# The correlation between cardiac and skeletal muscle pathology in animal models of idiopathic inflammatory myopathies

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**Idiopathic inflammatory myopathies (IIMs) represent a heterogeneous group of disorders in which skeletal muscle is inappropriately targeted by the immune system. IIMs are characterized by inflammation of muscle and varying degrees of muscle dysfunction. Extra-muscular manifestations may involve heart, skin, joints, lungs, and gastrointestinal tract. Cardiovascular involvement is a feared event because is one of the leading causes of mortality in IIM patients. As the myocardium shares many features with the skeletal muscle, it is supposed that it can be affected by the same inflammatory processes, which take place during the different forms of IIMs. However, the full extent of this link and the mechanisms behind it are still not fully understood. Animal models have greatly improved our understanding of IIM pathomechanisms and have proven to be a useful tool for discovering therapeutic drug targets. Here we report the evidence of heart muscle involvement in different animal models of spontaneous IIMs, assuming a common autoimmune mechanism and presenting them as study models for human pathology.**

**Key words:** neuromuscular disorders, myocarditis, myositis, idiopathic inflammatory myopathy, animal models

## Introduction

Professor Giovanni Nigro (Fig. 1) in his research and diagnostic activity has strongly supported the relationship between skeletal muscle and cardiac muscle diseases [1]. Skeletal and cardiac muscle share many structural and functional features; what affects one type of muscle is often associated with damage to the other as well [2]. This assumption is today well defined [3-6], but often still underestimated. Moreover, the treatment usually focuses on one of these tissue without addressing the other tissue involved in the same disease process; this can make general treat- ment less effective [2].

The study of cardiac pathology in animal with idiopathic inflammatory myopathies (IIMs) is an input that came from Prof. Giovanni Nigro and his determination to understand the correlation between heart and muscle pathology in both dystrophies and inflammatory diseases.

IIMs represent a heterogeneous group of disorders in which skeletal muscle is inappropriately targeted by the immune system [7]. The mean global prevalence of the IIMs in humans range from 4.27 to 7.89/100,000 [8].

**Figure 1.** From the left side: Lucia Ines Comi, Serenella Papparella, Valerie Askanas, Orlando Paciello, Giovanni Nigro and Corrado Angelini, at the XI International Congress on Neuromuscular Diseases in Istanbul (Turkey), 2006.

This group of diseases was typically divided into several subtypes such as dermatomyositis (DM), polymyositis (PM), inclusion body myositis (IBM), immune-mediated necrotizing myopathy (IMNM) and overlapping syndromes, a common feature of which is muscle inflammation leading to their progressive weakness; however, the skin and internal organs can also be affected [8]. The autoimmune origin is often regarded as the autoantibodies specific for myositis are detected in the serum of 50-70% patients [8].

Cardiac involvement in IIM was first reported by Oppenheim in 1899 [9]. Currently, more and more evidence has accumulated in support of a link between IIMs and cardiac involvement [10]. Since the myocardium shares many features with skeletal muscle, it is assumed that it can be affected by the same inflammatory process, which occur during the different forms of IIMs. However, the full extent of this link and the mechanisms behind it are still not fully understood [11].

Cardiac involvement is more commonly reported in patients with PM/DM, while patients with IBM have a lower risk [10]. Morphologically, in PM/DM the myocardium shows an inflammation similar to that of skeletal muscle [10]. An increased risk of myocardial infarction and venous thromboembolism has also been reported in patients with IIMs [4]. However, cardiac disease in IIM is most commonly subclinical and data in the literature suggest that rhythm disturbances are the most common subclinical cardiac manifestation of PM/DM, while congestive heart failure is the most frequently reported cardiac complication, and occurs in 10-15% of patients [10]. Congestive heart failure can develop at any time in the course of skeletal muscle disease, and even in remission state [10].

Cardiac involvement in IIMs is a feared event because it is one of the most common cause of patient death [4,10].

Increasing evidence suggests that the IIMs result from certain environmental exposures in genetically susceptible individuals [12,13]. The HLA 8.1 ancestral haplotype is a key risk factor for IIM in humans, and several genetic variants associated with other autoimmune diseases have been identified as IIM risk factors. Environmental risk factors are less well studied than genetic factors but could include viruses, bacteria, ultraviolet radiation, smoking, occupational and perinatal exposures and a growing list of drugs (including biologic agents) and dietary supplements [13].

Investigations have shown that a variety of infections not only cause infectious myopathies but could also be

possible triggers for IIM [12,13]. The link between infectious agents and IIM development has been determined through case reports, epidemiological investigations and animal models [12,13]. Examples include hepatitis B virus in PM and DM; hepatitis C virus in IBM; retroviruses, in particular human immunodeficiency virus (HIV) and human T-lymphotropic virus-1, in PM, DM and IBM; Toxoplasma spp. and Borrelia spp. in PM and DM; and influenza, picornaviruses and echoviruses in PM, DM and juvenile dermatomyositis [12,13].

Here we report the evidences of cardiac muscle involvement in different animal models of spontaneous IIMs, assuming a common autoimmune mechanism and presenting them as study models for human pathology.

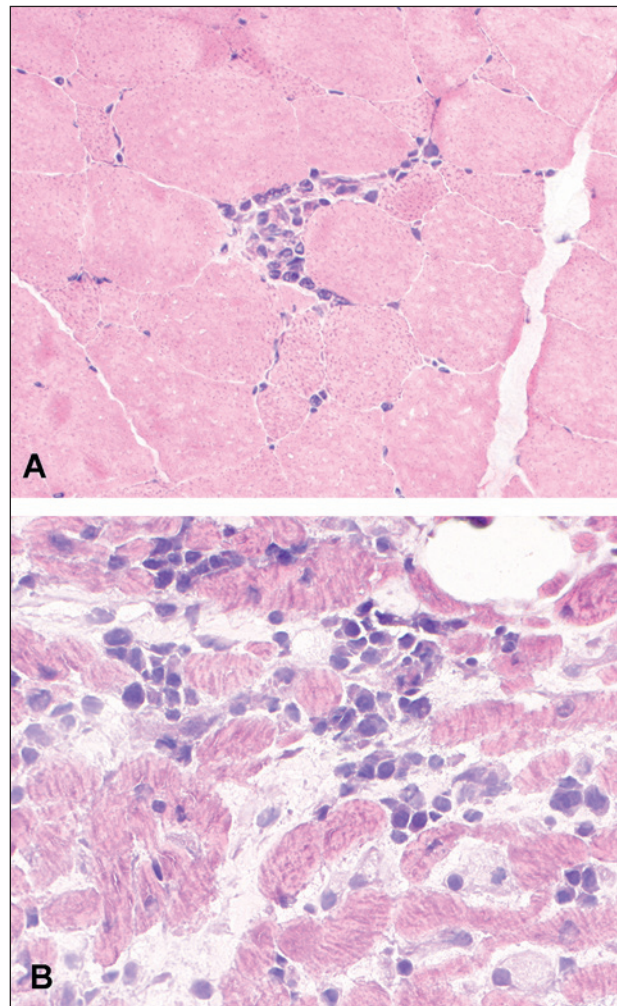## Inflammatory myopathy and cardiomyopathy in cats associated with Feline Immunodeficiency Virus (FIV) infection

FIV infection is associated with an inflammatory myopathy (IM) and myocarditis in adult cats [14,15]. HIV is similarly implicated in a form of IM and myocarditis in humans [15-17]. This model has so far been poorly characterized and there is little information in the literature, however, the characteristics of this myopathy are comparable to human PM [14,15].

No clinical signs have been associated with this IM, however, an increase in serum CK values has been reported [14]. Needle electromyography may be characterized by mild to moderate abnormal spontaneous activity. Furthermore, a mixture of positive acute waves and fibrillation potentials can be detected in a multifocal pattern [14].

The pelvic limb muscles are more frequently affected than the thoracic limb muscles. The vastus lateralis is the most frequently affected muscle while the brachial triceps is the least affected muscle [14].

Histologically, this IM is characterized by perivascular and endomysial multifocal infiltration of CD8+ T lymphocyte (Fig. 2A). Sometimes, these lymphocyte infiltrate non-necrotic myofibers. Myofiber necrosis and phagocytosis has also been reported [14].

FIV infection in adult cats has also been associated with myocarditis characterized by a coalescing multifocal inflammatory infiltrate mainly composed of lymphocytes and, to a lesser extent, of macrophages, neutrophils, and plasma cells (Fig. 2B). A variable degree of interstitial fibrosis has also been reported [15]. Hypertrophic cardiomyopathy (HCM) is also described in cats associated with FIV infection. Clinical manifestations included dyspnea, lethargy, anorexia and vomiting [15].
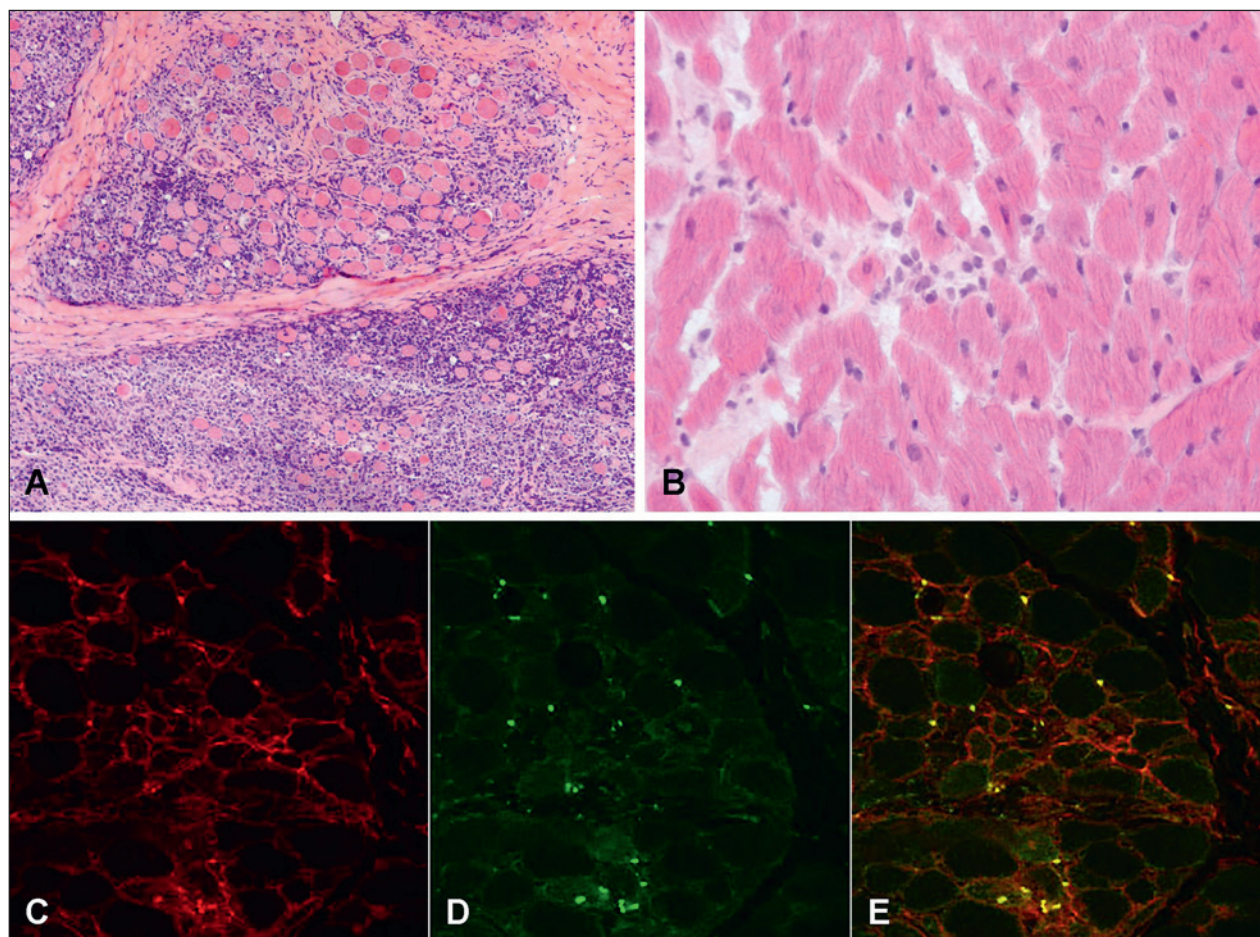


**Figure 2.** Histopathological findings in feline immunodeficiency virus (FIV) associated myositis and myocarditis, cat, hematoxylin and eosin (magnification 400 X). A) Skeletal muscle: the endomysium of the skeletal muscle is expanded by a lymphocytic infiltrate; B) Myocardium: the myocardium is infiltrated by numerous lymphocytes and plasma cells.

## Canine inflammatory myopathy and cardiomyopathy associated with Leishmania infantum infection

In dogs, *Leishmania infantum* infection is associated with an IM with many similarities with the human PM (Fig. 3A-B) [18,19].

IM in these dogs is often subclinical [20]. The most common clinical signs of leishmaniasis are skin lesions, lymphadenopathy, hepatosplenomegaly, weight loss, onychogryphosis and ocular lesions. Rarely, these signs can be associated with clinically evident neuromuscular signs, such as paraparesis [20]. With electromyography,

**Figure 3.** Histopathological findings in *Leishmania infantum* associated myositis and myocarditis, dog, hematoxylin and eosin. A) Skeletal muscle is effaced by a severe and diffuse lymphocytic and macrophagic infiltrate (magnification 100 X); B) Myocardium focally infiltrated by lymphocytes (magnification 400 X); C) Immunofluorescence detection of MHC I (red, TRIC, magnification 400 X); D: Immunofluorescence detection of *Leishmania spp.* (green, FITC, magnification 400 X); E: Colocalization of MHC I and *Leishmania spp.* (magnification 400 X).

fibrillation potentials, positive sharp waves and complex repetitive discharges can generally be detected even in asymptomatic dogs [20].

Morphologically, the inflammatory infiltrate in skeletal muscle is mainly perivascular in the perimysium and multifocally surrounds the muscle fibers in the endomysium (Fig. 3A). The inflammatory cells are mainly CD8+ T lymphocytes and macrophages with fewer CD4+ T lymphocytes [18,20]. Marked variation in fiber size, including atrophic and hypertrophic fibers, and different disseminated necrotic muscle fibers can usually be observed. Aspects of muscle regeneration can also be observed, including myotubes and type 2C fibers [18]. In the chronic stages, endomysial and perimysial thickening due to fibrosis can be observed. Usually, many muscle fibers show immunohistochemical sarcolemmal expression of Major Histocompatibility Complex (MHC) class I (Fig. 3C-D)

and class II [18]. Moreover, CD8+ T lymphocytes invade histologically normal muscle fibers expressing MHC class I antigens (CD8/MHC-I complexes) supporting an immune-mediated pathogenetic hypothesis [18].

Dogs infected with *Leishmania infantum* also show myocarditis characterized by an inflammatory infiltrate similar the that reported in skeletal muscle (Fig. 3B). Interstitial fibrosis and sarcolemmal expression of MHC class I and MHC class II antigens have also been reported [19].

Several pathogenetic mechanisms have been hypothesized [18]; however, the most supported is an antibody-mediated autoimmune mechanism [21]. We hypothesize that the autoantibodies produced by dogs with leishmaniasis may be directed against one or more proteins shared by skeletal and cardiac muscle, triggering immune-mediated damage in both tissue [18,19].

## Inflammatory myopathy in Syrian hamster associated with Leishmania infantum infection

Syrian hamster infected with *Leishmania infantum* develop an IM that shows close similarities to human PM [22].

Clinically, during the chronic phase of the disease (> 3 months after infection) infected hamsters have lost weight and are weaker. They are usually asthenic, have reduced activity and peeling in the extremities. Skeletal muscles usually appear moderately atrophic. In addition, an increase in serum muscle enzymes has been reported, with an increase in LDH and AST values more than 5 times and an increase in CK even more than 50 times [22].

This IM is histologically characterized by a multifocal inflammatory endomysial infiltrate composed mainly of CD8+ T lymphocytes. In addition, numerous perivascular aggregates of macrophages and CD4+ T lymphocytes have also been reported. Numerous muscle fibers with class I and II MHC sarcolemmal positivity has also been reported. Furthermore, the presence of some CD8+ lymphocytes invading histologically healthy muscle fibers expressing MHC class I antigens (CD8/MHC I complex) support an autoimmune hypothesis [22].

The Syrian hamster infected with *Leishmania infantum* also develops myocarditis characterized by an inflammatory infiltrate similar the that reported in skeletal muscles. (Paciello et al., personal observation).

## Inflammatory myopathy in sheep associated with *Sarcocystis tenella* infection

For years, it has been argued that Sarcocystis infection almost always did not cause injury to the ruminant's muscle or is associated with an eosinophilic myositis in the case of cyst rupture. We have defined that *Sarcocystis tenella* infection is associated with an IM in sheep [23].

In intermediate hosts, such as sheep, infection is commonly asymptomatic and the presence of muscle cysts is considered an incidental finding [23]. However more that 95% of infected animals show subclinical myopathy [23].

Histologically, this myopathy is characterized by a multifocal inflammatory endomysial infiltrate mainly composed of CD8+ lymphocytes, occasionally centered around parasitic and non-parasitic fibers and rarely arranged in perivascular cuffs (Fig. 4A). Variability of fiber diameter and different disseminated necrotic muscle fibers invaded by macrophages are reported [23].

We also reported widespread sarcolemmal immunopositivity for MHC I and MHC II in almost all cases and variable expression of MHC I antigen on the cyst wall [23]. Moreover, occasionally CD8+ cells invade non-necrotic parasitized and non-parasitized fibers [23].

Sheep infected with *Sarcocystis tenella* also show myocarditis characterized by an inflammatory infiltrate similar the that found in skeletal muscle (Fig. 4B).

Inflammatory CD8+ T lymphocytes infiltrate, sarcolemmal immunopositivity to MHC I and CD8+ T lymphocytes invading non-necrotic muscle fibers suggest that parasitized muscle fibers might play an active role in antigen presentation and stimulating inflammatory response [23].
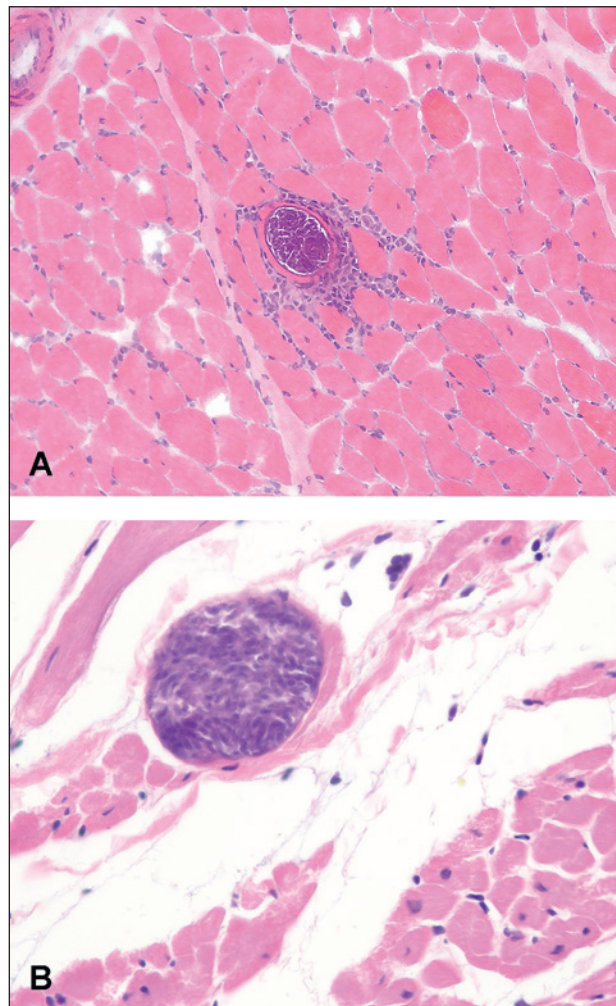


**Figure 4.** Histopathological findings in *Sarcocystis spp.* associated myositis and myocarditis, sheep, hematoxylin and eosin (magnification 200 X). A) Skeletal muscle: a lymphocytic infiltrate surrounds and infiltrate a parasitized muscle fiber; B) Myocardium: thin-walled septate sarcocyst containing myriad of banana-shaped bradyzoites in the myocardium with scattered inflammatory cells.

## Inflammatory myopathy in horses associated with piroplasmosis

Equine piroplasmosis is a protozoal disease caused in horses by two apicomplexan hemoprotozoa, *Theileria equi* and *Babesia caballi* [24]. Equine piroplasmosis has been associated with an IM characterized by circulation of anti-muscle antibodies [24].

Clinically, horses with chronic piroplasmosis can develop poor performance and muscle atrophy. The serum activity of CK, AST and LDH is usually slightly elevated [24].

Histologically, the main myopathic change is a multifocal lymphocytic infiltrate often organized in cuffs around the perimysial and endomysial blood vessels and less frequently expanding multifocally into the endomysium. The inflammatory infiltrate is mainly composed of both CD8+ and CD4+ T lymphocyte, fewer macrophages and rare scattered CD79α+ B lymphocytes [24].

Various degrees of atrophy of nonangular fibers, necrotic fibers invaded by macrophages, mild perimysial fibrosis were also observed. Mitochondrial abnormalities include ragged blue fibers with SDH and fibers with a moth-eaten appearance with COX and NADH stains [24].

Muscle fibers immunohistochemically overexpress MHC I and MHC II [24].

Increased mRNA levels of IL-12, TNF-α, and IFN-γ were found in the muscles of affected animals, while no changes in IL-10 mRNA levels were observed. Moreover, DNA from *Theileria equi* or *Babesia caballi* was not detected in muscle samples affected, by RT-PCR [24].

Involvement of the heart during piroplasmosis has been well established in dogs, but there is little sporadic information in the literature on heart involvement during piroplasmosis in horses [25,26]. Further studies are needed to better clarify the involvement of the heart in this species.

## Conclusions and perspectives

In this review we have summarized our studies and observations on the heart muscle involvement during inflammatory myopathies. We have shown in previous papers that various pathogens such as viruses and protozoa responsible for myositis in animals can also be responsible for myocarditis in the same animals. This possibility should always be considered during the diagnostic process and in the therapy of myositis in animal and humans [4,10].

The pathogenesis of most immune-mediated diseases is related to chronic organ inflammation that can be caused by specific interactions between genetic and environmental risk factors. In these diseases, immune activation often involves both innate and adaptive and non-immune mechanisms; however, the details and interactions of the various pathways are generally unclear and new animal models can be very valuable in elucidating these mechanisms [7,13].

Infections are the main actors in the environmental factors which modulate the development of autoimmune diseases. The underlying mechanisms are multiple and complex, probably different according to pathogens [27]. In these cases, as in the studies we reported, the most interesting observation is that even in the absence of the pathogen, the damage to the muscle persisted as immune-mediated damage [7]. We hypothesized that the pathogenetic mechanism underlying this form of IMs, due to the numerous shared structural features, may also involve the myocardium.

Therefore, these animals may be useful study models for IIM and myocarditis in humans. Furthermore, these observations lead to the hypothesis that in IIM it must always be considered that an etiological agent may have been involved as a primary or secondary cause of the disease. These pathogenetic mechanisms could be common to animals and humans and should be considered during both the diagnostic and therapeutic process for patients suffering from myositis and myocarditis.

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