# Eosinophilic Polymyositis in a Mouse

LISA A. KRUGNER-HIGBY, DVM, PHD,[1] ANNETTE GENDRON, DVM, PHD,[1] THEODORE GARLAND, JR., PHD,[2] PATRICK A. CARTER, PHD,[2] JOHN G. SWALLOW, PHD,[2] AND JAMES J. LEE, PHD[3]

**Abstract | Idiopathic eosinophilic polymyositis has been described in human beings and dogs, but has not been previously diagnosed in a rodent. A Swiss-derived mouse was submitted for necropsy because of weakness, anorexia, and difficulty righting itself when rolled onto its back. No gross lesions were apparent. Microscopically, there were accumulations of eosinophils in skeletal muscle and heart. Focal, myofiber necrosis and degeneration were associated with the cellular infiltrates in these tissues. No parasites were found associated with these lesions. The disease in this mouse may have had a genetic or common-exposure component.**

The term eosinophilic myositis or polymyositis is used to describe a diverse, heterogeneous group of diseases in human beings and animals. One form of the disease may be associated with parasites that invade muscle tissue, such as Trichinella or Sarcocystis (1–4). In human beings, it has been described as a reaction to drugs (5) or nutritional supplements, as has occurred with particular suppliers of L-tryptophan (6, 7). Eosinophilic myositis also occurs as a disease of unknown origin (idiopathic eosinophilic polymyositis) (1, 8–12). In human beings, idiopathic eosinophilic myositis can occur as a localized lesion (9, 13) and has even been described as a pseudotumor (14), or as a generalized condition involving multiple skeletal muscles and the heart (8, 9, 11). The disease can occur with or without concurrent increase in the number of eosinophils in the peripheral blood (9, 11, 15). A form of idiopathic eosinophilic myositis has been described in dogs (16), in which the lesions are frequently confined to the muscles of mastication, but cases of generalized disease have also been described (16). This report describes a case of eosinophilic polymyositis involving skeletal as well as cardiac muscle in a mouse.

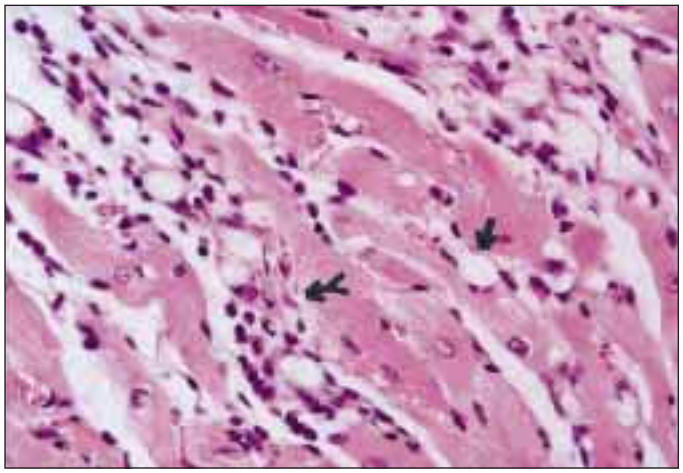

FIG. 1A. Section of left ventricle with cardiac myofiber degeneration and vacuolization (arrow), minimal fibrosis and inflammatory cell infiltrate (double arrow). 40X

## Case Description

A mouse from a campus breeding colony (identification #157116) (17, 18), which was founded by stock from outbred Swiss mice (Hsd:ICR) from a commercial vendor (Harlan Sprague Dawley, Indianapolis, IN), was presented to the diagnostic laboratory. The colony from which the mouse in this case was derived was conventionally housed in polycarbonate cages without microisolators, provided wood chip bedding, and fed commercial rodent chow (Harlan Teklad Laboratory Rodent Diet (W)-8604; Harlan Sprague Dawley, Indianapolis, IN) and water ad libitum. The colony was maintained at 22° C with a constant 12:12-h light-dark cycle. All procedures, both experimental and clinical, were done in accordance with the ILAR Guide for the Care and Use of Laboratory Animals. Since acquisition, one-half of the colony had undergone 12 generations of genetic selection for high frequency of locomotor behavior as measured by voluntary wheel-running. The breeding selection procedures described by Swallow et al. (17) avoid sibling matings but result in approximately 1.5% inbreeding per generation. This mouse was from a litter comprising five animals at weaning (21 days of age), which is about half the normal litter size in this colony. All four siblings were male; the studied mouse was a 1.6-month-old male. At 6 weeks of age, the mice were transferred from their home cage to a cage where they had free access to a stainless steel running wheel and the frequency and duration of use of the running wheel were measured. No external stimuli were used to induce the mice to run on the wheel.

On days 5 and 6 of wheel access, two of the siblings died, prompting close examination of all animals from this litter. At this time, the female mouse was thin, ataxic, dehydrated, had a ruffled hair coat, and had difficulty righting itself when turned over. Prior to wheel access (42 days of age), his body mass was 18.32 g. Body mass prior to wheel access was 23.76 g and 29.05 g in the two siblings that died; the two apparently non-affected males were larger (33.52 g and 39.86 g). The amount of wheel running (total revolutions per day) performed by the three affected individuals was somewhat lower than that of the two non-affected siblings and was also considerably lower than the gender-specific mean for the selected line from which they derived.

Blood was drawn by cardiac puncture after a deep pentobarbital overdose (150 mg/kg, administered intraperitoneal), effectively euthanizing the animal in accordance with the recommendations of the 1993 AVMA Panel on Euthanasia. The carcass was then submitted for gross and histopathologic evaluation. No gross lesions were noted in any organ. The following tissues were evaluated by histopathologic examination by using routine hematoxalin and eosin staining procedures: heart, lung, thymus, spleen, pancreas, kidneys, liver, lymph nodes, skeletal muscle, femur, stomach, intestines, hardarian gland, eye, testes, accessory sex glands, salivary gland, cross sections through the spinal cord, vertebrae and paravertebral muscle, and cross sections through the head, including the brain. In histologic sections of the heart (Figures 1a and 1b) and the right leg (Figure 2), numerous eosinophils were present both within and between

[1]*Research Animal Resources Center and the* [2]*Department of Zoology, University of Wisconsin, Madison, Wisconsin, and the* [3]*Department of Biochemistry and Molecular Biology, Mayo Clinic, Arizona, Scottsdale, Arizona.*

  

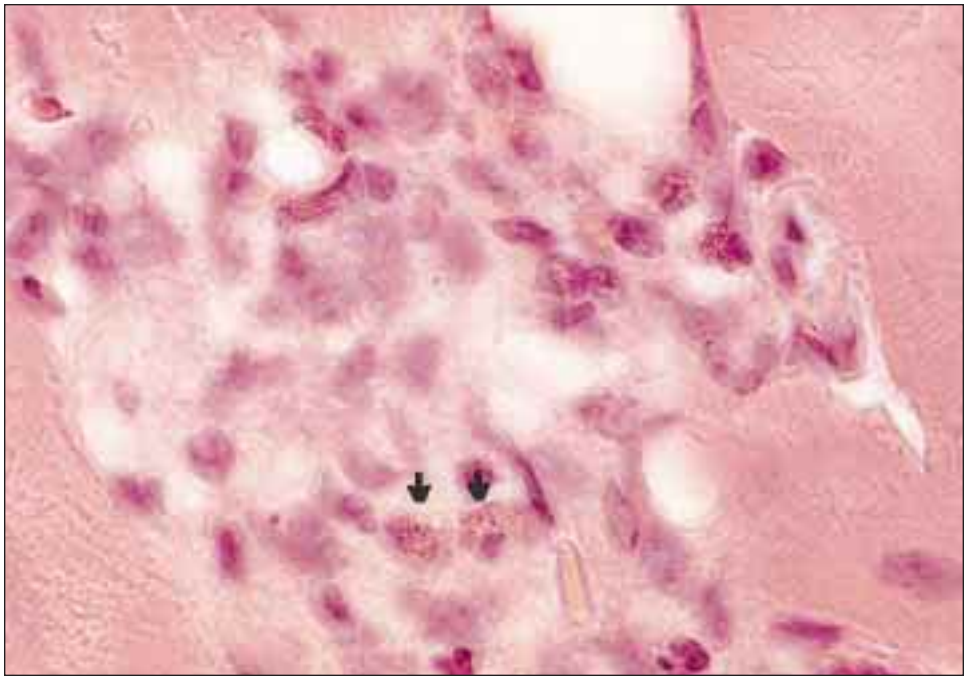

FIG. 1B. Inflammatory cell infiltrate in the left ventricle with numerous eosinophils (arrows). 100X.

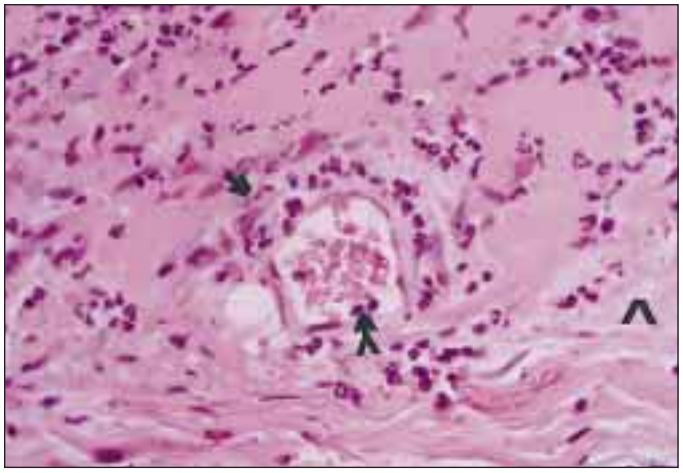

FIG. 2. Section of skeletal muscle with eosinophilic, neutrophilic, and lymphocytic infiltrates (arrow), muscle degeneration (arrowhead), and eosinophils in a small vessel (double arrow). 40X.

the muscle fibers. In the heart, there were multiple foci of eosinophilic infiltration within the myocardium in both the atria and the ventricles. A mixed inflammatory cell infiltrate with predominant eosinophil populations as well as lymphocytes and histiocytes was present in the papillary muscle and the wall of the left ventricular myocardium (Figure 1b). A cellular infiltrate comprising larger numbers of lymphocytes and histiocytes and fewer eosinophils was found in an inflammatory focus in the atrial wall. Within the ventricular myocardium, there were two coalescing foci of inflammatory cell infiltrates between the myofiber bundles consisting predominantly of eosinophils, with smaller numbers of vesicular mononuclear cells with abundant amphophilic cytoplasm. In the ventricular foci, there was also mild to moderate fibrosis associated with the eosinophilic infiltrates (Figure 1a). In a section of skeletal muscle from the right leg, a central area of hemorrhage was associated with a focus of mixed eosinophilic and neutrophilic infiltrates. The cellular infiltrates were clustered around a small artery in the center of the muscle bundle, but also extended between adjacent muscle fibers. There was degeneration, fragmentation, and vacuolization of muscle fibers but no fibrosis (Figure 2). Sections of skeletal muscle from the head and paravetebral sites were normal.

Other lesions in this animal included enlarged, hyperplastic pancreatic lymph nodes and hyperplastic germinal follicles in the spleen. No parasites were found in multiple silver- or Gram-stained sections of the affected muscles. The bone marrow from both femurs contained active hematopoesis, and eosinophils were common but not overrepresented. Eosinophils were seen in pulmonary hilar veins but, although they were more frequent than normal (2–3 per 40X field), they were not numerous. The cecal contents of this animal were negative for pinworms by direct examination, but murine pinworms have been found in other animals in this colony. The animal's serum was tested for the following murine pathogens: Mycoplasma pulmonis, Sendai virus, mouse hepatitis virus, and pneumonia virus of mice and found to be negative for these agents. This mouse was not tested for other agents. The colony from which this mouse was derived has been tested twice a year for the pathogens mentioned above and has been found to be serologically negative. In addition, over the past 3 years, selected serum samples from this colony have been tested for reovirus type III, Theiler's Virus, Ectromelia, mouse adenovirus, polyoma virus, Lymphocytic Choriomeningitis Virus, murine rotavirus, murine parvovirus, and Cilia-Associated Respiratory bacillus and found to be negative. Because mice in this colony are housed in conventional open-topped, wire-lidded cages, pathogen status should be generally uniform throughout the colony. Analysis of serum for IL5 concentration was done by ELISA. Serum concentration of IL5 was at or below the level of detection of the assay (10 pg./ml). This mouse had no history of exposure to drugs or to nutritional supplements containing L-tryptophan.

The two male littermates of this animal that died unexpectedly were not submitted for necropsy. However, the carcasses of the parents and one normal littermate were available for necropsy diagnosis. The parents and littermate had no accumulations of eosinophils in the multiple sections of cardiac and skeletal muscle examined.

## Discussion

This case report constitutes the first description of idiopathic eosinophilic polymyositis in a mouse. In this mouse, the lesions in the cardiac muscle were associated with pronounced infiltrates of eosinophils as well as other inflammatory cells. In addition to the inflammation, early myocardial fibrosis was present. The cardiac lesions in the mouse were more severe than those in the right leg, but the cause of the animal's weakness and incoordination, as well as the pathogenesis of the lesions in the heart and right leg, remains undetermined. Histologic data are adequate to rule out muscle-invading parasites, and there was no other apparent cause for the eosinophilic infiltrates in this animal. Cases of eosinophilic myositis with involvement of skeletal and cardiac muscle, similar to that seen in this mouse, have been

described in humans (8, 9, 11). In fact, the disease in this mouse appears to be more similar to the human disease than the idiopathic eosinophilic myositis described in the dog, because the disease in the dog is frequently confined to the muscles of mastication, with no cardiac muscle involvement (16). However, at least one dog is reported to have had severe, generalized involvement of skeletal and cardiac muscle. This dog did not have peripheral eosinophilia (16).

In Fauci's review of the hypereosinophilic syndrome in humans, the defining characteristic is peripheral blood eosinophilia (1500 eosinophils/$mm^3$) with or without eosinophilic infiltrates in tissues. However, tissue involvement in the hypereosinophilic syndrome is commonly documented; 12% of the cases had skeletal muscle, and 54% had cardiac muscle involvement (19). It is not known for certain if this mouse had peripheral eosinophilia. There was no conclusive histologic evidence of peripheral or bone marrow eosinophilia in this case. Post-mortem evaluation is not as accurate as a total white cell and differential count performed antemortem. Eosinophilia may have preceded the development of the muscle lesions, but may have no longer been present when the mouse was submitted for necropsy. Human cases of eosinophilic polymyositis have been described which did not have concurrent peripheral eosinophilia (9, 11, 15).

Very little is known about the pathogenesis of eosinophilic polymyositis in humans or animals. No familial predilection for the disease has been documented in humans, and although the majority of canine cases have been described in German Shepherds (16), individual family lines have not been studied. Three cytokines are known to be involved with eosinophil differentiation and maturation, including GM-CSF, IL3, and IL5 (1, 9). It is possible that some derangement in the production or distribution of these factors could mediate the accumulation of eosinophils seen in this disease. In the mouse we evaluated, serum IL5 concentration was not elevated, indicating that the focal lesions in the heart and the skeletal muscle of the right leg were not severe enough to increase the amount of IL5 in the systemic circulation. In addition, eosinophils themselves produce two inflammatory mediators, eosinophil cationic protein and major basic protein (MBP) (1, 9). Recently, positive staining for MBP has been found in sections of skeletal muscle from human patients with eosinophilic polymyositis (9). Therefore, it is hypothesized that the eosinophils are at least partly responsible for the tissue damage seen in this disease. In one experimental study, serum antibodies were found in a dog with eosinophilic myositis of the masticatory muscles; these antibodies were directed against components of canine temporalis muscles but not against canine triceps brachii muscles (20). This finding indicates that other components of the immune system, such as antibody-producing B cells, are involved in the pathogenesis of the disease. However, the degree of physical debility in people with eosinophilic disease is often greater than would be predicted by the extent of the histologic lesions (19). Similarly, this mouse was very weak, but although the cardiac lesions were focally prominant, they were not extensive enough to cause gross or histologic evidence of overt cardiac failure. The systemic debility found in this mouse could have been associated with soluble factors produced by the eosinophils themselves or by the other inflammatory cells in the lesions, including neutrophils, lymphocytes, and macrophages rather than products from the eosinophils themselves (19). Eosinophilic myositis has been described as a reaction to an asthma drug, Tranilast (5), and has also been associated with ingestion of a specific lot of L-tryptophan supplement (6, 7). This mouse had no history of exposure to drugs or supplemental L-tryptophan, but we cannot definitively rule out exposure of this animal, and perhaps the two clinically affected littermates, to some unknown chemical agent that initiated the pathologic condition.

Eosinophilic myositis is a rare disease in humans; the only model described to date for this disease is in dogs. Cases of eosinophilic myositis in dogs are also rare. This circumstance makes animals, serum, or other specimens difficult to obtain for experimental studies. Mice with eosinophilic myositis which occurs within a family line would be a useful model of a rare human condition. Future studies will include monitoring this breeding colony for additional cases of muscle weakness or neurologic disease, as well as periodic evaluation of the high-running lines for peripheral eosinophilia.

## Acknowledgments

We would like to thank Maureen Decorah for the histopathology. This work was partially supported by National Science Foundation grants # IBN-9111185 and IBN-9157268 to T.G.

## References

1. **Trueb, R. M., P. Becker-Wegerich, J. Hafner, *et al.*** 1995. Relapsing eosinophilic perimyositis. Br. J. Dermatol. **133:**109–114.
2. **Van Den Enden, E., M. Praet, R. Joos, *et al.*** 1995. Eosinophilic myositis resulting from sarcocystosis. J. Trop. Med. Hyg. **98:**273–276.
3. **Gajadhar, A. A. and W.C. Marquardt.** 1992. Ultrastructural and transmission evidence of Sarcocystis cruzi associated with eosinophilic myositis in cattle. Can. J. Vet. Res. **56:**41–46.
4. **Terrell, T. G. and J. L. Stookey.** 1972. Chronic eosinophilic myositis in a rhesus monkey. Vet. Pathol. **9:**266–271.
5. **Arase, S., H. Nakanishi and Y. Sadamato.** 1990. Eosinophilic polymyositis induced by Tranilast. J. Dermatol. **17:**182–186.
6. **Dicker, R. M., N. James and B. A. Cunha.** 1990. The eosinophilia-myalgia syndrome with neuritis associated with L-tryptophan use. Ann. Intern. Med. **112:**957–958.
7. **Kaufman, L. D., R. J. Seidman, and B.L. Gruber.** 1990. L-Tryptophan-associated eosinophilic perimyositis, neuritis and fasciitis. Medicine. **69:**1287–1299.
8. **Hall, F. C., T. Krausz, and M. J. Walport.** 1995. Idiopathic eosinophilic myositis. Q. J. Med. **88:**581–586.
9. **Kaufman, L. D., G. M. Kephart, R. J. Seidman, *et al.*** 1993. The spectrum of eosinophilic myositis. Clinical and immunopathogenic studies of three patients and review of the literature. Arthritis Rheum. **36:**1014–1024.
10. **Layzer, R.B., M.A. Shearn and S. Satya-Murti.** 1976. Eosinophilic polymyositis. Trans. Am. Neurol. Assn. **101:**137–141.
11. **Layzer, R. B., M. A. Shearn, and S. Satya-Murti.** 1977. Eosinophilic polymyositis. Ann. Neurol. **1:**65–71.
12. **Engfeldt, B. and R. Zetterstrom.** 1956. Disseminated eosinophilic "collagen disease". A clinical and pathological study of a clinical entity related to Loffler's syndrome. Acta Med. Scand. **153:**337–353.
13. **Murray-Leslie, C. F., R. C. Quinnell, R. J. Powell, *et al.*** 1993. Relapsing eosinophilic myositis causing acute muscle compartment syndrome. Br. J. Rheumatol. **32:**436–437.
14. **Pye, M., R. Pope, H. Jones, *et al.*** 1987. Eosinophilic myositis presenting as a pseudotumor in the hypereosinophilic syndrome. Br. J. Rheumatol. **26:**384–385.
15. **Behari, M., P. Saha, A. Dinda, *et al.*** 1992. Eosinophilic polymyositis without peripheral eosinophilia. J. Assoc. Physicians India. **40:**132.
16. **Scott, D.W. and A. de Lahunta.** 1974. Eosinophilic polymyositis in a dog. Cornell Vet. **64:**47–56.

17. **Swallow, J. G., P. A. Carter, and T. Garland, Jr.** 1998. Artificial selection for increased wheel-running behavior in house mice. Behav. Genet. **28:**227–237.
18. **Swallow, J. G., T. Garland, Jr., P. A. Carter, *et al.*** 1998. Effects of voluntary activity and genetic selection on aerobic capacity in house mice (Mus domesticus). J. Appl. Phys. **84:**69–76.
19. **Fauci, A. S., J. B. Harley, W. C. Roberts. *et al.*** 1982. The idiopathic hypereosinophilic syndrome. Ann. Intern. Med. 97P 78–92.
20. **Shelton G. D., G. H. Cardinet III, E. Bandman, *et al.*** 1985. Fiber type-specific autoantibodies in a dog with eosinophilic myositis. Muscle Nerve. **8:**783–790.