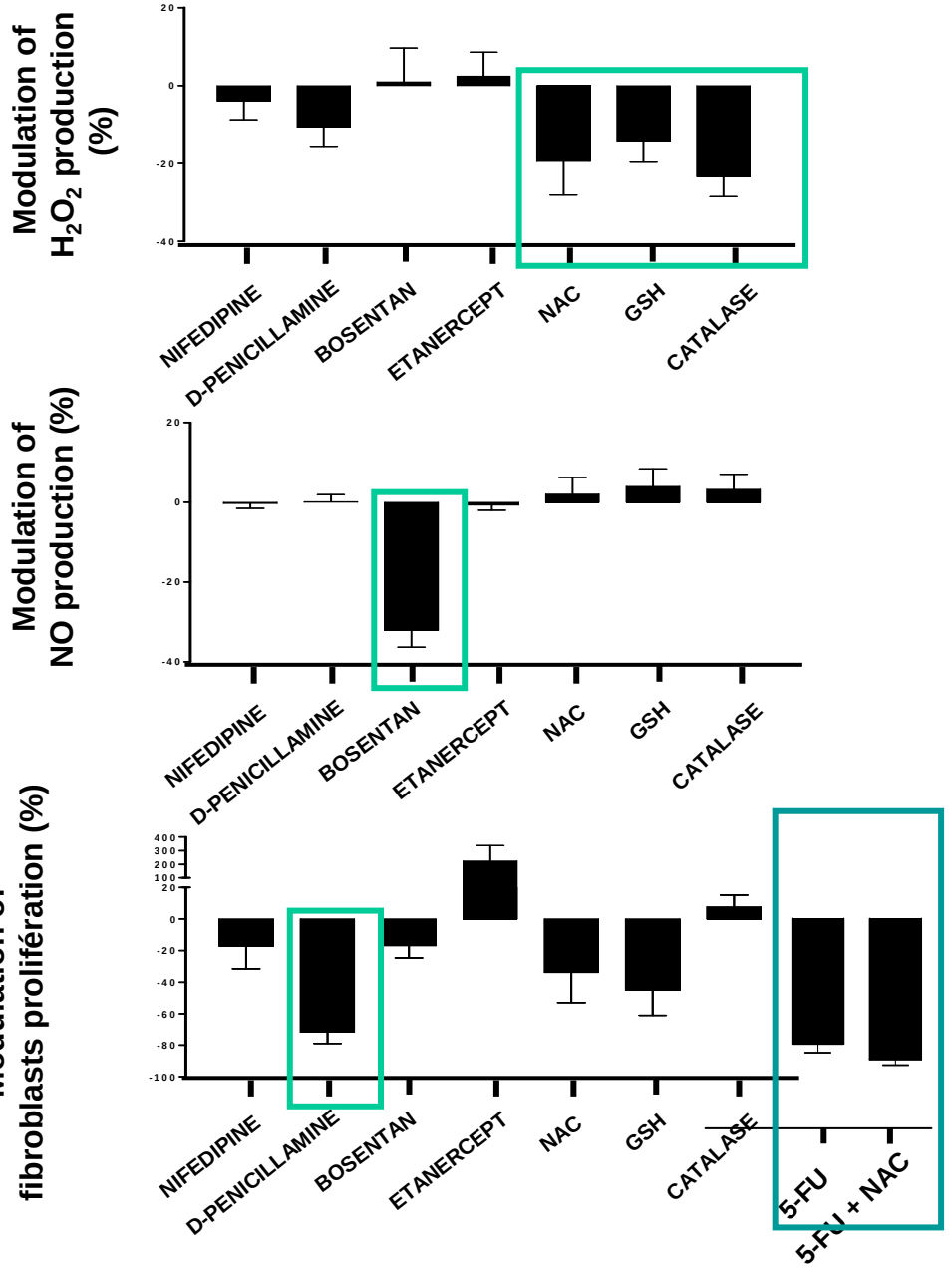
marqueurs de peroxydation lipidique dans le sérum (SOLANS, Arthritis Rheum. 2000)

Generation of H2O2 in the presence of SSc serum



Servettaz A et al, Ann Rheum Dis 2007

Effect of drugs and anti-oxydizing molecules



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)*

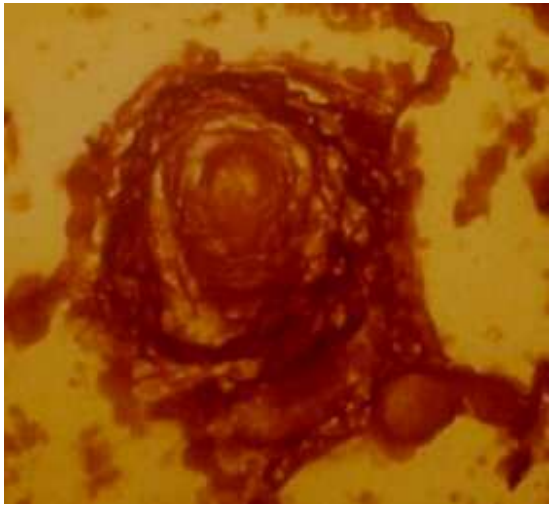
Perturbed angiogenesis: VEGF decreased of not detectable *(Distler O., Circ Res, 2004)*

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

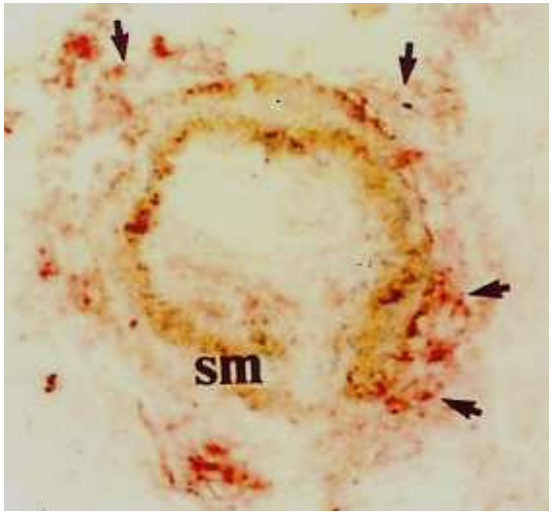
Synthesis of TGFβ and PDGF: activation of fibroblasts *(Cotton,J Pathol, 1998)*

Endothelin-1 expression in pulmonary and renal vasculature

PAH

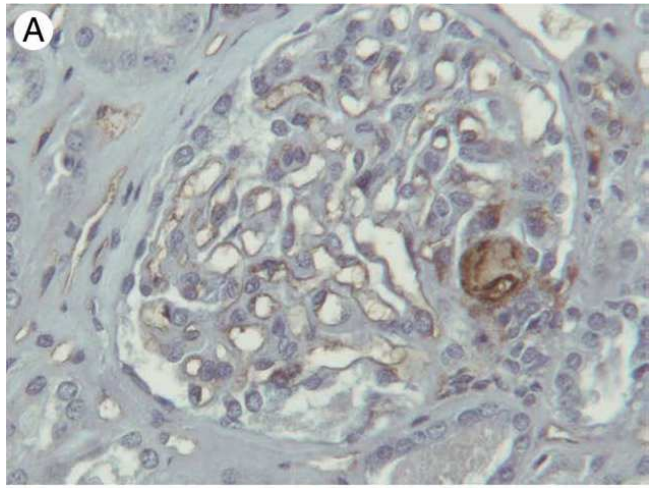


Sirius red stain - collagen

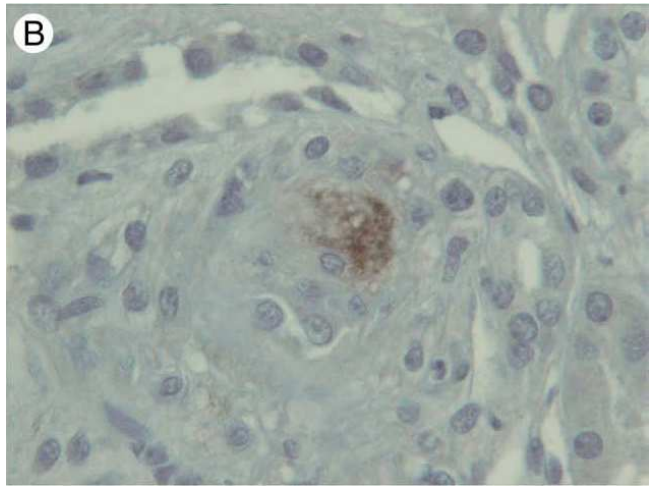


Immunolocalisation of ET-1 ligand

Scleroderma renal Crisis



ET-1 in glomerular thrombosis and along glomerular basement membranes



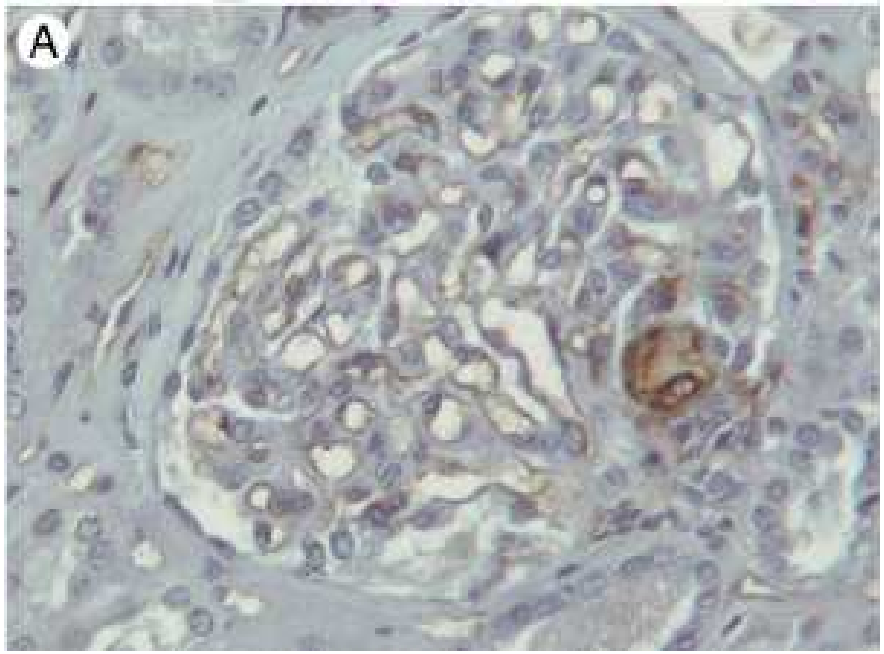
ET-1 in arteriolar thrombosis

Mouthon et al. Human Pathol 2010

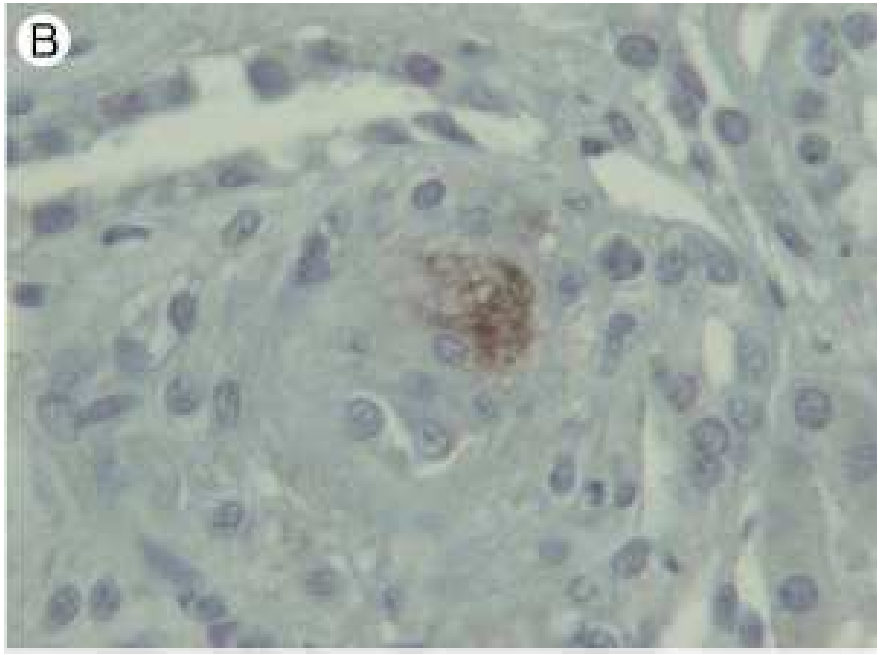
Endothelin 1 expression in scleroderma renal crisis

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)



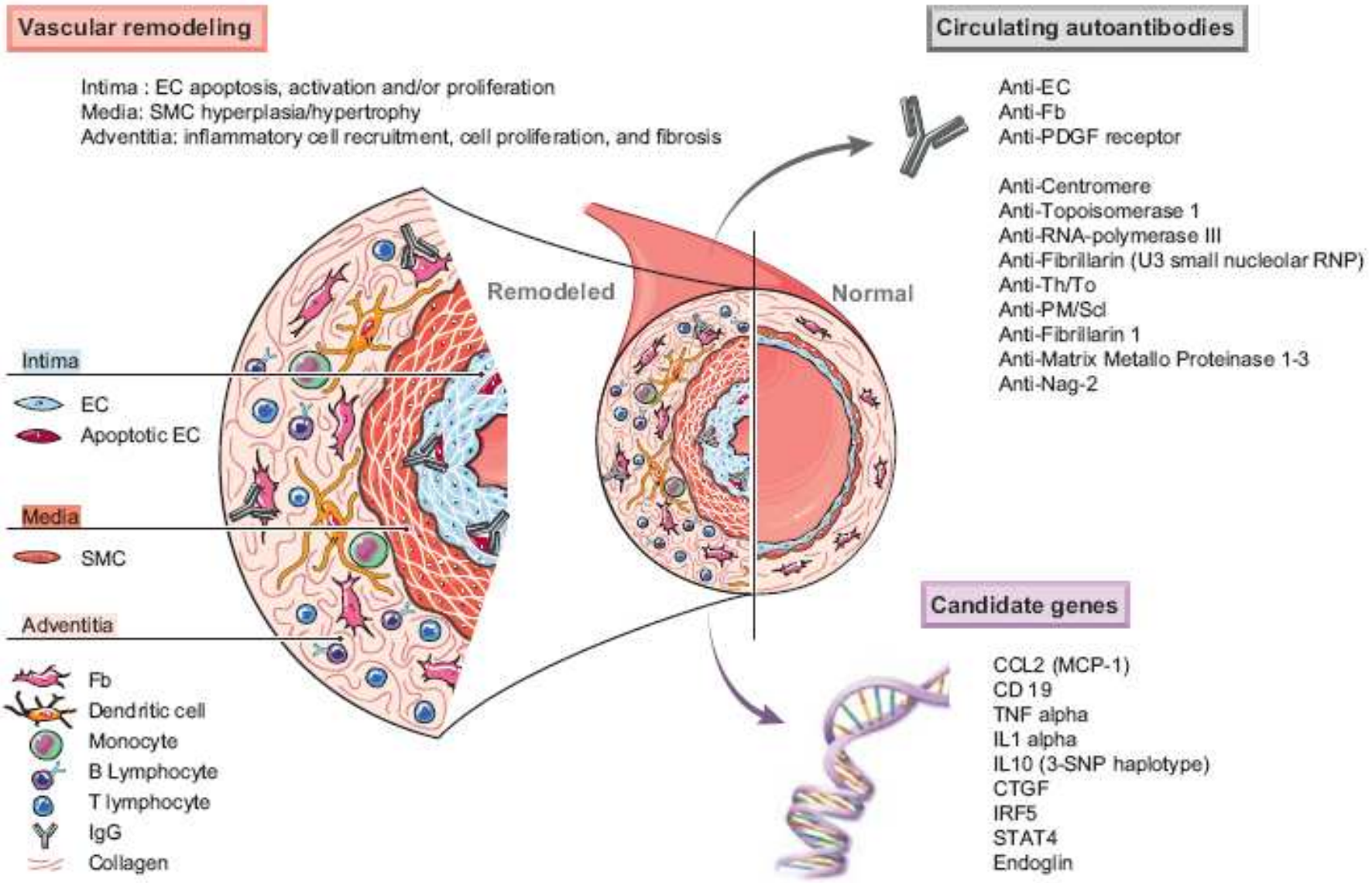
ET-1 in glomerular thrombosis and along glomerular basement membranes



ET-1 in arteriolar thrombosis

Mouthon et al. Human Pathol 2010

Pulmonary vascular remodeling in SSc-PAH



Le Pavec J et al 2010 AJRCCM

FIBROBLASTES SCLÉRODERMIQUES

Acquisition d'un phénotype activé en myofibroblastes *(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)*

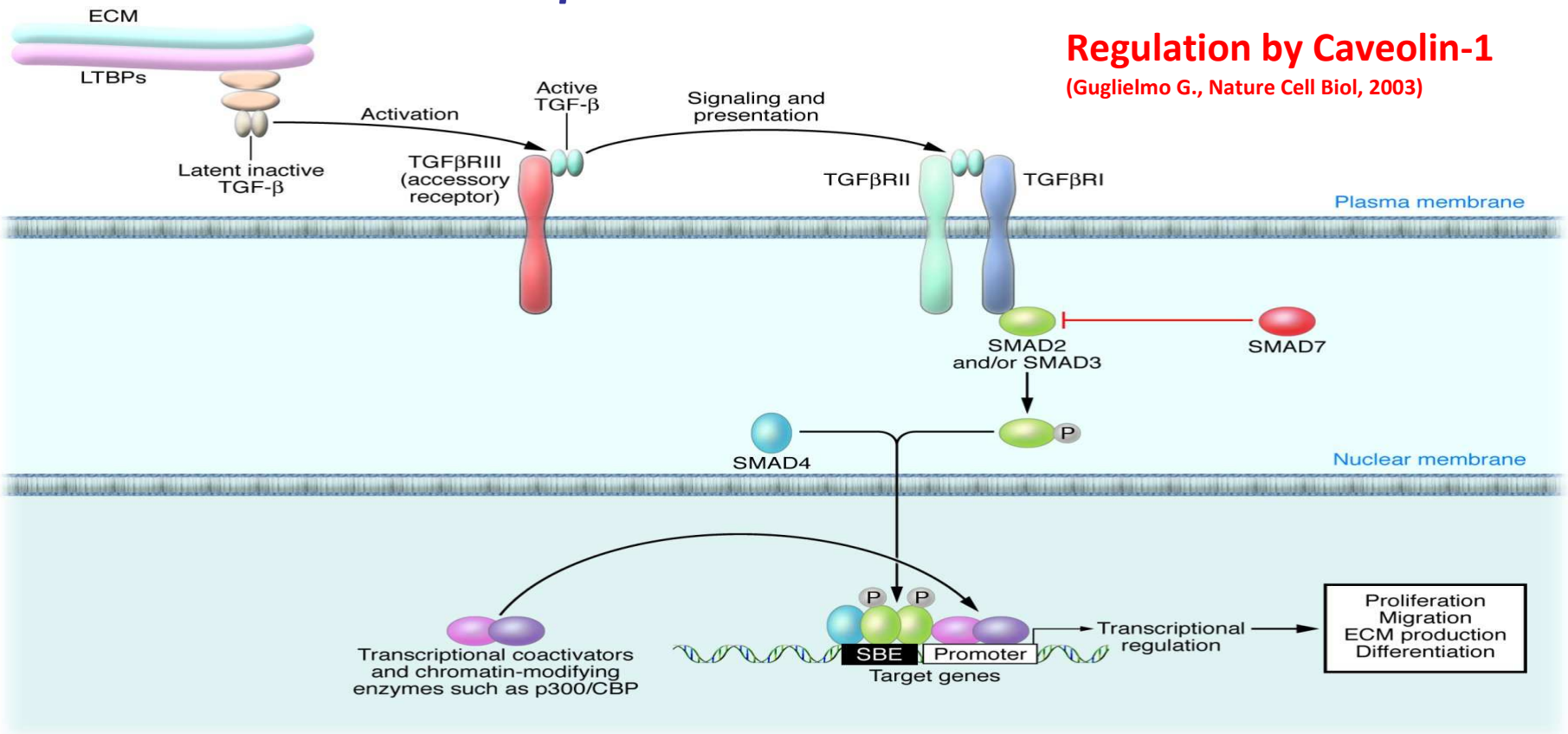
Défaut d'apoptose par Fas/Fas-ligand *(Santiago B., Arthritis Rheum 2001)*

Défaut de synthèse des régulateurs de la MEC (métalloprotéinases) *(VAN DER SLOT, J Biol Chem. 200*

Activation et synthèse excessive de collagène sous le contrôle de

- **IL-4: prolifération** *(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)*
- **Formes Réactives de l'Oxygène (FRO)** *(Sambo P., Arthritis Rheum.,2001)*
- **Anticorps anti fibroblastes et anti-PDGFR** *(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



Regulation by Caveolin-1
(Guglielmo G., Nature Cell Biol, 2003)

produced by EC, dermal perivascular macrophages

Activation of Smads

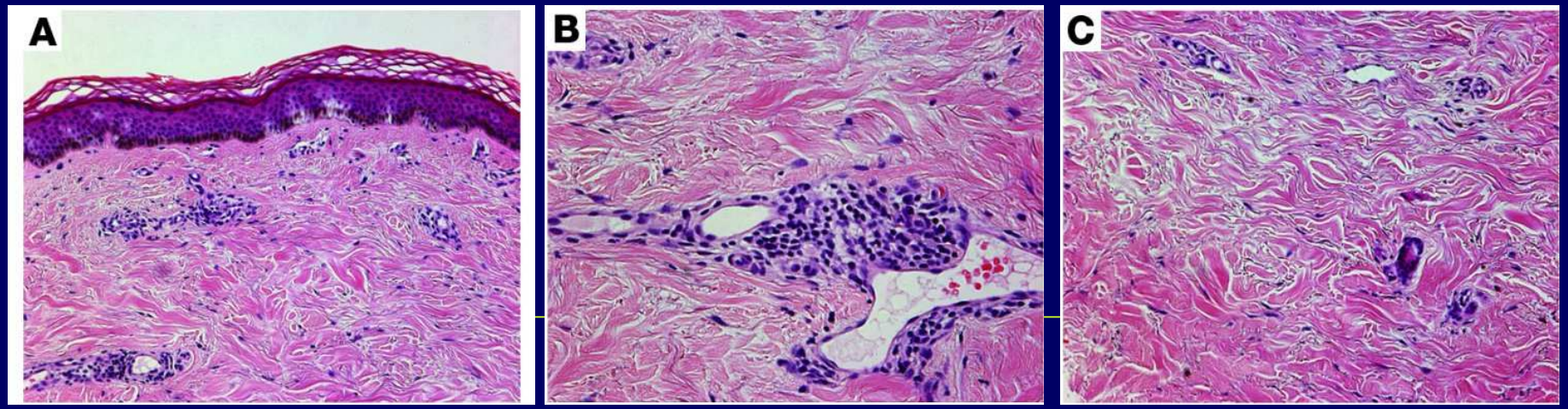
Activation of non-smads pathways: p21 activated kinase 2, Rho associated Kinase, …

Transcription of genes encoding for:

Type I collagen
PDGF
CTGF

Varga J., JCI, 2007

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

Varga J, Abraham D. *J Clin Invest* 2007; 117:557–67.

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.

CYTOKINES I

TGF-β

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

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

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

PDGF

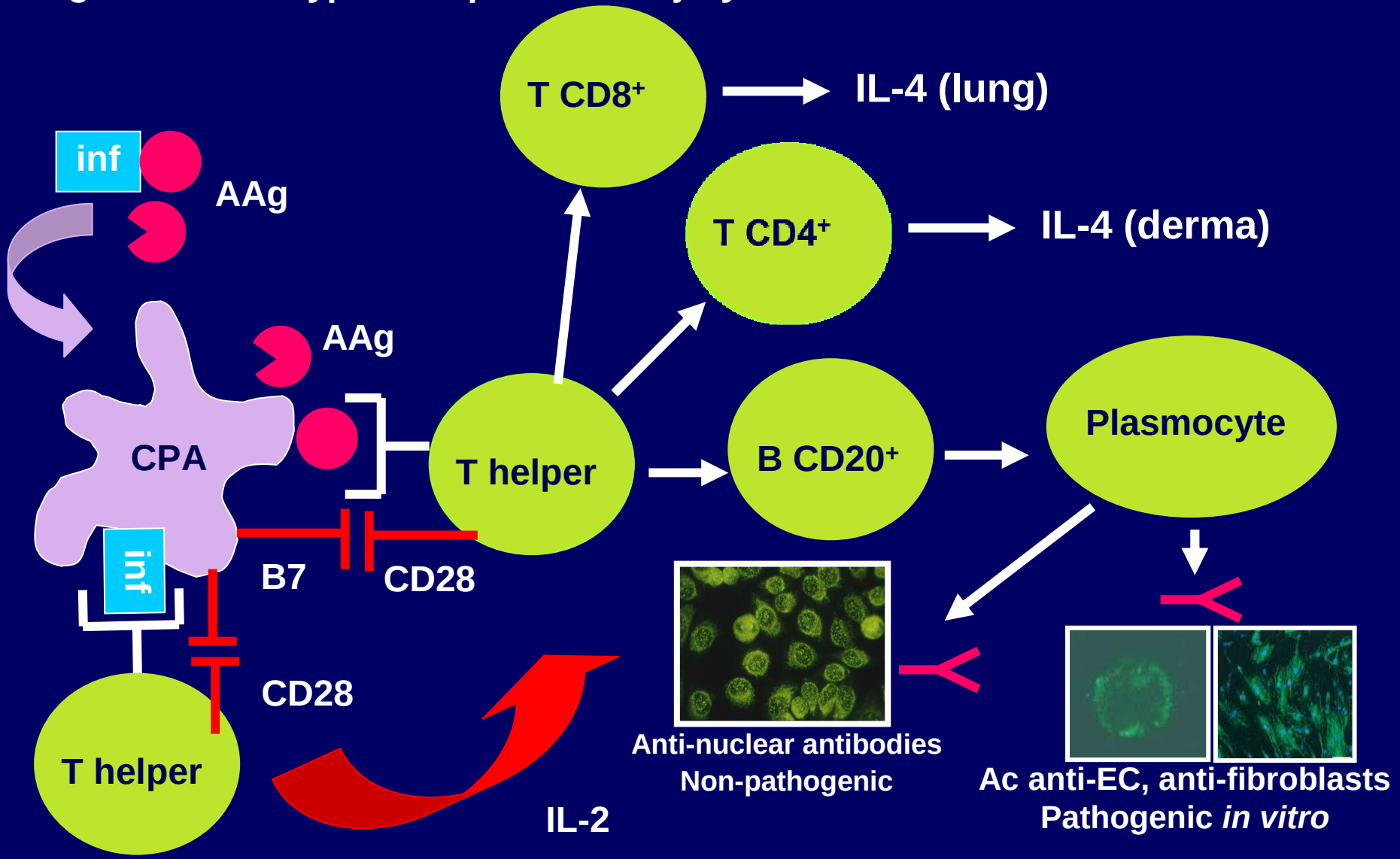
PDGF produits par plaquettes, macrophages, CE, fibroblastes

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

SSc: involvement of the adaptative immune system

Infectious agent: topoisomerase 1 and cytomegalovirus

Fragmentation: hypoxia-reperfusion injury



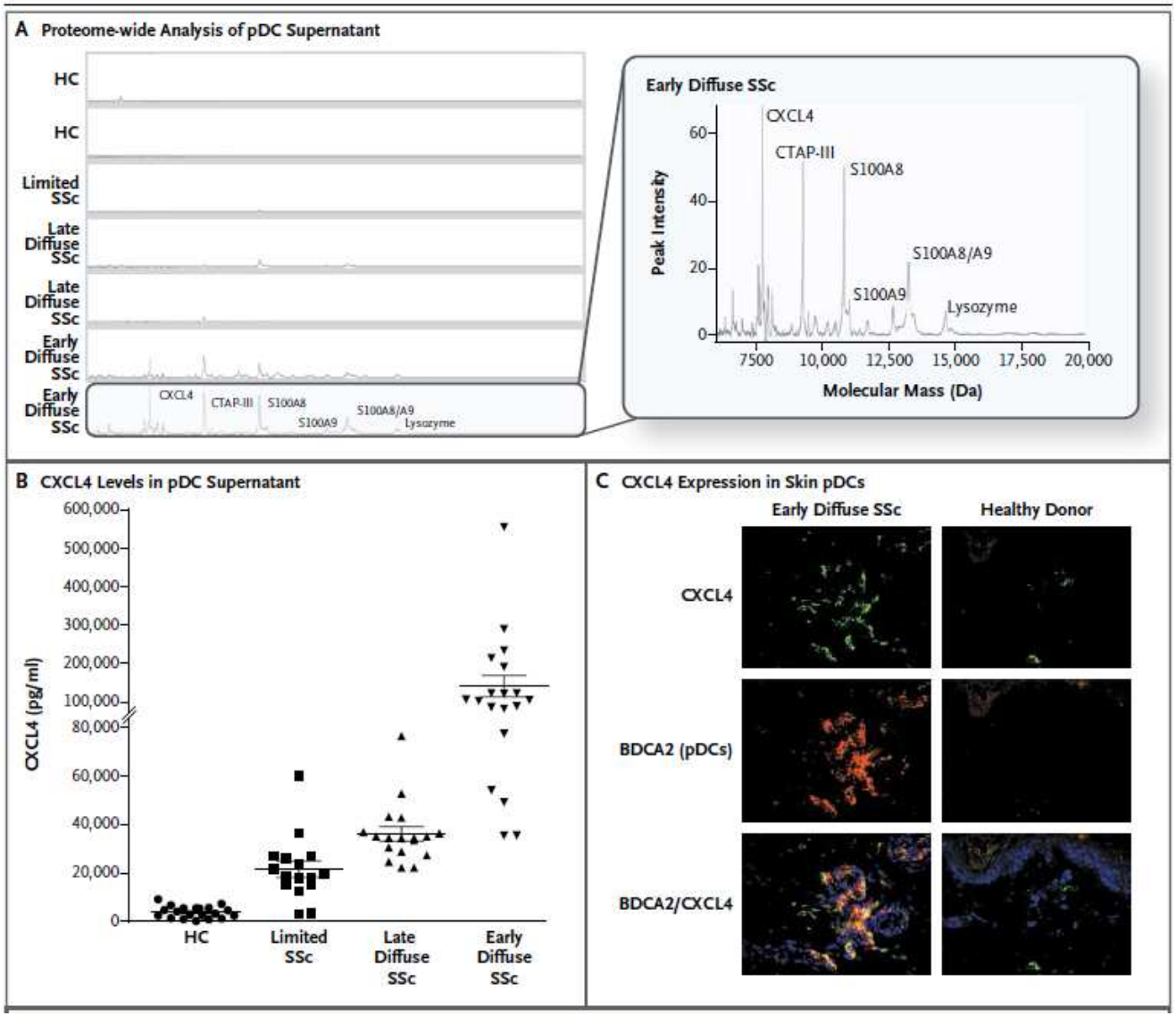
ORIGINAL ARTICLE

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

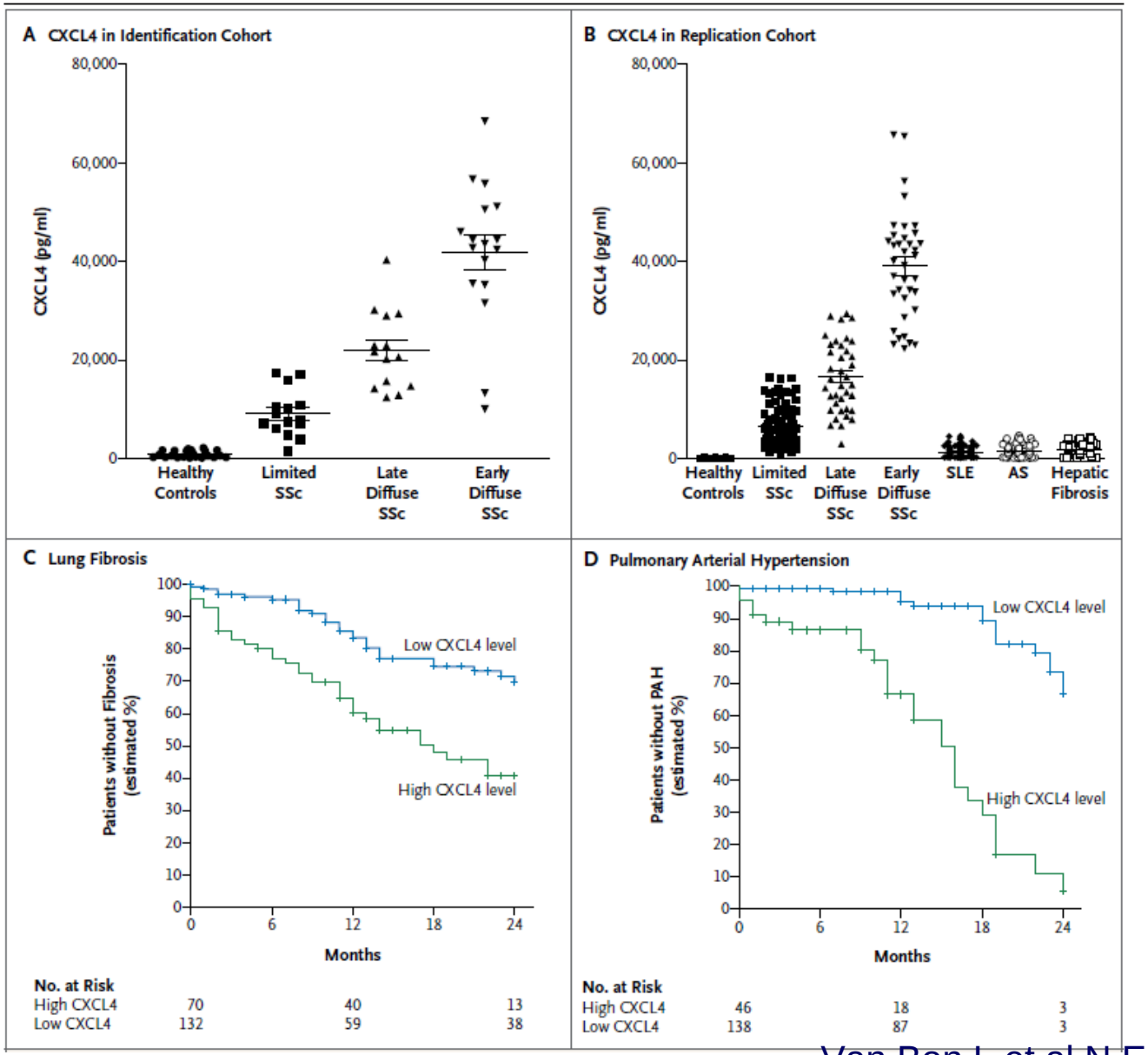
N Engl J Med 2014;370:433-43.

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



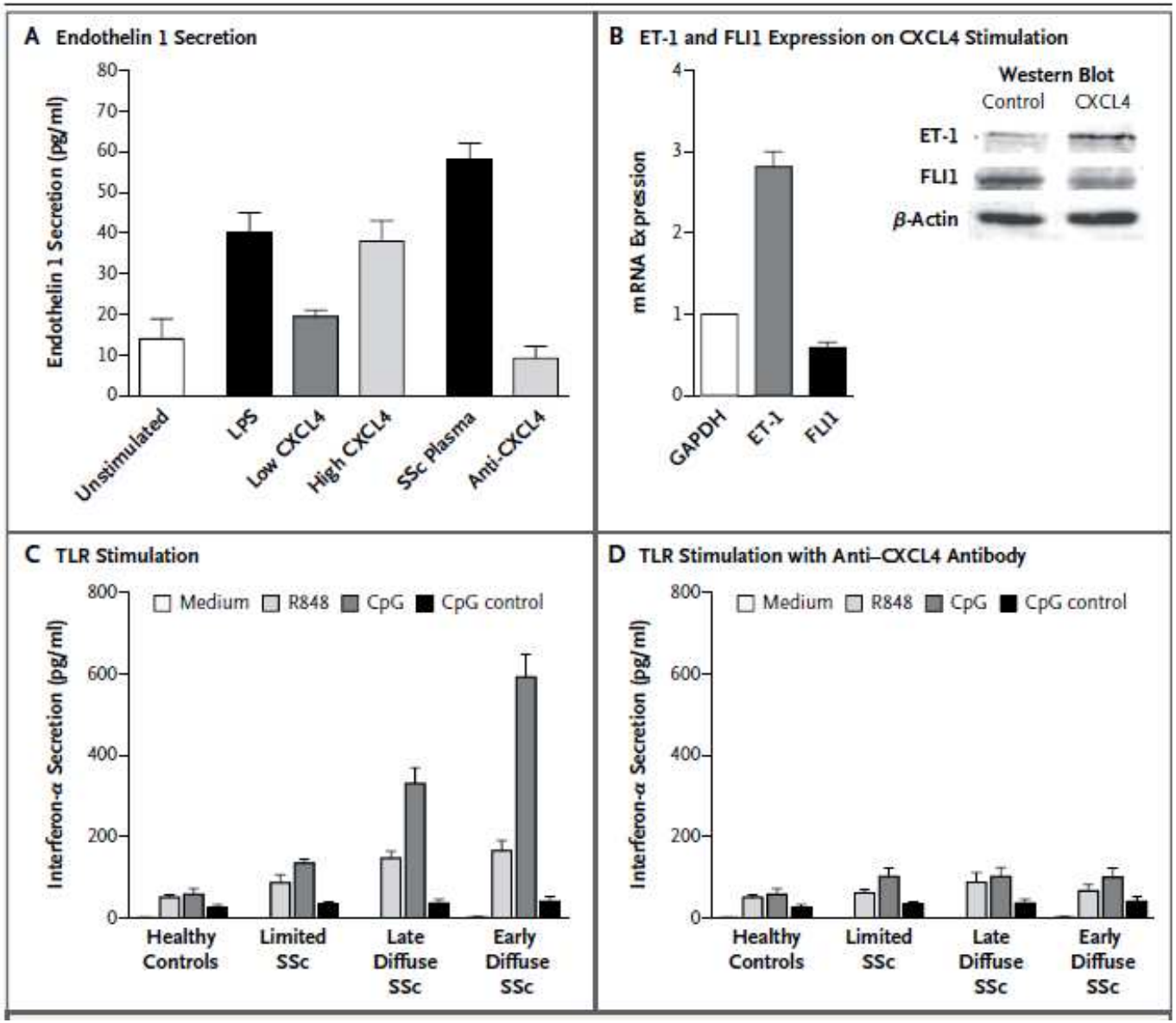
Van Bon L et al N Engl J Med 2014

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



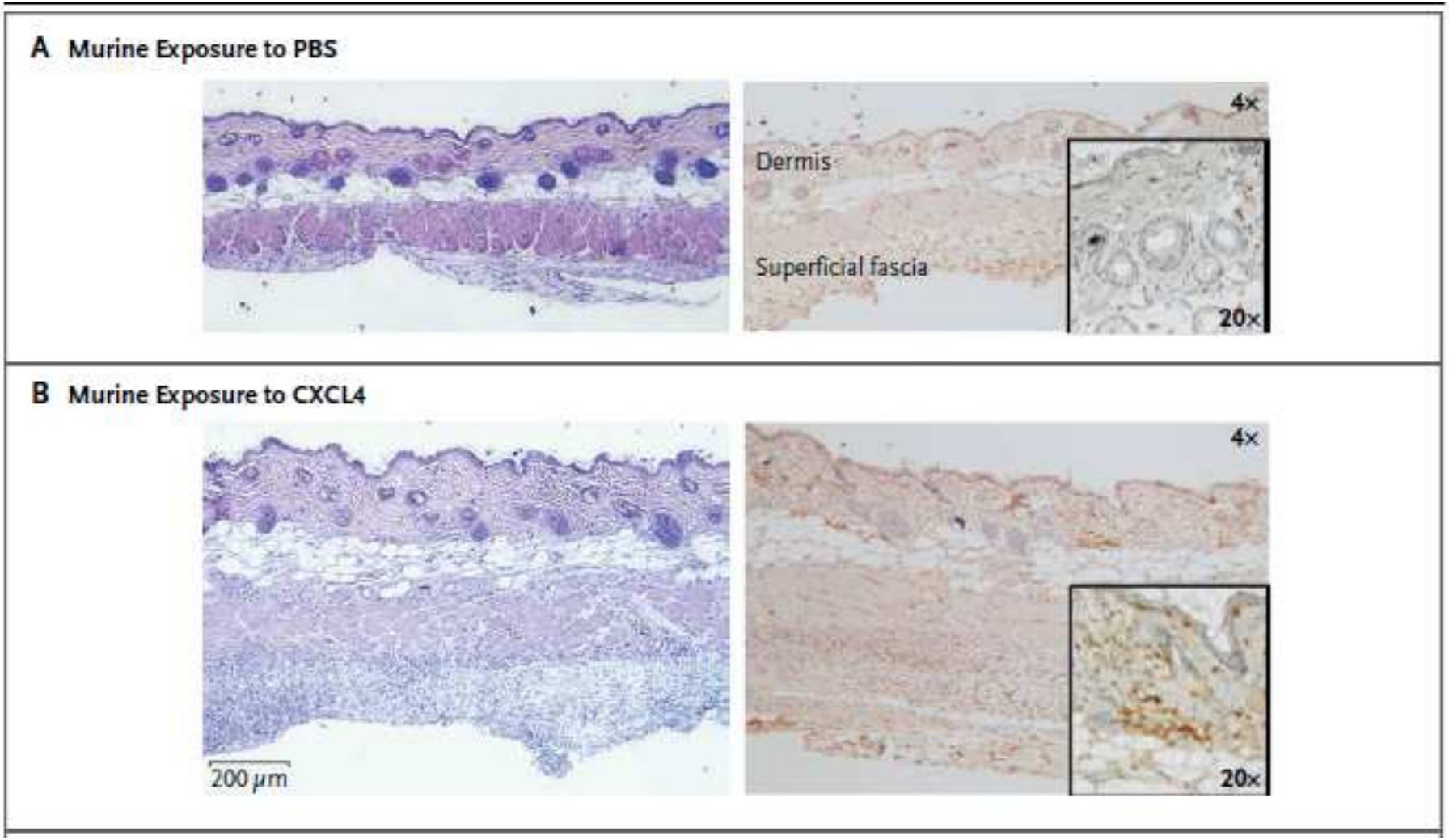
Van Bon L et al N Engl J Med 2014

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



Van Bon L et al N Engl J Med 2014

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



Van Bon L et al N Engl J Med 2014

T cell activation in SSc

- T cell activation in blood
  - Soluble IL-2R level correlated with the extent of skin fibrosis[1]
  - Clonal expansion of blood T cells[2]
- T cell activation in skin
  - Oligoclonal T cell expansion in the skin[3]
  - Enhanced transendothelial migration of $CD4^+$ T cells[4]
- Pronounced Th17 profile in SSc; intracellular expression of TGFβ and IFNg 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.

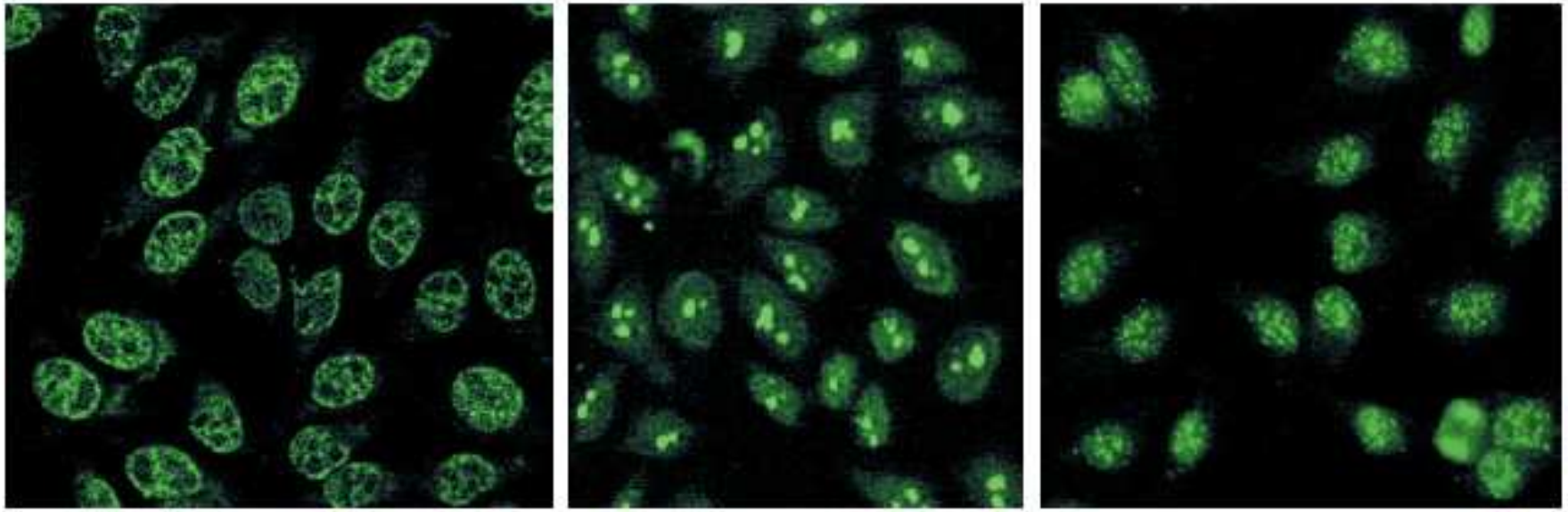
SSc: involvement of B lymphocytes

- Abnormal B cell signalling in TSK/+ mice[1]
- Presence of B cells in skin[2] and in lungs from SSc patients[3]
- Expanded naive B cells and diminished but activated memory B cells[4]
- 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 severity[5]
- 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
Antifibrillarin (U3RNP)	Diffuse cutaneous scleroderma, internal-organ involvement	Anti–Nag-2	Induce endothelial-cell apoptosis
Anti-Th/To	Limited cutaneous scleroderma, pulmonary fibrosis		

Gabrielli A, *et al. N Engl J Med* 2009

SSc: origin of autoantibodies

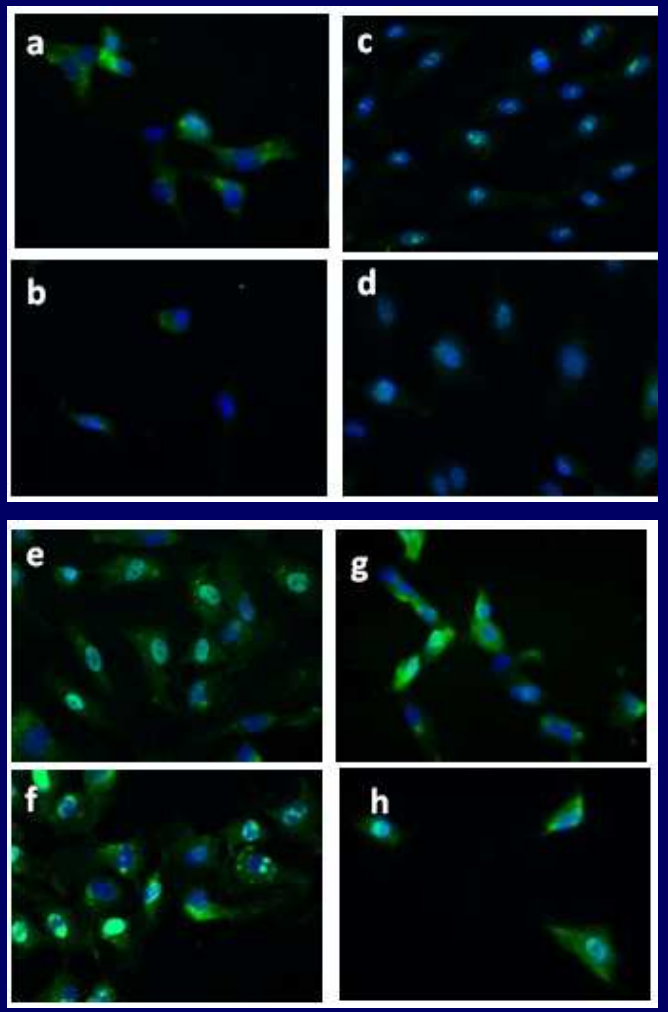
- Molecular mimicry (topo I and CMV)[1]
- Polyclonal B cell activation with excess of **IL-4**
- **Fragmentation of autoantigens** by metalloproteinases, favoured by hypoxia[2] and by mercury chloride[3]
- Selective **oxidation** of DNA topoisomerase 1 induces SSc in the mouse[4]
- A subset of SSc patients shows a “lupus-like” high **IFN-α** inducible gene expression pattern[5]

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-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 cells[2]
- Cross-reactivity of AECA with a CMV protein[3]
- Target antigens unknown except ”scleroderma specific” autoantigens[4,5]

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



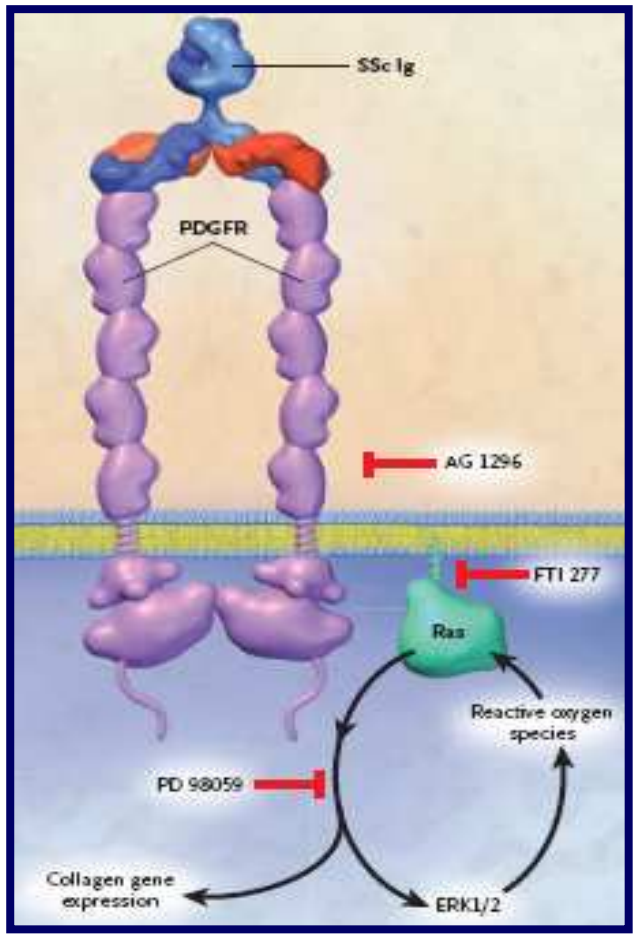
1. Carvalho D. *Arthr Rheum* 1999. 2. Bordron A. *J Clin Invest* 1998.
3. Lunardi C, *et al. Nat Med* 2000. 4. Garcia de la Pena *et al. Clin Immunol* 2004.
5. Servettaz *et al. Clin Immunol* 2006. 6. Dib H, *et al.* Eur Resp J 2011

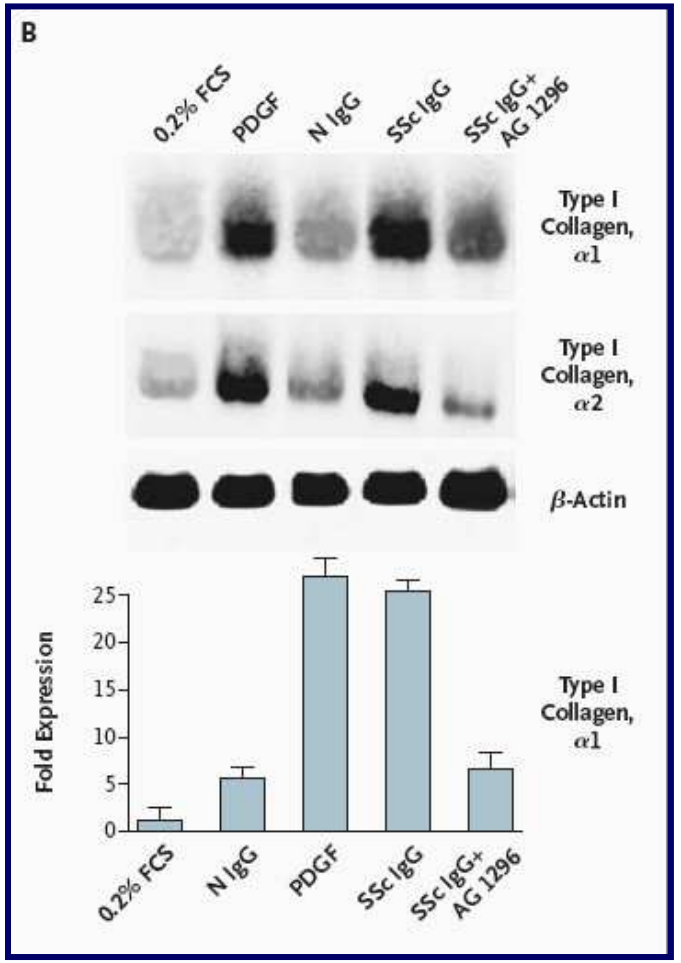
Anti-fibroblast Abs in SSc

- Anti-fibroblast antibodies (AFA) are present in the serum of 20 to 80% of SSc patients[1]
- AFA can activate fibroblasts and induce extracellular matrix proteins synthesis[2]
- 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 TLR4[3]
- Target antigens
  - DNA topoisomerase 1[4]
  - PDGF receptor[5]

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.

ANTICORPS ANTI-PDGFR





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

L'induction de ERK1/2 entraine 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

Svegliati Baroni, NEJM, 2006

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

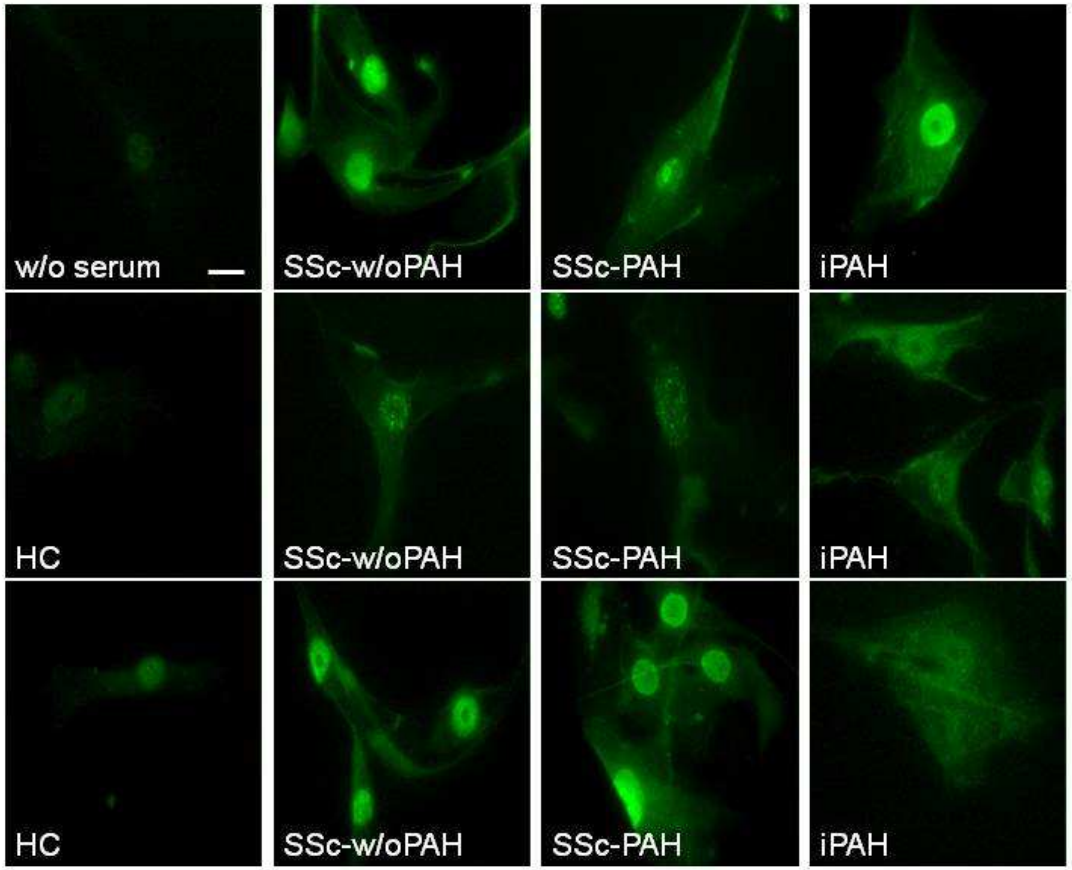
- **Oxydative stress**
  - ✓ G6PD
  - ✓ HSP27
  - ✓ HSP70

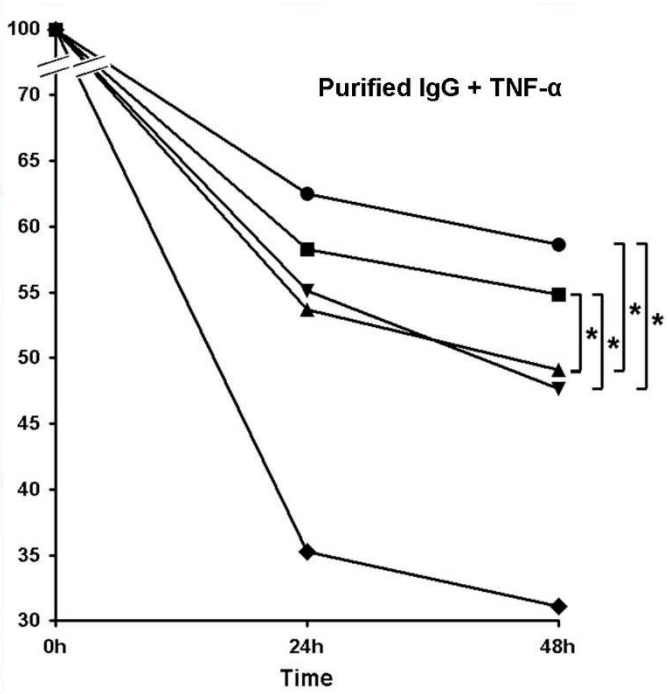
- **Protein metabolism**
  - ✓ Glutaminase
  - ✓ alanine-glyoxylate amino-transferase2
  - ✓ glutamate carboxy-peptidase

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

Terrier B et al AJRCCM 2008

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.

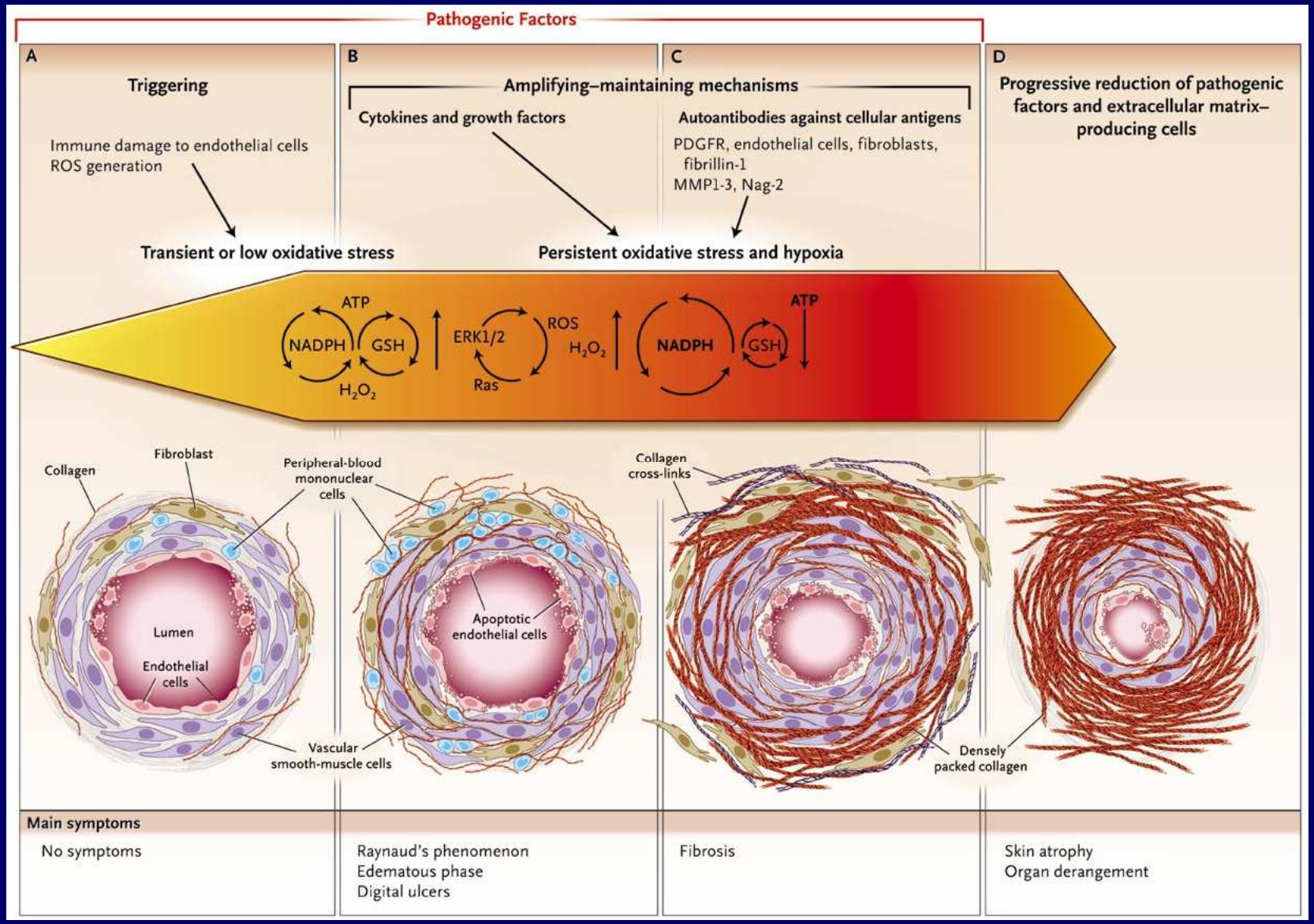




Inhibition of contraction

Bussone et al.Ann Rheum Dis 2011

Systemic sclerosis: lesions at different stages



Gabrielli A. *NEJM* 2009

Conclusion

- Major work has been done in order to improve the understanding of SSc pathogenesis.
- A number of new experimental models have been set up, that should help to understand the disease pathogenesis and test new therapeutic targets.
- ROS represent a hallmark of the pathogenesis of SSc
- Besides endothelial cells and fibroblasts, major development has been made in the understanding of the role of B cells and autoantibodies in the pathogenesis of SSc.
- Plasmacytoid dendritic cells seem to play a major role in the pathogenesis of SSc through the secretion of CXCL4, although these data will need to be confirmed in the near future.

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