

Biopsy-Documented Myopathy and Vasculitis in Essential Mixed Cryoglobulinemia

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Abstract

A skeletal muscle involvement associated with skin, renal and liver pathology has been described in patients with essential mixed cryoglobulinemia. In some cases the muscle atrophy was consequence of a peripheral neuropathy but in such patients the muscle pathology has not been defined. Skin and skeletal muscle biopsies were performed in 6 patients with type II essential mixed cryoglobulinemia. They showed skeletal muscle weakness and atrophy and slight increase of CPK. Two patients showed clinical signs of peripheral neuropathy. Morphological studies revealed vasculitis and lymphoid perivascular infiltration in the skin and fascial connective tissues. Vasculitis, perivascular lymphoid infiltration and muscle fibre necrosis were seen in all cases. Immunofluorescence showed IgM and IgG components and complement fractions in the arteriolar and capillary walls. At immunophenotyping, mononuclear T cells expressing CD4 and CD8 antigen were intermingled with few polytypic B cells. This study suggest that necrotizing changes in skeletal muscle of patients with type II mixed cryoglobulinemia result from a cryoglobulin-mediated vasculitis and/or intravascular precipitation of cryoglobulins with consequent ischaemic changes on muscle fibres. These alterations may be overimposed on denervation atrophy seen in patients with documented peripheral neuropathy.

Key words: essential mixed cryoglobulinemia, vasculitis, myopathy, morphology, immunohistochemistry.

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Cryoglobulinemias are a group of immunopathologic diseases recognized and classified according to immunochemical properties of cryoglobulins based on their constituent immunoglobulin proteins [4]. Three major groups of cryoglobulins are described. Type I cryoglobulins are characterized by a single monoclonal immunoglobulin, usually IgM but sometimes IgG or IgA. Type II cryoglobulins consist of mixed monoclonal immunoglobulins: a monoclonal component (often IgM k) that has rheumatoid factor activity and a polyclonal immunoglobulin. The type III cryoglobulins are also mixed cryoproteins molecules. Because of clinical and pathogenetic differences most authors tend to separate type I cryoglobulinemias which are often associated with overt or low-malignancy lymphoproliferative disorders [16, 17] from type II and III cryoglobulinemias that are related to hepatitic viruses B and C [1, 12] and to a variety of autoimmune and hepatic diseases [10]. Some authors regarded type II and III cryoglobulinemias as an unique entity called essential mixed cryo-

globulinemia [10, 12, 15] but other authors do not give confirmation to this unitary view. Type II essential mixed cryoglobulinemias are clinically characterized by asthenia, purpura and arthralgia with possible hepatic and renal involvement [7]. In addition to these manifestations, the patients show clinical signs of skeletal muscle involvement, a finding interpreted as a consequence of the frequently associated peripheral neuropathy [1, 13, 18].

The aim of the present study is to investigate the skeletal muscle involvement in cryoglobulinemic patients with or without peripheral neuropathy, and to obtain informations as to the pathogenetic mechanisms responsible for muscle damage. To this end, biopsies from skin, fascia and skeletal muscle were taken from 6 selected patients with type II cryoglobulinemia showing clinical and laboratory findings of myopathy.

Myopathy in essential mixed cryoglobulinemia

Patients and Methods

Six patients (3 males and 3 females) with cryoglobulinemia associated to myopathy were studied. All the patients entering the study were affected by type II essential mixed cryoglobulinemia demonstrated by the method of Meltzer et al [15]. In all cases the cryoglobulins were characterized using immunofixation (IFE) and classified as type II (monoclonal IgM with kappa light chain) according to Brouet et al [4].

Clinical and laboratory findings of patients are summarized in table 1.

All patients had cutaneous manifestations like purpura, erythematous patches or ecchymoses in association with skeletal muscle atrophy and weakness, mainly in the lower limbs. The patients showed electrophysiological signs of myopathy and had slight increase of CPK. Two patients showed an asymmetric sensory-motor neuropathy with paresthesia and sensory loss in the feet with slight increase of serum CPK levels. Electrophysiological investigation showed signs of myopathy in 4 cases and mixed signs of myopathy and denervation in 2 cases. Bone marrow biopsies obtained from each patient with Jamshidi's needle showed monotypic infiltrates composed by small lymphoid elements with plasmacytoid appearance in 4 cases, the remaining 2 having only a mild hyperplasia of the hemopoietic series. There was no cerebral or cranial nerve involvement. All patients suspended any pharmacological treatment 1 month before muscle biopsy.

Quadriceps femoris muscle, fascial and skin biopsies were obtained under local anaesthesia with open surgical methods. Skin and fascial biopsies were divided into two parts. One was fixed in formalin and embedded in paraffin and stained according the following methods: hematoxylin and eosin, PAS, Gomori' silver impregnation for reticulin. The second specimen was frozen in liquid nitrogen and cryostat sections were processed for a direct immunofluorescence method for the following monoclonal antibodies: anti IgA; anti IgG; anti IgM; anti-kappa, anti-lambda (all obtained from Becton-Dickinson and Dako).

Skeletal muscle biopsies were divided into three parts. Of these, one was fixed in formalin for routine histology. The second part was frozen in isopentane cooled in liquid nitrogen at -170°C and cryostat transverse sections were

stained for the following histological, enzyme-histochemical and histochemical methods: hematoxylin and eosin, Giemsa, modified Gomori' trichrome stain, PAS, Oil red O, ATPase at pH 9.6, 4.6 and 4.35; DPNH-d; COX, acid and alkaline phosphatase, non-specific esterase. Moreover cryostat sections were fixed, incubated in normal horse serum (Vectastain TM ABC Kit, PK 4002) and the following monoclonal antibodies were applied: anti-IgA; anti IgG; anti IgM, anti-kappa; anti-lambda; Leu 14 (CD22, pan-B cells); Leu 3a + 3b (CD4, helper-inducer T cells); Leu 3a + b (CD8, cytotoxic / suppressor T cells); Leu 4 (CD3, pan T cells); Leucocyte common antigen (LCA, CD45); Kp 1 (CD 68, macrophages) (all obtained from Biotest AG, Becton-Dickinson and DAKO). After washing the sections were incubated in horse anti-mouse serum pre-adsorbed with normal human serum. Then the sections were counterstained with Mayer' haematoxylin and mounted in glycerin. Morphometry of the fibre diameter and fibre types was performed with an automatic computerized imaging analyzer (IBAS I and II, Kontron).

Results

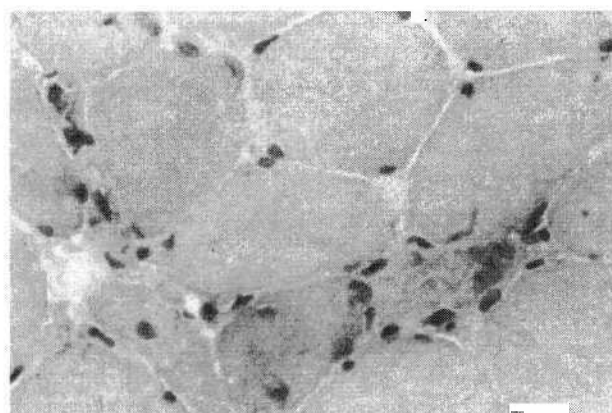
Skeletal muscle biopsy

All patients showed necrotizing myopathy of variable severity. In some patients the number of necrotic fibres was consistent and patterns of phagocytosis were observed (fig. 1A). Scattered and dispersed inflammatory infiltrates composed by lymphocytes and histiocytes were seen in endomysial and perimysial connective tissues and around muscle fibres, as well as around intramuscular arterioles and capillaries (fig. 1B and 1C). Vasculitis was sometimes observed in form of damage of vascular wall with infiltration of neutrophils, lymphoid cells and histiocytes. Immunocytochemical studies on frozen sections are summarized in table 2. Infiltrates around blood vessels and muscle fibres consisted of T cells of both helper/inducer (CD4) and cytotoxic/suppressor (CD8) phenotype (ratio 3:1), and of CD68 positive histiocytes intermingled with a slight number of polytypic CD 22 positive B cells. In two cases these changes were associated with denervation patterns, consisting in groups of atrophic angular fibres and in enzyme-histochemical patterns of "type grouping", indicating denervation with collateral reinnervation.

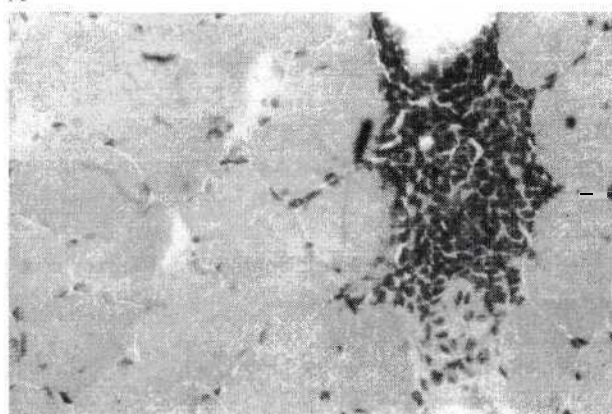
Table 1. Clinical and laboratory characteristics of patients with type II essential mixed cryoglobulinemia and myopathy.

Case/age/sex	CrioIg	Purpura	Arthralgia	Weakness	Sensory loss	Serum CPK	EMG
1/64/F	II	+++	+++	+++	-	380	Myopathic
2/68/M	II	++	++	+++	-	400	Myopathic
3/58/M	II	++	++	++	-	360	Myopathic
4/57/F	II	++	++	++	-	420	Myopathic
5/64/M	II	+++	++	++	+	280	Mixed
6/60/F	II	++	++	+++	++	360	Mixed

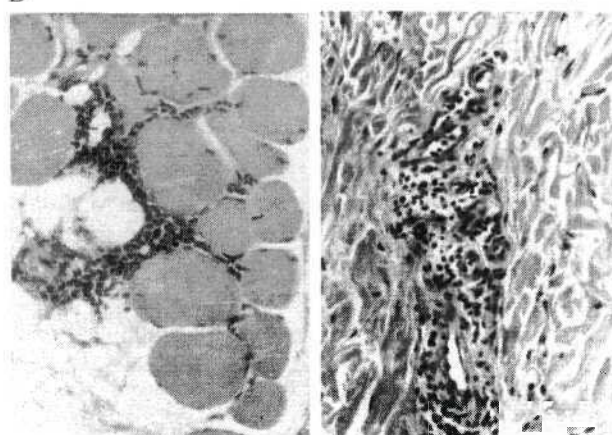
Myopathy in essential mixed cryoglobulinemia



A



B



C

D

Figure 1. A. Variation in the muscle fibre diameters, fibre necrosis and phagocytosis. Case 2: Hematoxylin and eosin, X800. B, C. Intramuscular infiltration by mononuclear lymphoid and histiocytic cells in muscle biopsies. Case 1 and 5: Hematoxylin and eosin, X750. D. Perivascular mononuclear infiltrates in dermal tissues from a skin biopsy. Case 2: Hematoxylin and eosin, X400.

Skin biopsy

All patients showed variable patterns of dermal vasculitis, involving capillary, arteriolar and venular walls. Endo-

Table 2. Summary of immunophenotyping analysis of mononuclear cells in skeletal muscle biopsies of 6 patients with cryoglobulinemia.

Cell type marker	Range (%)	Mean (%)
Leucocyte common antigen (CD45)		100
B cells, CD22	2-6	4.0
T cells, CD3	48-70	57.0
T cells, CD4a		70.0*
T cells, CD8a		23.3*
NK cells, Leu 7	0.5-3	1.2
Macrophages, CD 68, Kp1	20-44	36.0
Dendritic cells, RFD14-12		10.0
Ratios CD4/CD8		3.1

* CD4 and CD8 cells recorded as percentage of total T cells (CD3).

thelial swelling and perivascular infiltration by lymphocytes, histiocytes and rare neutrophils were observed (fig. 1 D). At immunofluorescence, numerous arteriolar walls were positive for IgM and IgG components and sometimes for complement fractions.

Fascial biopsy

Chronic active fasciitis with diffuse inflammatory infiltration by lymphocytes, histiocytes and neutrophils was seen in four cases.

Discussion

Peripheral neuropathy with denervation muscle atrophy was found to be present in the majority of patients with mixed cryoglobulinemias [8]. Sural nerve biopsy showed vasculitis of vasa nervorum and axonal degeneration [5, 21]. Biopsy-documented muscular lymphocytic vasculitis was recently described in two patients with cryoglobulinemic peripheral neuropathy of primary Sjogren's syndrome [11]. The present study shows for the first time a microvascular and inflammatory involvement of skeletal muscle in a series of patients with type II cryoglobulinemia. The patients of the present series were more often middle aged subjects showing asthenia, purpura and arthralgia; they showed type II cryoglobulins, according to Brouet and Meltzer et al [4, 15] and presented clinical and electrophysiologic signs of myopathy with increase of serum CPK. Two of patients showed clinical and neurophysiological signs of peripheral neuropathy. The main pathological alterations in the skeletal muscle biopsies were myopathic alterations i.e. degenerative and necrotizing patterns in muscle fibres and vasculitis with mononuclear infiltrates composed by CD4 helper-inducer and CD 8 cytotoxic-suppressor T cells and by CD 68 positive histiocytes. The predominant cell type invading necrotic

muscle fibres was the CD 68 positive histiocyte with patterns of phagocytosis. Infiltration around normal fibres and among interstitial connective tissues was sustained by T-cells and rare histiocytes, these two cell types representing more than 70% of the infiltrating mononuclear elements.

Previous studies have characterized the nature of the mononuclear infiltrate in skeletal muscle biopsies from patients with inflammatory and non-inflammatory myopathies. Immunocytochemistry indicated that the main cell types in juvenile dermatomyositis and X-linked muscular dystrophies were histiocytes and T lymphocytes with striking predominance of CD 4 helper/inducer T cells [2, 9, 14]. These cells coupled with an aberrant class I MHC antigen expression may be sufficient to induce cell damage in the inflammatory myopathies [14].

In the present series vasculitis and perivascular infiltration composed by T cells and histiocytes was seen. Deposition of IgM, IgG and complement fractions in the vascular walls were also observed together with a variable inflammatory chronic infiltration in fascial tissues. A study on a case of paraneoplastic cryoglobulinemic neuropathy with occlusive microangiopathy by immunoglobulin precipitation interpreted the mononuclear infiltrates made of T lymphocytes around the blood vessels as a moderate inflammatory response secondary to the vascular occlusion [19]. It is assumed that different mechanisms are involved in the pathogenesis of the cryoglobulinemic pathology: i) aggregation and precipitation of cryoproteins within the vascular lumen interfering with microcirculation [5, 10, 19]; ii) a possible autoimmune activity of the cryoglobulinemia and/or vasculitis by circulating immune complexes [3, 6, 10]. In the present series, vasculitis and/or permanent intravascular cryoglobulin precipitation may be of pathogenetic relevance for the development of necrotizing ischaemic alterations in the skeletal muscle and skin biopsies. They may be considered a cryodependent manifestation also responsible for articular, renal and peripheral nervous manifestations and for the ischaemic multiinfarctual encephalopathy [20]. The myopathic changes may be overimposed on denervation muscle atrophy in patients with documented cryoglobulinemic peripheral neuropathy.

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References

- [1] Apartis E, Leger JM, Musset L, Gugenheim M, Cacoub P, Lyon-Caen O, Pierrot-Deseilligny C, Hauw JJ, Bouche P: Peripheral neuropathy associated with essential mixed cryoglobulinemia: a role for hepatitis C virus infection? *J Neurol Neurosurg Psychiatry* 1996; 6: 661-666.
- [2] Arahata K, Engel AG: Monoclonal antibody analysis of mononuclear cells in myopathies. I, Quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibres invaded by T cells. *Ann Neurol* 1984; 16: 193-208.
- [3] Armin Ar, Rosenbaum L, Chapman-Winokur R: Intravascular immunoglobulin crystalloids in monoclonal cryoglobulinemia-associated dermatitis: ultrastructural findings. *J Cutan Pathol* 1993; 20: 74-78.
- [4] Brouet JC, Clauvel JP, Danon F: Biologic and clinical significance of cryoglobulins: a report of 86 cases. *Am J Med* 1974; 57: 775-788.
- [5] Chad D, Parisier K, Bradley WG: The pathogenesis of cryoglobulinemic neuropathy. *Neurology* 1982; 32: 725-729.
- [6] Clemmensen I, Jensen BA, Holund B: Circulating monoclonal IgM lambda cryoglobulin with collagen type I affinity in vasculitis. *Clin Exp Immunology* 1986; 64: 587-589.
- [7] Geltner D: Therapeutic approaches in mixed cryoglobulinemia. *Springer Semin Immunopathol* 1988; 10: 105-113.
- [8] Gemignani F, Pavesi G, Fiocchi A: Peripheral neuropathy in essential mixed cryoglobulinemia. *J Neurol Neurosurg Psychiatry* 1992; 55: 116-120.
- [9] Giorno R, Barden MT, Kohler PF, Ringel SP: Immunohistochemical characterisation of the mononuclear cells infiltrating muscle of patients with inflammatory and non-inflammatory myopathies. *Clin Immunol Immunopathol* 1984; 30: 405-412.
- [10] Gorevic PD, Kassab JH, Levo Y, Meltzer M, Franklin E: Mixed cryoglobulinemia: clinical aspect and long-term follow up of 40 patients. *Am J Med* 1980; 69: 287-308.
- [11] Hebbbar M, Hebbbar-Savean K, Hachulla E, Brouillard M, Hatron PY, Devulder B: Participation of cryoglobulinemia in the severe peripheral neuropathies of primary Sjogren's syndrome. *Ann Med Interne. Paris* 1995; 146: 235-238.
- [12] Levo Y, Gorevich PD, Kassab HJ, Franklin EC: The association between hepatitis B vims and essential mixed cryoglobulinemia. *New Engl J Med* 1977; 296: 1501-1504.
- [13] Lippa CF, Chad DA, Smith TW, Kaplan MH, Hammer K: Neuropathy associated with cryoglobulinemia. *Muscle and nerve* 1986; 9: 626-631.
- [14] MacDouall RM, Dunn JM, Dubowitz V: Nature of mononuclear infiltrate and the mechanism of muscle damage in juvenile dermatomyositis and Duchenne muscular dystrophy. *J Neurol Sci* 1990; 99: 199-217.

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- [15] Meltzer M, Franklin EC, Elias K, McCluskey RT, Cooper N: Cryoglobulinemia. A study of 29 patients. I: IgG and IgM cryoglobulins and factors affecting cryoprecipitability. *Am J Med* 1966; 40: 828-836.
- [16] Monteverde A, Rivano MT, Allegra GC, Monteverde AI, Zigrossi P, Baglioni P, Bordin G, Pileri S: Essential mixed cryoglobulinemia, type II: a manifestation of a low grade malignant lymphoma? Clinical morphological study of 12 cases with special reference to immunohistochemical findings in liver frozen sections. *Ada Haematol* 1988; 79: 20-25.
- [17] Mussini C, Ghini M, Zanni G: Cryoglobulinemia: bone marrow histological investigations. *Conn Tiss Dis* 1992; 11: 25-29.
- [18] Nemni R, Corbo M, Fazio R, Quattrini A, Comi G, Canal N: Cryoglobulinemic neuropathy. A clinical, morphological and immunocytochemical study of 8 cases. *Brain* 1988; 111: 541-552.
- [19] Prior R, Shober R, Sharffetter K, Wechsler W: Occlusive microangiopathy by immunoglobulin (IgM kappa) precipitation: pathogenetic relevance in paraneoplastic Cryoglobulinemic neuropathy. *Acta Neuropathol* 1992; 83: 423-426.
- [20] Serena M, Biscaro R, Moretto G, Recchia E: Peripheral and central nervous system involvement in essential mixed cryoglobulinemia: a case report. *Clinical Neuropathology* 1991; 10: 177-180.
- [21] Tredici G, Petruccioli MG, Cavalletti G: Sural nerve bioptic findings in essential Cryoglobulinemic patients with and without peripheral neuropathy. *Clin Neuropathol* 1992; 121-127.