

Cutaneous epitheliotropic T-cell lymphoma with dissemination to the liver in an eastern chipmunk (*Tamias striatus*)

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Conflict of Interest

No conflicts of interest have been declared.

Abstract

Cutaneous epitheliotropic T-cell lymphoma with liver metastasis was diagnosed in a 10-year-old eastern chipmunk (*Tamias striatus*). Physical findings included intracutaneous swellings, ulcerated plaques and nodules, hypotrichosis and erythema of the skin. Fine needle aspiration from the skin lesions showed a population of large lymphocytes and lymphoblasts, and was helpful in establishing the diagnosis ante-mortem. The post-mortem examination revealed epitheliotropic lymphoma with liver metastasis. Immunohistochemistry proved the T-cell origin both in the liver and skin tumours. Electron microscopy did not reveal any viral particles within the tumour. To the authors' knowledge, this is the first case of lymphoma described in this species.

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Introduction

This report describes a case of cutaneous epitheliotropic lymphoma of T-cell origin with dissemination to the liver in an eastern chipmunk (*Tamias striatus*). Chipmunks are becoming more popular as pets. They are rodents of the genus *Tamias*. Most originate from North America but one species originates from Eurasia. In nature, they survive for approximately 3 years, but live for up to 5–10 years in captivity.

Cutaneous lymphomas (CLs) account for 3–8% of all lymphomas in dogs.^{1,2} CLs are also described in cats,¹

horses,^{3,4} cattle,^{5,6} rabbits,⁷ mice,⁸ a squirrel,⁹ a coati,¹⁰ syrian hamsters¹¹ and a mongolian gerbil¹². Small mammals (rabbits, mice, rats) with nonepitheliotropic lymphoma may provide useful models for the human disease.¹³ The pathogenesis of lymphomas has been reported to be multifactorial, involving chronic antigen stimulation, infectious diseases (e.g. viral diseases), genetic and environmental factors, and/or immunosuppression (by disease or medication).¹⁴

Epidermotropism is an important criterion in veterinary and human medicine for CL as the prognostic and treatment modalities vary between the epitheliotropic and nonepitheliotropic forms.² Nonepitheliotropic CL is uncommon, accounting for 1% and 2.8% of all cutaneous tumours in dogs and cats respectively.¹ Most CLs in dogs exhibit epidermotropism,² but most feline CLs are nonepitheliotropic lymphomas, predominantly of T-cell origin.^{1,15} In human medicine, approximately 65% of CLs are of T-cell origin, 25% of B-cell origin and 10% have mixed phenotypes.¹⁴

Retroviruses have been shown to play a role in the development of lymphomas. In humans, involvement of a herpes virus (Epstein Barr Virus) in the development of endemic African Burkitt cell lymphoma CL is documented.¹⁴ HTLV1 (human T-lymphotropic virus) causes adult T-cell lymphocytic leukaemia (ATLL), which frequently involves skin, but does not seem to be a primary aetiological factor in cutaneous T-cell lymphoma, as these tumours are often negative for antibodies to ATLL.¹⁴ In veterinary medicine, bovine leukaemia virus is recognized as a cause of some B-cell lymphomas in cattle¹⁶ and feline leukaemia virus is associated with the development of malignant lymphoma.¹⁷ Using PCR and immunohistology, FeLV virus was also identified in CL from a serologically negative cat.¹⁷ A C-type retrovirus was isolated from lymphocytes of a dog with large granular cell leukaemia.¹⁸ Despite this, a viral aetiology cannot be identified in many patients. Using electron microscopy, for example, viral particles could not be detected in equine lymphoma.⁴ No known retro-, papilloma-, herpes- or other viral skin diseases associated with malignant transformation have been reported in chipmunks, in contrast to humans.¹⁹ However, several other viral diseases such as La Crosse Virus encephalitis (Bunyaviridae),²⁰ West Nile Virus (Flaviviridae),²¹ Parvovirus (Parvoviridae)²² and Rabies (Lyssa Virus, Rhabdoviridae)²³ are described in chipmunks.

Case report

A 10-year-old, male chipmunk weighing 174 g was presented with a 3-week history of progressive nonhealing

wounds, ulcerated nodules and plaques, and depression. Prior to referral, bacterial culture of the lesions revealed the Gram-negative bacillus *Pantoea agglomerans*, fungal culture was negative, and treatment with ivermectin (Ivomec®; Merial GmbH, Hallbergmoos, Germany) 500 µg kg⁻¹ once weekly and enrofloxacin (Baytril® 2.5% ad us. vet., orale Lösung; Bayer Healthcare GmbH, Leverkusen, Germany) 20 mg/kg once daily orally for 8 days was ineffective.

General physical examination revealed an alert chipmunk with pale mucous membranes. Asymmetric, erythematous, hypotrichoic and oedematous blepharitis, an erythematous, mildly oedematous and crusted cheilitis, and a serous discharge from both external nares were noted. Intracutaneous ulcerative plaques and nodules were present in both axillae, on the ventral abdomen and thorax (Figures 1 and 2). Large ulcerated nodules (1 × 2 × 2 cm) were found bilaterally in the inguinal region.

Cytology of Diff-Quik® stained fine needle aspiration biopsies from plaques and nodules revealed lymphocytes and lymphoblasts (Figure 3). Further diagnostic work-up (including blood analysis and diagnostic imaging of body cavities) was recommended, but not permitted. Similarly,



Figure 1. A 10-year-old chipmunk with plaques, ulceration and crusting due to epitheliotropic T-cell lymphoma.



Figure 2. Erythema and swelling of the muzzle in the affected chipmunk.

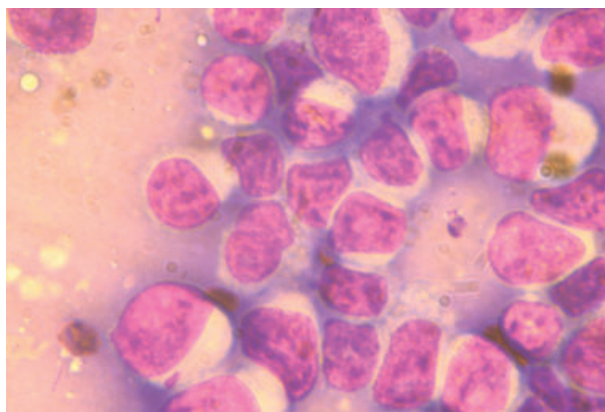


Figure 3. Lymphocytes and lymphoblasts on fine needle aspiration from plaques and nodules (Diff-Quik®). Magnification ×1000.

the owner did not consider chemotherapy or glucocorticoids, but continued with antibiotic treatment. Three days later, the chipmunk died and a post-mortem examination was performed.

Post-mortem examination performed by one of the authors (KH) revealed cutaneous changes comparable with the clinical findings described above. A swollen liver of homogeneous colour and structure was present, but no other macroscopic changes to internal organs were found. Multiple fresh skin samples were collected within 1 h of death either by excision with a scalpel blade or a 6-mm biopsy punch. Further specimens were obtained from the internal organs (heart, lung, liver, kidneys, gonads, spleen, stomach and intestines). The samples were fixed in 10% neutral-buffered formalin, and stained with haematoxylin and eosin, periodic acid Schiff and Giemsa. Immunohistochemical staining with CD3 and CD79a antibodies (Monoclonal Mouse Anti-Human CD3 and CD79a; Dako Cytomation; DK-Glostrup, Denmark) was also performed.

In the skin, there were large, multifocal, predominantly superficial nodules extending to the deep dermis and subcutis. These were comprised of a monomorphic population of mononuclear cells. Focal, subepidermal band-like infiltrates of mononuclear cells were also seen. Focal exocytosis of these cells into the epidermis was also present as well as Pautrier's microabscesses and apoptotic keratinocytes (Figure 4). The cells were of blastic type, showing mild anisocytosis, a high nuclear/cytoplasmic ratio, a medium mitotic index (five to six mitosis/high power field) and occasional nucleoli. The Giemsa stain was negative for mast cells within the nodules. In the liver, lymphoblastic cells, morphologically identical with those in the skin, were present perivascularly and within the portal spaces. The lymphoblastic cells in the skin and liver were CD3 positive (Figure 5), with only few cells CD79a positive.

For transmission electron microscopy, three excision biopsies of the skin were fixed in 6.25% glutaraldehyde (Serva, Heidelberg, Germany) in PBS (pH 7.4) at 8 °C for 48 h, post-fixed in 1% osmium tetroxide (OsO₄; Merck, Darmstadt, Germany), and then dehydrated and embedded in Epon (syn. 'glycid ether 100'; Serva) according to standard procedures. Azur II/Safranin or Toluidine blue-stained sections of the Epon-embedded samples were

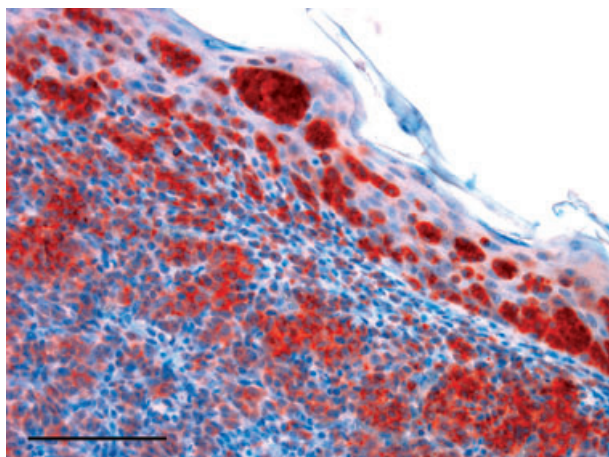


Figure 4. Subepidermal infiltration of lymphocytes with exocytosis, a Pautrier microabscess and apoptotic keratinocytes in the epidermis (H&E x200, Bar = 100 μm).

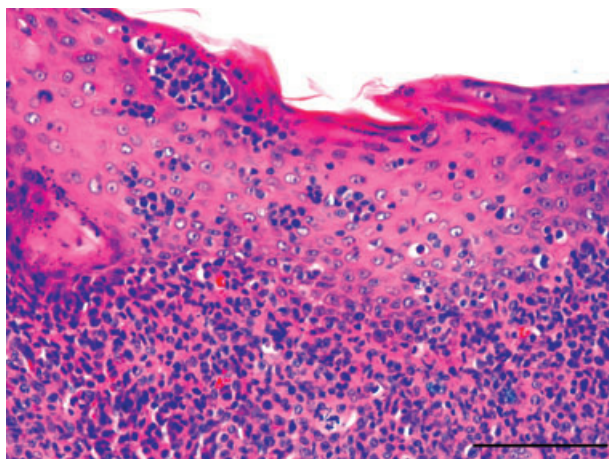


Figure 5. Multiple Pautrier microabscesses in the epidermis and dermal infiltration of CD3 positive T cells (x200, Bar = 100 μm).

surveyed to locate areas of interest within the block. Ultra-thin sections were cut with an Ultracut E microtome (Leica, Solms, Germany) and stained with lead citrate and uranyl acetate. TEM was performed using a Zeiss EM 10 electron microscope (Carl Zeiss, Oberkochen, Germany). A number of images of areas of interest from each of the specimens were photographed at $\times 8000$ magnification in a predetermined manner (by full turns of the stage handle). For calibration, photographs of a standard cross-grating grid (S107; TAAB, Aldermaston, UK) with 2160 lines mm^{-1} were taken with every set. Photographs were developed to a final print magnification of $\times 22\,500$. Intracellular viral particles were not identified in skin or liver biopsies.

A diagnosis of epitheliotropic T-cell lymphoma with liver metastases was made. Neither electron microscopy nor histopathology showed any evidence of viral involvement.

Discussion

This report describes a case of cutaneous epitheliotropic T-cell lymphoma with liver metastases in a chipmunk.

Spread to lymph nodes and other organs have also been reported with more advanced tumour stages in dogs. A viral aetiology could not be documented by using histopathology and electron microscopy, although more data on viral diseases and tumours need to be collected in this species. Historical information regarding exposure to chemicals, drugs and irradiation did not reveal any clues for the cause of the disease. It was not possible to evaluate neoplastic lymphatic cells in peripheral blood as haematology was not permitted. Histopathology of the bone marrow could have provided more information, as post-mortem differentiation is possible, but the bone marrow was not sampled. Antemortem tumour staging and a search for metastases is difficult in small mammals because of the risk of sudden death due to stress during the examination. Cytology from fine needle aspirates of the intracutaneous nodules was helpful in the diagnosis of CL, and with the owner's assistance was achieved without undue stress in this chipmunk.

The efficacy of medical treatment for lymphoma in chipmunks is not known, although treatment options such as glucocorticoids or even chemotherapy could be considered to prolong survival in affected animals. However, at presentation this chipmunk was already very old, possibly testament to the excellent housing care received from its owner, and treatment was declined.

Cutaneous lymphoma has been diagnosed in many different animal species, but to the authors' knowledge, this is the first described case of presumed cutaneous T-cell lymphoma with liver metastasis in a chipmunk.

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Résumé Un lymphome cutané T épithéliotrope avec métastase hépatique, a été diagnostiqué chez un Ecureuil de Corée (*Tamias striatus*) de 10 ans. L'examen clinique révélait une tuméfaction cutanée, des nodules et plaques ulcérées, une hypotrichose et un érythème cutané. Une cytoponction à l'aiguille fine des lésions cutanées a révélé une population de large lymphocytes et lymphoblastes et permis d'établir un diagnostic ante mortem. L'examen post mortem a révélé un lymphome épithéliotrope avec métastase hépatique. L'examen immunohistochimique a révélé la présence de lymphocytes T à la fois dans le foie et dans les lésions cutanées. La microscopie électronique n'a pas révélé la présence de particule virale dans la tumeur. A la connaissance de l'auteur, il s'agit du premier cas de lymphome décrit dans cette espèce.

Resumen Se diagnosticó un linfoma de linfocitos T epiteliotrópico con metástasis en el hígado en una ardilla terrestre (*Tamias striatus*). Los hallazgos clínicos incluyeron hinchazones cutáneas, placas ulceradas y nódulos, hipotricosis y eritema de la piel. Un aspirado de aguja fina de las lesiones de la piel mostró una población de linfocitos de gran tamaño y linfoblastos, y fue de ayuda para establecer un diagnóstico antes de la muerte. El examen post mortem mostró un linfoma epiteliotrópico con metástasis en el hígado. La técnica de inmunohistoquímica demostró linfocitos T neoplásicos tanto en el hígado como en los tumores de la piel. Mediante microscopía electrónica no se observaron partículas virales en las células tumorales. A nuestro entender este es el primer caso de linfoma descrito en esta especie.

Zusammenfassung Bei einem 10 Jahre alten Streifenhörnchen (*Tamias striatus*) wurde ein kutanes epitheliotropes T-Zell Lymphom mit Metastasen in der Leber diagnostiziert. Die klinischen Befunde bestanden aus intrakutanen Schwellungen, ulzerierten Plaques und Knoten, Hypotrichose und einem Erythem der Haut. Eine Feinnadelaspiration der Hautveränderungen zeigte eine Zellpopulation bestehend aus großen Lymphozyten und Lymphoblasten, und war hilfreich beim Erstellen der Diagnose ante mortem. Die Post-mortem Untersuchung zeigte ein epitheliotropes Lymphom mit Lebermetastasen. Mittels Immunhistochemie wurde sowohl in der Leber als auch in den Haut-Tumoren die Herkunft der Zellen als T-Zellen bewiesen. Mittels Elektronenmikroskop konnten keine Viruspartikel innerhalb des Tumors festgestellt werden. Nach dem Wissen des Autor's handelt es sich hierbei um den ersten Fall eines Lymphoms, dass bei dieser Spezies beschrieben wurde.