Case Report: Lymphosarcoma in Adult African Green Monkeys (*Chlorocebus Aethiops*)

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Summary

The clinical observations and pathological manifestations of lymphosarcoma in two African green monkeys are described. Monkeys had been caught from the wild. Prior to the development of neoplasms one monkey had been experimentally infected with *Trypanosoma brucei rhodesiense* as a model of human trypanosomiasis and subsequently treated with a proprietary trypanocidal drug and observed for any after-effects. Th e other monkey was used to test for the safety of another trypanocidal drug.During the monitoring period, terminated by euthanasia, monkey became dull, unable to perch, and hunched. In the same animal the facial skin became hypersensitive and nodular skin lesions developed. In the other animal used in safety study, skin lesions, weight loss, and swollen eyelids were observed prior to euthanasia. During the terminal stages of the experimental protocol, the superficial lymph nodes of both animals became swollen, and the white blood cell count increased. Lesions disclosed during necropsy and subsequent histopathology revealed classical signs of nodular multicentric lymphosarcoma. In both animals the neoplastic infiltrates were dominated by large lymphocytes with anisokaryosis and megakaryosis. In several organs (lungs, liver and kidneys) of one of the animals, the neoplastic infiltrates were accompanied by compression and degeneration of bordering tissues. The cause of the neoplasms remains unknown, but stress-induced immunosuppression associated with captivity, to a lesser extent and, more importantly, the induction and treatment of experimental trypanosomiasis may, have triggered the onset of neoplastic proliferation, which is frequently associated with simian T-cell leukemia virus 1 (STLV-1).

Introduction

The inability to satisfy the demand for captive-bred non-human primates for biomedical research (*Hau & Schapiro, 2006*) results in the use of captive non-human primates in various countries including Kenya. The African green (vervet) monkey (*Chlorocebus aethiops* syn *Cercopithecus aethiops*) model for human African trypanosomosis has been well established at the Kenyan Trypanosomiasis Research Centre since the 1980s (*Schimdt & Sayer, 1982*). Many drug and pathogenesis studies have been undertaken using this induced laboratory animal model (*Farah et al, 2005; Gichuki &Brun 1999; Maina et al, 2004*). However, this is the first report on the occurrence of lymphosarcomas in non-human primates used in this model. Indeed, the incidence of naturally occurring lymphoma in non-human primates is low and has been reported mainly in cynomolgus monkeys (*Macaca fascicularis*), rhesus monkeys (*Macaca mulatta*) and African green monkeys (*Feichtinger et al, 1990; Habis et*

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al, 1999; Jayo et al, 1990). An increased frequency of lymphomas has been observed in non-human primates with retroviruses, suggesting an important pathogenic role of virally induced immunomodulation for the development of lymphoma (*Feichtinger et al, 1990*). In this report, we describe the clinical and pathological observations in two cases of spontaneous lymphoma in adult African green monkeys kept in captivity.

Clinical history

The two vervet monkeys were part of a group (n=80) of wild caught African green monkeys, which underwent an initial quarantine period of 90 days during which they were screened for diseases, including zoonotics. After the 90 days, the animals were transferred to the experimental holding rooms. In both quarantine and experimental rooms the animals were housed singly in squeeze back stainless steel cages and the cage layout ensured that the animals had visual contact with conspecifics. The two vervet monkeys were used in two different experimental protocols, approved by the Institutional Animal Care and Use Committee, at different times when the lymphosarcomas were detected and documented.

The animals were fed twice daily with a ration comprising commercial monkey pellets (Monkey Cubes®, Unga Feeds Ltd, Kenya), green maize, carrots, tomatoes, and bananas. Water was provided *ad libitum*. The ambient temperatures varied between 23-25°C. Clinical appraisal was done daily. The animals were regularly anaesthetized using diazepam (1mg/kg) and ketamine hydrochloride (15mg/kg) for sampling and clinical examination. Stabilized EDTA blood samples were drawn by inguinal venipuncture and used for haematological analyses. The hematological parameters analyzed included: red blood cell count (RBC), hematocrit (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemogolobin (MCHC), red cell distribution width (RDW), platelets counts, mean platelets volume, and white blood cell counts (WBC).

Case 1

An adult female vervet was part of a group (n= 4) of non-human primates used to test the efficacy of diminazene aceturate (Berenil®, Bayer, Germany) against acute experimental infection with *Trypanosoma brucei rhodesiense*. The drug, which was used at a dose rate of 5mg/kg and administered intramuscularly between days 14-16 of infection, led to successful clearance of parasitaemia. All the clinical signs associated with experimental trypanosomosis were resolved within one week of the drug application. However, nine months later, an enlargement of superficial lymph nodes was observed, which persisted until the animal was euthanised, one month later. The animal was inactive and less playful compared to peers. Two days before euthanasia by an overdose of 20% pentobarbitone sodium (Euthatal®), Rhone Merieux, Ireland), the animal developed clinical signs which included: inability to perch, dullness, hunched, pale mucous membranes, hypersensitive facial skin, and nodules-like lesions in the skin.

Case 2

An adult female vervet was part of a group (n=3) of non-human primates used to its evaluate the safety of pentamidine isethionate (Pentacarinat®, Aventis Pharma Ltd, Auckland, New Zealand) for treatment of acute experimental trypanosomiasis. The drug was applied intramuscularly at a dose rate of 4 mg/kg for 5 days. Based on the clinical picture, haematology and biochemical analysis of blood parameters, the drug was declared to be non-toxic at the doses tested. However, two years after this protocol, the animal began to develop clinical signs indicative of ill health. These included enlarged and disfigured superficial lymph nodes, enlarged spleen, stool inconsistencies (pelleted to soft faeces), weight loss (from 2.8 kg to 2.5 kg), swollen eyelids, and small-circumscribed wounds on the skin. The subject was euthanised using 20% pentobarbitone sodium (Euthatal®), Rhone Merieux) when the clinical condition deteriorated.

Pathology

Following euthanasia, both monkeys were subjected to a complete necropsy. Tissues were sampled from major organs and fixed in 10% neutral buffered formalin, processed and stained with hematoxylin and eosin for histopathology.

Results

Hematology

Case 1

During the pre-infection period, the total WBC was within the normal range (4200-9200/µl). There was an increase in WBC after experimental infection, which was followed by a decline and normalization after treatment. The hematocrit levels declined after treatment and this was followed by a recovery following the treatment with diminazene aceturate. A rise in WBC counts (from 6000 to 12000/µl) was observed in the animal, three weeks before it developed the adverse clinical signs which eventually led to its sacrifice. All other haematological parameters were within the normal ranges.

Case 2

High leucocyte counts were observed between the 14^{th} and 18^{th} month after experimentation. These counts, although gradually decreasing, remained above the normal levels until euthanasia. The leucocytosis was mainly due to an increase in the lymphocyte counts. The granulocytes and monocyte levels were also higher than normal and increased during the nine months prior to the animal being euthanised.

Throughout the period of captivity, the RBC counts were within normal ranges (5.1-5.7 x 10^6/µl). However, the RBC counts increased from 5.5 x 10^6/µl to 7.5 x 10^6/µl between the 10^{th} and 14^{th} month post experimentation. There was a significant increase in MCV (from 59.2 to 74.5 fl) and a decrease in MCHC (from 36.5 to 30 g/dl) between the 16^{th} and 25^{th} month post experimentation. All other hematological parameters were within the normal ranges.

Gross pathology

Case 1

Skin wounds with formation of granulation tissue covered major parts of the lower abdomen and all four limbs. The liver was friable and enlarged. The spleen and the inguinal and axillary lymph nodes were enlarged and had lost the normal architecture.

Case 2

The animal had copious amounts of straw-coloured peritoneal fluid, which covered most of the abdominal organs. The stomach was filled with dark fluid. Multiple ulcers were observed distributed throughout gastric and intestinal mucosa. The colon was filled with dark pelleted contents while the liver was markedly enlarged and oedematous with widespread pale patches on the liver surface.

The kidneys were enlarged and hemorrhagic. The capsule adhered to the cortex and on the cut surface there were large white patches, which extended from the cortex to the medullary regions. The uterus and ovaries had numerous nodules that caused the organs to be disfigured.

The tonsils were enlarged and hemorrhagic leading to a narrowed throat opening. Dark red areas with consolidation were observed in the lungs giving the organs a variegated appearance. Brownish nodules of 0.5- 2 cm in diameter were observed in all the lobes. On cut surfaces, the hemorrhagic and brownish nodules were extending deep into the lung parenchyma. Hydrothorax and hydropericardium made up of straw-coloured fluid was a prominent feature. The heart was pale and flabby. Some pinpoint to ecchymotic haemorrhages were observed mainly on the epicardium in both sides of the heart. Most of the lymph nodes were enlarged, brownish and hemorrhagic and had lost the normal architecture. The affected nodes included the axilla, inguinal, sub-mandibular, retropharyngeal, bronchial, mediastinal, mesenteric, and iliac lymph nodes. Some of the internal lymph nodes were attached to the adjacent tissues. The spleen was also enlarged up to 1.5 times the normal size and had white to brown multiple nodules (up to 1cm diame-

ter), protruding from the surface.

Histopathology

Histologically, neoplastic lymphocytes infiltrated and disfigured several organs. The most prominently affected organs were the kidneys, lymph nodes, spleen, liver, kidneys and the lungs. The infiltrating neoplastic cells varied in morphology. The majority of the cells were large and had a round or oval to irregular shape. The nucleus to cytoplasm ratio was high and mitotic figures could be observed. Most of the nuclei had reduced basophilia and marginating chromatin. Interspersed among the neoplastic cells were inflammatory cells consisting of neutrophils, macrophages and plasma cells.

The spleen and the lymph nodes were made up of uniform monotonous sheets of the neoplastic cells. There were only a few organised follicles, while the red pulp of the spleen was drastically reduced. The capsule of the spleen was virtually obliterated by the infiltrating tumour cells. Haemorrhages and pinkish exudates were observed in the spleen parenchyma.

In the liver, the neoplastic lymphocytes appeared either as diffuse infiltrates or as localised tumour nests. The infiltrating cells were mainly located along the sinusoids and around blood vessels. The liver capsule was also obliterated and what was left consisted of hemorrhagic areas, neoplastic cells and normal lymphocytes. Cellular degeneration of hepatocytes was evident and included ballooning and fatty degeneration, and hepatic necrosis. Other features included congestion, haemorrhages and thrombi especially along the sinusoids.

In the kidneys, the tumour cells were found in the interstitium, around tubules, blood vessels and within the capsule (Fig. 1). The tubular cells were in different stages of degeneration. In the lungs the main features included neoplastic infiltrations along the inter-alveolar septae, around bronchioles and blood vessels (Fig 2). Large nodules of tumor cells were scattered in the lung parenchyma and

Figure 1. Infiltration of a monkey (No. 469) kidney with neoplastic cells.
Key: A = Masses of neoplastic cells around a gromerulus

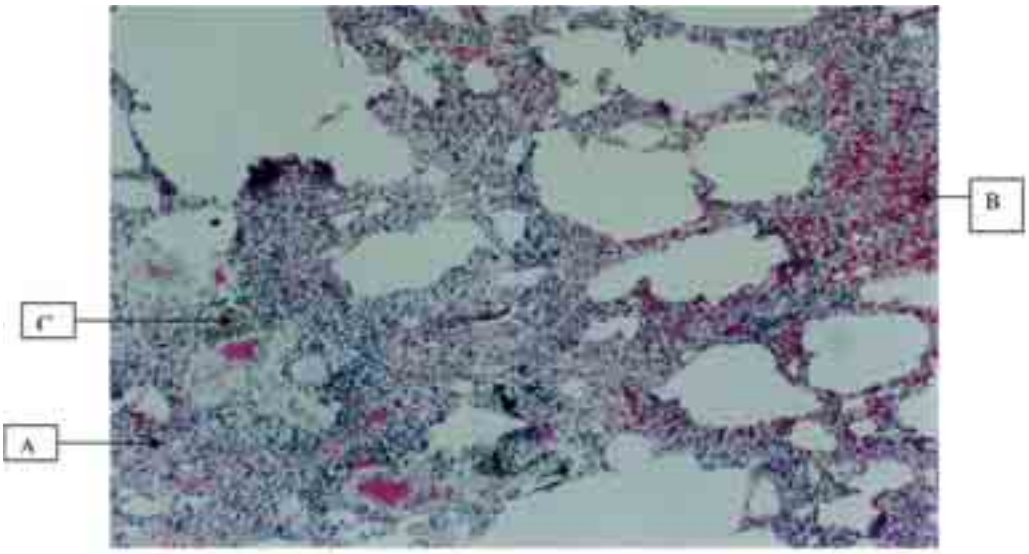

Figure 2. Infiltration of monkey (No. 469) lung with neoplastic cells.
Key: A = Masses of neoplastic cells, B = Hemorrhages, C = Hemosiderosis

caused atelectasis of surrounding tissues. Areas with extensive haemorrhages, congestion and inflammatory cells, mainly neutrophils, plasma cells, and macrophages, were common in the lungs, too. Gastro-intestinal erosions and ulcers extending up to submucosa were observed. The lamina propria and the submucosa were infiltrated with neoplastic lymphocytes and inflammatory cells, mainly neutrophils. In most of the affected organs, haemosiderosis, congestion, and haemorrhages were common features.

Discussion

Spontaneous lymphoma with accompanying haematological changes is generally considered rare in the African green monkey (*Ishida et al, 1986; Mwenda et al, 1999; Sato et al, 1999)*. The two present cases differed in terms of severity but most of the characteristics of malignant cancer were evident in both cases. These included invasion of several organs and extensive haemorrhages. The histological features in both animals were similar, with proliferation of neoplastic lymphocytes being observed in most organs. Leucocytosis, which was observed for a long period in Monkey 2 was mainly characterised by an increase of lymphocytes. This agrees well with what has previously been reported in African green monkeys (*Sato et al, 1999)*. The observed increase in the neutrophils- and monocyte counts as the disease progressed (in Case 2) may indicate the presence of a secondary infection but leukocytosis is not necessarily an integral feature in all lymphomas (*Sato et al, 1999)*.

Both animals had previously been exposed to diamidines as part of the experimental protocols, but these drugs have been used for decades without reports on any association with lymphoma or other malignancies (*Abaru et al, 1984; Matz-Rensing et al, 1999; Schimdt & Sayer, 1982*). The drugs are known to interact with trypanosome DNA to produce the trypanocidal effect (*Pepin & Milord, 1994*), but their effect on the DNA of the host has not been well elucidated. Pentamidine has been shown to have myelotoxic effect in patients with compromised bone marrow reserve, whereby it causes megaloblastic anaemia demonstrating that a

compromised immune system increases the toxicity of the drug (*Wing et al, 2002*). Wild African green monkeys are significantly stressed and immunosuppressed when housed singly in captivity (*Suleman, et al, 2000; Suleman et al, 1999*). Additionally, sleeping sickness in humans is characterized by immunosuppression in patients. The increase in WBC counts in Case 1 just before the animal was euthanised, may have been an indication of imminent relapse of trypanosome infection. Although not clinically diagnosed in the present cases, perhaps due to the length of time that had elapsed since treatment, relapse is common in non-human primates treated with Berenil® (*Pepin & Milord, 1994)*

Retroviruses, including simian T-cell lymphotropic viruses (STLVs), simian immunodeficiency virus (SIV) and Epstein Barr virus have been implicated in cases of spontaneous lymphoma and in non-human primates (*Matz-Rensing et al, 1999; Schatzl et al, 1993)*. Although the prevalence of these viruses in non-human primates is reportedly high, infected animals rarely develop lymphoma (*Allan et al, 2001; Hunsmann et al 1983; Ishida et al, 1986)*. Stress and a period of immunological inadequacy may render retrovirus-infected animals more susceptible to development of lymphoma (*Feichtinger et al, 1990*). In the present case, the non-human primates were only screened for SIV, and whether other retroviruses were present in the two monkeys is unknown.

The present study showed that the lesions of lymphosarcoma, here seemingly spontaneous and unconnected with the periods treated, can be found in practically any system in the body, and thus clinical signs will depend on the organ(s) involved. The severe destruction of the kidneys and liver parenchyma was probably the cause of the observed exudation into body cavities, while the ulcers in the gastrointestinal tract likely caused the haemorrhages and the dark faeces.

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