Simian immunodeficiency virus infections in vervet monkeys (*Clorocebus aethiops*) at an Australian zoo

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A number of monkey species, including African green monkeys and African vervet monkeys (*Chlorocebus aethiops*), are frequently infected in the wild and in captivity with a Simian immunodeficiency virus strain, SIVagm, a primate lentivirus.[1-3] Up to 50% of African green monkeys are estimated to be infected with SIVagm.[4] SIV strains are very closely related to HIV-2 strains, which are a cause of AIDS in humans, predominantly in western Africa, although cases in Australia have also been reported.[5] It is generally thought that SIV is non-pathogenic in several natural hosts, including African green monkeys.[6] Nevertheless many SIV strains induce a profound immunodeficiency virtually identical to HIV-1 induced AIDS in humans when administered to Asian macaque species such as rhesus (*Macaca mulatta*) or pigtailed macaques (*M nemestrina*).[7] SIV infection of Asian macaque species is frequently employed as an animal model for AIDS vaccine studies.[8]

In November 1996 a group of 10 African vervet monkeys were imported from the USA for display at Victoria's Open Range Zoo in Werribee. Two animals in this group of monkeys later developed a fatal gastroenteric illness. These diagnoses led us to initiate SIV testing of the colony.

AIDS	Acquired Immunodeficiency Syndrome
HIV	Human immunodeficiency virus
PBMC	Peripheral blood mononuclear cells
SIV	Simian immunodeficiency virus

Case reports

In April 1998, one of the animals originally imported from the USA, M653, a 6-year-old female, developed persistent profuse watery diarrhea. This progressed to emaciation and dehydration. Serial haematology and biochemistry revealed persistent hypochromic anaemia, hypoalbuminaemia, and elevated LDH. Ring forms and occasional developing trophozoites of *Hepatocystis* spp were seen on blood smears. Multiple faecal samples were cultured for *Salmonella* spp, *Shigella* spp, *Campylobacter* spp and *Yersinia* spp. *Salmonella typhimurium* was isolated from one sample and the animal successfully treated for this. Protozoa including *Balantidium coli*, *Giardia* spp, *Entamoeba histolytica* and *Blastocystis hominis* were identified intermittently and treated with metronidazole (140 mg/d). Diarrhoea persisted despite these treatments.

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Biopsies of stomach and duodenum taken via endoscopy revealed lymphoplasmacytic gastroenteritis with no apparent infectious aetiology, and an allergic or autoimmune cause was proposed. A 3-week course of prednisolone resulted in further deterioration of the animal's condition. Necropsy revealed an emaciated carcase. Further gross lesions were confined to the distal 20 cm of jejunum and comprised focal regions 1 to 2 cm in length of saccular dilations of the bowel associated with thickening of the bowel wall. These regions were separated from each other by grossly normal sections of bowel 2 to 3 cm in length. Histology demonstrated thickening of the muscular layer of lesional jejunum, especially the inner circular layer; crypt hyperplasia with villous fusion and atrophy to the point of focal ulceration; increased mixed inflammatory cells, including neutrophils, in the lamina propria; inflamed submucosa as well as serosal oedema and inflammatory cell infiltration. Many neutrophils were present in the luminal exudate. Silver stains were negative for Lawsonia-like organisms in enteric epithelium. There was also chronic typhlitis and chronic active colitis with atrophy of luminal epithelium. Faecal culture on selective media was negative for *Salmonella* spp, *Shigella* spp, *Campylobacter* spp and *Yersinia* spp. On ultrastructural examination, *Giardia* spp trophozoites were identified on duodenal epithelium. The morphologic diagnosis was a regional enteritis interspersed with more normal sections of bowel, possibly leading to pseudo-obstruction, of undetermined aetiology.

In November 1998 another of the original animals, K086, an 8-year-old female developed a similar condition of chronic diarrhoea, but progressed more rapidly to severe dehydration, emaciation and anorexia until her death 3 months later. Haematology and biochemistry revealed a hypochromic anaemia and hypoalbuminaemia. No *Hepatocystis* were present on blood smears. Faecal sample analysis was similarly positive for protozoan species, which were appropriately treated, however pathogenic bacteria were not isolated. Diarrhoea persisted and the animal was euthanased. The primary histopathological finding was a lymphoplasmacytic or granulomatous gastroenteritis. Gram, PAS, ZN and silver stains were negative.

Serology

Based on the findings of a chronic diarrhoea without clear bacterial or protozoan aetiology, a viral or immunosuppressive aetiology was contemplated. Non-human primates are susceptible to a number of viral infections that cause either lymphoproliferative disease or immunodeficiency.[9] These are primarily retroviruses such as simian type D retroviruses, simian T-cell lymphotropic virus, and simian immunodeficiency viruses (SIV).[10] SIV infections frequently result in fatal gastroenteric illnesses in experimentally inoculated Asian macaques.[11] There are no specific serologic tests available in Australia for any of these viruses, however SIV strains are almost identical to HIV-2, a pathogen of humans present primarily in

western Africa, and very good serologic cross-reactivity between an HIV-2 and SIV is present.[12] A particle agglutination assay containing HIV-2 antigens (Serodia-HIV1/2, Fujirebio, Japan) used for HIV serology in Australia was utilised for the SIV serology. Twenty-four serum samples which had been stored at –70°C from the 10 original animals were assayed. To determine the magnitude of the antibody response, positive sera were diluted with serial 10-fold dilutions and re-assayed (Table 1). Both animals with gastroenteritis (M653 and K086) were strongly seropositive. One additional animal (N704) was also seropositive upon arrival. Two other animals (M652 and M657) seroconverted to SIV while held at the zoo (albeit with lower titres), with another animal (M587) recording an indeterminate result. The three monkeys born in captivity were seronegative (Table 1).

Immunological and virological investigations

The serologic findings suggested that SIV might be at least a co-factor in the gastroenteritis of the two vervet monkeys. We therefore performed additional investigations to establish if a severe immunodeficiency characteristic of AIDS was present and to assess the levels of SIV in animal M653. We first analysed the CD4^{+} T lymphocyte numbers in PBMC by flow cytometry as previously described. We found that 8% of gated lymphocytes were CD4^{+}. Although this was lower than the expected normal range for non-human primates (30-60%), it was not characteristic of simian or human AIDS which typically has a CD4^{+} T-cell count of <1%.

We attempted to recover SIV by co-culture of PHA-stimulated PBMC from monkey M653 with a T-cell line CEMx174 in vitro and analysis of reverse transcriptase activity in the supernatants as previously described.[13] No reverse transcription activity was detected from 10[6] PBMC from animal M653, in contrast to similar experiments with Asian macaques experimentally inoculated with wild type SIV, where SIV can routinely be recovered from 10[4] or 10[5] PBMC.[14]

We also analysed SIV RNA and DNA levels in animal M653. Plasma was assayed for SIV RNA using a commercially available SIV RNA assay (bDNA, Chiron, USA) which uses multiple probes that hybridise to conserved sequences of SIV RNA and has a limit of detection of 1500 copies of RNA per mL of plasma.[15] No SIV RNA was detected in the plasma of M653 (<1500 RNA copies/mL). Although low levels of SIV RNA could not be detected by this assay, >10[6] copies of SIV RNA are typically detected in Asian macaques experimentally infected

with SIV progressing to AIDS. It is also important to note that the sensitivity of this SIV RNA assay for detecting uncharacterised SIVagm strains in vervet monkeys, in comparison to experimental SIV strains used to infect macaques (such as SIVmac), is not known but may be limited.[16]

SIV DNA in PBMC was examined by PCR for the Nef gene utilising a nested PCR method with conserved primers developed for studying SIV infection of *M nemestrina*. Briefly, DNA was extracted from 3 x 10^{6} PBMC as described and PCR performed using first round primers Odp.205 (position 9162-9181, where 1 = the start of SIV U3 from Genbank accession M33262), and Odp.193 (position 10016-10036) for 30 cycles, and then nested round primers Odp.191, (position 9191-9208), and Odp.192 (position 9872-9890), for 30 cycles. PCR was performed with 1U of Amplitaq (Perkin Elmer Cetus, Norwalk, CA), 0.8mM dNTPs and 1μM of primers in 10mM Tris-HCl, 50 mM KCl, 1.5mM $MgCl_2$, 0.001% gelatin, together with 2μL of the PBMC DNA in the first round, or 2μL of first round product diluted 1:10[3] for the second round PCR. DNA from vervet monkey was amplified along side negative controls of DNA alone and primers alone and a positive control of a plasmid expressing SIVmac239 DNA obtained from the AIDS research reagent repository of the National Institutes of Health, USA. Using these conditions, SIV *nef* was detected in a nested PCR from DNA obtained from PBMC of animal M653 (Figure 1).

Discussion

A gastroenteritic illness was described in two of a group of 10 African vervet monkeys housed at Victoria's Open Range Zoo. A lymphoplasmacytic gastroenteritis was found at necropsy with no infective aetiology identified. Both monkeys which became ill, as well as two others of the group, were seropositive to SIV (Table) and PBMC from animal M653 contained SIV DNA. However, immunological studies did not demonstrate a severe CD4^{+} lymphopenia characteristic of AIDS, and quantitative virological studies did not demonstrate high levels of SIV replication. Although SIV could have played a role in the illnesses observed, a convincing case that SIV was implicated in the disease was not demonstrated. It is possible that co-infection with other simian retroviruses (for example simian type D retrovirus or simian T-cell lymphotropic virus) could have played an additional role in the disease of these animals.[9,10,17] However, in the absence of validated serologic assays and quantitative virologic assays to demonstrate high levels of

Table 1. SIV antibody testing of African vervet monkeys.

Time folloiwing acquisition	Monkey													
	M653	K086	N704	M652	M657	M587	K427	L660	F204	J151	Gable[a]	Tribitarki[a]	Tumbili[a]	M12[b]
Week 0#	+(10[4])	+(10[4])	+(10[4])	-	-	-	-	-	-	-				-
Week 30										-				-
Week 57-67			+(10[4])	+(10[1])	+(10[1])	±	-	-						+(10[7])
Week 83			+(10[4])											+(10[6])
Week 109-113	+(10[4])	+(10[3])		+(10[1])										
Week 132	+(10[4])										-	-	-	-

[a]Born in captivity at Victoria's open range zoo.
[b]M12 is a pigailed macaque (M nemestrina) experimentally inoculated with SIV (at time week 0),shown as a positive control.
[c]Week 0 = November 1996, when the imported animals were first tested. Sera were stored at -70°C and retrospectively batch tested.
- = negative result, + = positive (titre in parentheses), ± = indeterminate result.
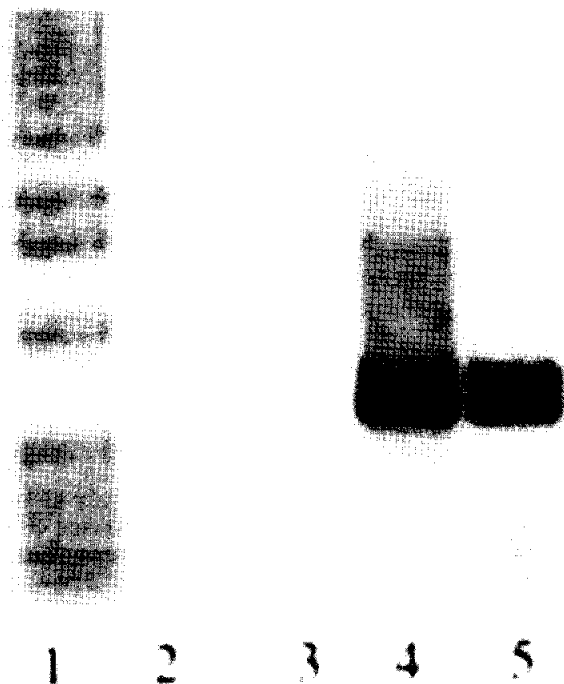
Figure 1. SIVnef DNA detected in a vervet monkey PBMC. A nested PCR for SIVnef was performed as described in the text. Lane 1 is a molecular weight marker, lane 2 extracted DNA without added primers, lane 3 primers alone without DNA, lane 4 a positive control plasmid of SIVmac239, and lane 5 DNA from PBMC from vervet monkey M653.

replication of other retroviruses, the aetiological role of additional retroviruses in the disease observed remains speculative.

Infection of imported or zoologically housed monkeys with SIV has not, to our knowledge, been previously reported in Australia. The potential for monkeys to harbour simian retroviruses presents a potential zoonotic risk to handlers, since transmission of these viruses to humans is possible.[18,19] Although cases of seroconversion to SIV$_{mac}$ have been reported in humans, SIV infection of humans has not yet been associated with AIDS. However, since the HIV-2 is highly related to SIV and can cause AIDS in humans, persons in close contact with monkeys should be aware of the potential risks.

Acknowledgments

References

1. Allan JS, Kanda P, Kennedy RC, et al. Isolation and characterization of simian immunodeficiency viruses from two subspecies of African green monkeys. *AIDS Res Hum Retrov* 1990;6:275-85.
2. Muller MC, Saksena NK, Nerrienet E, et al. Simian immunodeficiency viruses from central and western Africa: evidence for a new species-specific lentivirus in tantalus monkeys. *J Virol* 1993;67:1227-35.
3. Rensburg van EJ, Engelbrecht S, Mwenda J, et al. Simian immunodeficiency viruses (SIVs) from eastern and southern Africa: detection of a SIVagm variant from a chacma baboon. *J Gen Virol* 1998;79:1809-14.
4. Johnson PR, Fomsgaard A, Allan J, et al. Simian immunodeficiency viruses from African green monkeys display unusual genetic diversity. *J Virol* 1990;64:1086-92.
5. Downie JC, Dwyer DE, Kazazi F, et al. Identification of infection of an Australian resident with the human immunodeficiency virus type 2 (HIV-2). *Med J Aust* 1992;157:415-7.
6. Norley SG. SIVagm infection of its natural African green monkey host. *Immunol Lett* 1996;51:53-8.
7. Letvin NL, King NW. Immunologic and pathologic manifestations of the infection of rhesus monkeys with simian immunodeficiency virus of macaques. *J Acquir Immune Defic Syndr* 1990;3:1023-40.
8. Kent SJ, Hu SL, Corey L, et al. Detection of simian immunodeficiency virus (SIV)-specific CD8+ T cells in macaques protected from SIV challenge by prior SIV subunit vaccination. *J Virol* 1996;70:4941-7.
9. Lowenstine LJ. Lymphotropic and immunosuppressive retroviruses of nonhuman primates: a review and update. *Proc American Assoc Zoo Vet* 1993;40:51-60.
10. Lowenstine LJ, Lerche NW. Nonhuman primate retroviruses and simian acquired immunodeficiency syndrome. In: *Zoo and wildlife medicine: Current therapy.* Fowler ME, editor. Philadelphia, Saunders, 1993:373-378.
11. Mansfield KG, Carville A, Hebert D, et al. Localization of persistent Enterocytozoon bieneusi infection in normal rhesus macaques (Macaca mulatta) to the hepatobiliary tree. *J Clin Microbiol* 1998;36:2336-8.
12. Chen Z, Luckay A, Sodora DL, et al. Human immunodeficiency virus type 2 (HIV-2) seroprevalence and characterization of a distinct HIV-2 genetic subtype from the natural range of simian immunodeficiency virus-infected sooty mangabeys. *J Virol* 1997;71:3953-60.
13. Kent SJ, Woodward A, Zhao A. Human immunodeficiency virus type 1 (HIV-1)-specific T cell responses correlate with control of acute HIV-1 infection in macaques. *J Infect Dis* 1997;176:1188-97.
14. Daniel MD, Kirchhoff F, Czajak SC, et al. Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene. *Science* 1992;258:1938-41.
15. Sodora DL, Lee F, Dailey PJ, Marx PA. A genetic and viral load analysis of the simian immunodeficiency virus during the acute phase in macaques inoculated by the vaginal route. *AIDS Res Hum Retrov* 1998;14:171-81.
16. Jin MJ, Hui H, Robertson DL, et al. Mosaic genome structure of simian immunodeficiency virus from west African green monkeys. *Embo J* 1994;13:2935-47.
17. Mansfield K, King N. Viral diseases. In: *Nonhuman primates in biomedical research: Diseases.* Bennet BT, Abee CR, Henrickson R, editors. Academic Press, 1998:1-57.
18. Khabbaz RF, Heneine W, George JR, et al. Brief report: infection of a laboratory worker with simian immunodeficiency virus. *N Engl J Med* 1994;330:172-7.
19. Roberts JA. Occupational health concerns with nonhuman primates in zoological collections. *Zoo Wildl Med* 1995;26:10-23.