but IL1 which has a pivotal role in the pathogenesis of AOSD[3] and systemic onset juvenile idiopathic arthritis.[9] In these diseases and in other rare disorders with a single amino acid mutation in the NALP-3 gene which results in increased IL1 secretion, IL1 blockade seems to be the preferred treatment.[10]

Furthermore, our case suggests that hypersensitivity to NSAIDs is not exclusively mediated by COX-1 blockade, but can also be provoked by selective COX-2 inhibitors that can function as haptens, resulting in anaphylaxis upon next exposure.[5] Our case shows that these reactions are not mediated by TNFα and not altered by TNFα neutralisation.

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REFERENCES

1 **Husni ME**, Maier AL, Mease PJ, Overman SS, Fraser P, Gravallese EM, *et al*. Etanercept in the treatment of adult patients with Still's disease. *Arthritis Rheum* 2002;**46**:1171–6.
2 **Fautrel B**, Sibilia J, Mariette X, Combe B. Tumour necrosis factor alpha blocking agents in refractory adult Still's disease: an observational study of 20 cases. *Ann Rheum Dis* 2005;**64**:262–6.
3 **Vasques Godinho FM**, Parreira Santos MJ, Canas da Silva J. Refractory adult onset Still's disease successfully treated with anakinra. *Ann Rheum Dis* 2005;**64**:647–8.
4 **West PM**, Fernandez C. Safety of COX-2 inhibitors in asthma patients with aspirin hypersensitivity. *Ann Pharmacother* 2003;**37**:1497–501.
5 **Stevenson DD**, Simon RA. Lack of cross-reactivity between rofecoxib and aspirin in aspirin-sensitive patients with asthma. *J Allergy Clin Immunol* 2001;**108**:47–51.
6 **Berkes EA**. Anaphylactic and anaphylactoid reactions to aspirin and other NSAIDs. *Clin Rev Allergy Immunol* 2003;**24**:137–48.
7 **Quartier P**, Taupin P, Bourdeaut F, Lemelle I, Pillet P, Bost M, *et al*. Efficacy of etanercept for the treatment of juvenile idiopathic arthritis according to the onset type. *Arthritis Rheum* 2003;**48**:1093–101.
8 **Horneff G**, Schmeling H, Biedermann T, Foeldvari I, Ganser G, Girschick HJ, *et al*. The German etanercept registry for treatment of juvenile idiopathic arthritis. *Ann Rheum Dis* 2004;**63**:1638–44.
9 **Pascual V**, Allantaz F, Arce E, Punaro M, Banchereau J. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. *J Exp Med* 2005;**201**:1479–86.
10 **Dinarello CA**. Blocking IL-1 in systemic inflammation. *J Exp Med* 2005;**201**:1355–9.

Distal degeneration of sensory and autonomic cutaneous nerve fibres in systemic sclerosis

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We studied innervation and dermal vasculature in affected and apparently normal skin of sclerodermic patients to evaluate the involvement of different nerve fibre groups and to determine a possible correlation with vascular damage in this disease. Immunohistochemical analysis and confocal microscopic examination of skin biopsy samples were used.

METHODS AND RESULTS

We obtained 3 mm punch skin biopsy samples from the distal thigh and distal leg in 11 consecutive 34–70 year old female patients with systemic sclerosis (SSc), identified by the American College of Rheumatology classification criteria.[1] We excluded patients who had been exposed to potentially neurotoxic exogenous or endogenous conditions. The skin appeared sclerotic in 4/11 patients in the leg and in 3/11 in the thigh (table 1). In four patients a further skin sample from fingertip was taken to evaluate myelinated fibres. None of the patients complained of sensory disturbances, and neurological and neurophysiological evaluations were normal except in two patients, in whom a conduction velocity study showed the presence of an entrapment syndrome. Patient morphological findings were compared with data from a group of 16 healthy volunteers (nine male, seven female, age range 34–65 years).

Skin biopsy specimens were processed according to previously published procedures.[2] Floating sections were immunostained using a panel of primary antibodies, including the pan-neuronal marker anti-protein gene product (PGP) 9.5, anti-myelin basic protein for myelinated fibres, anti-vasoactive intestinal peptide (VIP) to mark autonomic nerve fibres, and anti-collagen IV to visualise basement membrane and blood vessels.

We quantified, as previously described, epidermal nerve fibres (ENFs) per linear millimetre,[3] Meissner corpuscles (MCs), and myelinated papillary endings per square millimetre[4] on confocal images using image analysis software (Neurolucida, Microbrightfield Inc, Colchester VT, USA; ScionImage, Scion Corporation, Frederick, MD, USA). On the same images used to quantify ENF density, we measured blood vessel density in $\mu m^2/100\ \mu m^2$ of dermal tissue within 250 μm below the basement membrane.

We found a significant loss of ENFs in sclerodermic patients in all the examined sites (table 1) without a distal-proximal gradient, a poor subepidermal neural plexus, and a reduced innervation of sweat glands, blood vessels, and arrector pilorum muscles compared with controls. These findings, evident in apparently unaffected areas (figs 1E and F compared with 1A and B), were more severe in clinically involved skin (figs 1C and D compared with 1A and B) and affected both sensory and autonomic unmyelinated nerve fibres as demonstrated by PGP and VIP immunostainings.

The mean (SD) density of blood vessels measured in $\mu m^2/100\ \mu m^2$ of dermal tissue, was 6.4 (2.9) and 8.7 (4.7), respectively, in the thigh and leg of patients with SSc. These values significantly correlated with the density of epidermal nerve fibres in both sites ($r^2 = 0.51$; $p<0.05$ at the thigh and $r^2 = 0.58$; $p<0.05$ at the leg). In glabrous skin we found a significant reduction of MC density compared with controls, with a number of intrapapillary myelinated fibres still within the normal range. Moreover, evident structural abnormalities of the surviving mechanoreceptors and predegenerative

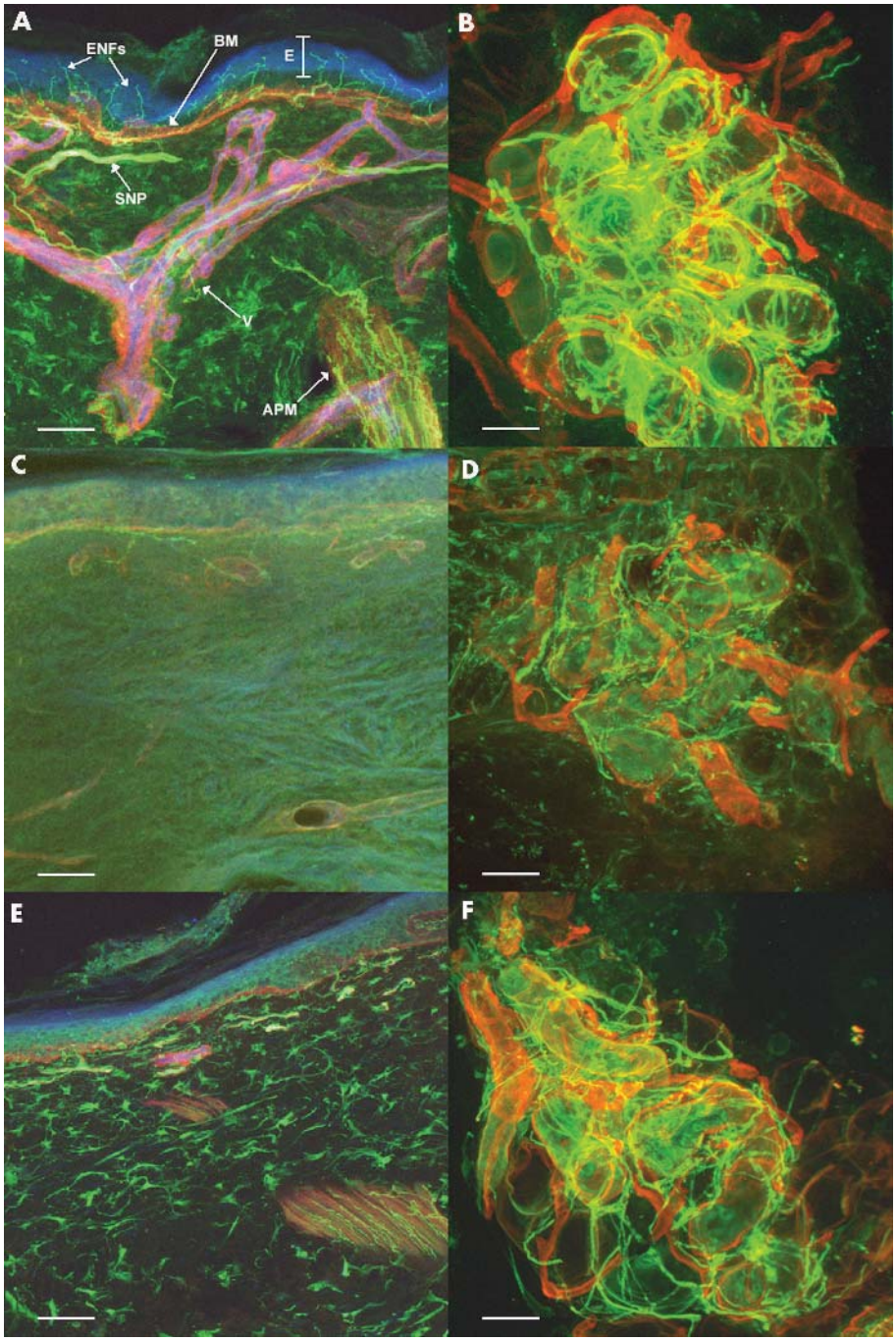

Figure 1 Confocal micrographs of cutaneous innervation in thigh skin samples. (A) Normal skin, (C) affected, and (E) apparently unaffected skin from a patient with SSc: samples are triple stained to visualise nerve fibres (rPGP in yellow-green), basement membrane and vessels (mCollV in red), and endothelium and epidermis (ULEX Europaeus in blue). Sweat gland images in a healthy subject (B) and in sclerodermic patients from affected (D) and apparently normal (F) skin, are double stained to visualise nerve fibres (rPGP in yellow-green) and vessels (mCollV in red). A derangement of dermal architecture, of subepidermal neural plexus, and a marked reduction of ENF density are evident in affected and, although to a lesser extent, in apparently normal skin of patients with SSc (C and E compared with A). A loss of nerve fibres innervating dermal annexes such as arrector pilorum muscles (E compared with A) and sweat glands (D and F compared with B) in skin from sclerodermic patients is also evident. Bar = 50 μm. E, epidermis; ENFs, epidermal nerve fibres; BM, basement membrane; V, blood vessels; SNP, subepidermal neural plexus; APM, arrector pilorum muscle.

aspects of myelinated fibres, such as swellings or vacuolisation, were present.

DISCUSSION

Our data indicate that the cutaneous nerves in SSc are impaired. This mainly involves the unmyelinated sensory and autonomic nerve fibres, but does not completely spare the large fibres. The observation that the loss of ENFs was more significant in subjects with an evident reduction of vascular bed suggests that ischaemia may have a role in determining the neuropathic process. However, we cannot rule out the possibility that early biohumoral changes, demonstrated in apparently unaffected skin,[5 6] may induce both neural and vascular damage. We speculate that the abnormalities of terminal innervation seen in the skin may be present in multiple organs in SSc. This neuropathic process, affecting

Table 1 Clinical and morphological data in sclerodermic patients compared with mean values in the control group

Patient	Age	Disease duration (years)	Subset	ENF thigh*	Dermal vessels density thigh†	ENF leg*	Dermal vessels density leg†	ENF fingertip*	Meissner corpuscles‡	Myelinated intrapapillary endings‡
1	62	1	lSSc	15.8	8.1	10.3	8.7	7.4	20.5	42.7
2	43	13	dSSc	7.3	7.8	9.0	7.1	–	–	–
3	63	1	lSSc	6.0	2.2	3.5	11.3	1.9	8.6	18.3
4	62	2	lSSc	16.6	9.6	9.3	9.2	–	–	–
5	34	6	dSSc	(0.0)	4.7	(0.0)	1.5	–	–	–
6	65	5	dSSc	2.0	11.8	(0.0)	3.4	–	–	–
7	46	2	dSSc	(0.0)	3.0	(3.1)	2.8	–	–	–
8	41	1	lSSc	18.7	8.0	10.5	12.0	–	–	–
9	60	8	dSSc	(0.0)	7.1	(0.0)	9.6	–	–	–
10	70	3	lSSc	20.5	8.3	23.8	16.2	3.5	15.1	63.9
11	70	5	lSSc	13.3	9.7	11.5	13.6	2.2	15.0	37.2
Mean (SD)										
Patients	56.0 (12.6)	4.3 (3.7)		9.1 (8.1)	6.4 (2.9)	7.4 (7.1)	8.7 (4.7)	3.8 (2.5)	14.8 (4.9)	40.5 (18.8)
Controls	52.6 (10.0)	–		27.2 (7.7)	–	19.1 (8.8)	–	7.5 (3.6)	29.7 (11.2)	51.9 (20.7)
Significance				$p<0.0001$§ $p<0.005$¶		$p<0.001$§ $p<0.05$*		$p<0.05$§	$p<0.005$§	$p=0.34$§

ENF density values from affected skin are shown in parentheses.
lSSc, limited cutaneous systemic sclerosis; dSSc, diffuse cutaneous systemic sclerosis.
*Expressed as the number of epidermal nerve fibres/mm; †expressed in $\mu m^2/100\ \mu m^2$ of dermal tissue; ‡ expressed as the number of structures/mm^2; §comparison of density values in the control group and in all skin samples from patients with SSc; ¶comparison of density values in the control group and in samples of apparently unaffected skin in patients with SSc.

primarily unmyelinated nerve fibres, may contribute to the production of abnormalities that are common in SSc, like visceral dysmotility and cardiac arrhythmias.

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REFERENCES

1 **ARA**. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980;**23**:581–90.
2 **Kennedy WR**, Wendelschafer-Crabb G. The innervation of human epidermis. *J Neurol Sci* 1993;**115**:184–90.
3 **Kennedy WR**, Wendelschafer-Crabb G, Johnson T. Quantitation of epidermal nerves in diabetic neuropathy. *Neurology* 1996;**47**:1042–8.
4 **Nolano M**, Provitera V, Crisci C, Stancanelli A, Wendelschafer-Crabb G, Kennedy WR, *et al*. Quantification of myelinated endings and mechanoreceptors in human digital skin. *Ann Neurol* 2003;**54**:197–205.
5 **Søndergaard K**, Stengaard-Pedersen K, Zachariae H, Heickendorff L, Deleuran M, Deleuran B. Soluble intercellular adhesion molecule-1 (sICAM-1) and soluble interleukin-2 receptors (sIL-2R) in scleroderma skin. *Br J Rheumatol* 1998;**37**:304–10.
6 **Claman HN**, Giorno RC, Seibold JR. Endothelial and fibroblastic activation in scleroderma. The myth of the ''uninvolved skin''. *Arthritis Rheum* 1991;**34**:1495–501.