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The HIV-1 Transgenic Nude Rat: A model of Pneumocystis Pneumonia

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Abstract

HIV-infected individuals are at increased risk for acute and chronic airway disease though there is currently no evidence that the virus can infect lung epithelium. Pneumocystisis a protozoan opportunistic pathogen that usually causes interstitial pneumonitis in patients with HIV/AIDS.We previously described the HIV-1 transgenic rat which bears a gag-pol –deleted HIV-1 genome that developed immunologic pathologies. In the current experiments, we studied the role of functional T cells in the development of Pneumocystis carinii(Pc) infection. We developed the HIV-1 Tg nude rat model by backcrossing onto thenude background from an HIV-1 Tg rat. Prior studies has determined that the HIV-1 transgene in rats results in a dysfunctional alveolar epithelial barrier and the alveolar macrophages from these HIV-1–transgenic rats have decreased intracellular expression of the GM-CSFRb subunit and decreased bacterial phagocytic function. We then hypothesizedthat having the HIV transgene on a nude background which lacksfunctional T cells will render them completely vulnerable to Pc infection even more so than the wild type rat and/or the HIV-1 Tg rat. After its development, the HIV-1 Tg nude rats showing signs of lethargy, dyspnea, and a dramatic weight losson gross pathology revealed generalized multifocal whitish – grey lung lesions. On microscopic examination the alveoli were filled with "foamy" material and interstitial pneumonitis. Silver methenamine stain demonstrated protozoan cysts and PCR of lung tissues confirmed the presence of Pneumocystis carinii. These observations substantiate that the HIV-1 transgenic nude rat model can be used as an appropriate small animal model to study the pathogenesis of HIV associated respiratory disease in patients.

Keywords: HIV-1(human immunodeficiency virus-1), Pneumocystis, PCR (Polymerase chain reaction), Tg (transgenic), AM (Alveolar macrophages)

Introduction

HIV-infected individuals are at increased risk for acute and chronic airway disease even though there is no evidence that the virus can infect the lung epithelium[1-4]. Pneumocystis pneumonia is a serious opportunistic infection among immunocompromised patients including malnourished newborns, infants with hypogammaglobulinemia, thymic dysplasia, or severe combined immunodeficiency, transplant recipients, patients receiving intensive chemotherapy, and persons infected with HIV-1 [5-7]. The laboratory rat (*Rattus norvegicus*) harborstwo Pneumocystis species, *P. carinii* and *P. wakefieldiae*,[8,9] with *P. carinii* being much common and the only species so far linked to infectious interstitial pneumonia[10] in rats.*Pneumocystis* are host-species specific organisms that are transmitted from animal to animal via the airborne route. The easy and consistent horizontal transmission of spontaneous Pc pneumonia to previously non-infected athymic rats and the similarity of the disease to human infection human infection indicates that this is an excellent model forstudying pulmonary pneumocystis of immunodeficient human patients[11]. The HIV-1 transgenic rat bears a replication deficient version of HIV as the integrated transgene on the F344 background. Specifically, a 3.1 kb deletion was made in the pNL4-3 HIV proviral construct spanning HIV-1 gag and pol from bp 1443 to 4551[12]. Research conducted in our lab and by others using this HIV-1 Tg rat has shown HIV -1 related conditions such as neurological deficits, kidney disease, cardiac disorders and mild to severe skin disease. The expression of HIV-1 viral proteins in different organs seen in these animals, is partly responsible for the pathogenesis and manifestation of some of these clinical conditions similar to those seen in HIV-1 infected patients.

Studies have shown that the HIV-1 transgene expression in these rats caused oxidant stress within the alveolar space and impaired epithelial barrier function even though there was no evidence of overt inflammation within the airways[1]. Additionally, the expression and membrane localization of the tight junction proteins zonula occludens-1 and occludin were decreased in alveolar epithelial cells[1]. Further studies determined that the HIV-1 transgenic rats have significantly lower level of Zinc(Zn) in the alveolar space and macrophages together with an impaired alveolar macrophage phagocytosis. Along similar lines, in humans, compared with healthy

individuals, alveolar macrophage phagocytosis of *P. carinii* from HIV+ persons was reduced to 74% (P = 0.02) [13].

As in humans where HIV infection causes an increase in pulmonary arterial hypertension [14,15], studies in these HIV-1 transgenic rats showed that the expression of the transgene also causes an increase in pulmonary hypertension[16].

To further study the effects of these HIV-1 proteins in an immunocompromised background, we created the HIV-1 nude transgenic rat taking cue from the creation of the athymic T26 HIV transgenic mouse[17] by crossing the nude rat (mutant: Hsd:RH-Foxn1^{rnu}) with the HIV-1 transgenic rat and backcrossing the offspring for 10 generations. Different reviews of animal models of pneumocystis have been reported [18] with the nude rat being described and reported as a model for pneumocystis pneumonia[11]. Creation of immunosuppression in rats by the administration of corticosteroids has been described for the creation of the necessary conditions for establishment of Pc in the lungs of rats[19]. Here we described the development and pulmonary infection of Pc in HIV-1 Tg nude rats. We hypothesized that having the HIV transgene on a nude background which lacks functional T cells will render these HIV Tg nudes completely vulnerable to Pc infection even more so than the wild type rat and/or the HIV-1 Tg rat. The predictability, high morbidity, and well-defined genetics of the HIV-1 Tg nude rat make this the animal model of choice for *in vivo* studies of pneumocystis in HIV infections.

Material and Methods

Animals: HIV-1 Tg and HIV-1 Tg nude rats, developed, bred and maintained at the Animal Core Facility (Institute of Human Virology, University of Maryland School of Medicine), F344/NHsd and Hsd:RH-Foxn1^{rnu} (Harlan, Frederick, MD), were housed in clear polycarbonate cages with Sani-chip bedding (Harlan Teklad) and maintained on 12:12-hlight: dark cycles at 22 ± 2°C temperature, controlled humidity (50% to 70%) with ad libitum access to rodent diet (Harlan Teklad 8604) and reverse-osmosis–treated water. These rats were housed with other specific pathogen-free rats in the Institute of Human Virology (IHV) Animal Core facility, University of Maryland School of Medicine, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All experimental work on theses rats was approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee. Three sentinel rats (Sprague-Dawley) were used for every 50-70 cages. These rats remained negative for the following microbial agents during the time period covered by this report: sialodacryoadenitis virus, rat parvovirus, Kilham rat virus, Toolan H1 virus, rat minute virus, *Syphacia* spp., *Aspicularis tetrapetra,* Sendai virus, pneumonia virus of mice, Theiler murine encephalomyelitis virus strain GDVII, reovirus type 3, *Mycoplasma pulmonis*, P*neumocystis carinii*, lymphocytic choriomeningitis virus, and mouse adenovirus.

Rat cages were changed bi-weekly within a Class II type A2 biosafety cabinet. Personal protective equipment included dedicated scrubs, a disposable tyvex gown, face mask, hair bonnet, shoe covers and latex gloves. Prior to use, the biosafety cabinet was turned on for 5 minutes and correct magnehelic gauge readings were confirmed. The interior of the cabinet was disinfected witha chlorine based disinfectant. Rats were transferred from soiled to clean cages by using gloves which were disinfected with a chlorine based solution (Alcide) before handling each cage and gloves were changed after each side of the ventilated cage rack had been changed or when obviously soiled.

Transgenic nude rat: We generated the HIV-1Tg nude rat taking cue from the generation of the HIV-1 Tg we had earlier constructed[17]. Briefly, Heterozygous HIV-1 transgenic rats (purchased from Harlan) (++,T+) were bred with athymic nude rats (Hsd:RH-Foxn1^{rnu}, ++). Dual heterozygotes were selected by identifying rats bearing the HIV transgene, either by noting the presence of cataract (present with 100% penetrance but difficult to detect by visual inspection in athymic nude rats) or by Southern blotting. Dual heterozygotes were then intercrossed to obtain the following genotypes: ++, ++ and *nu* +, ++ (which were not distinguished and are termed wild-type mice); r*nu/rnu,* ++ (termed athymic rats); ++, T+ and r*nu* +, T+ (termed HIV-1 transgenic rat). We then backcrossed the offspring to the HIV-1 transgenic nude rat for ten generations housed in micro isolator cages.

Animal Sample Collection and Histopathology: Rats were euthanized by carbon dioxide inhalation and cervical dislocation. Immediately after euthanasia, lung and spleen specimens for histopathology and Pneumocystis PCR testing were collected after gross pathological examinations. Lungs were gently inflated with 10% neutral-buffered formalin. All histopathology tissues were fixed by immersion in 10% formalin. After at least 72-hour fixation, representative samples were cut from the fixed tissues and processed into paraffin blocks. Sections approximately 5 microns thick were cut onto slides and stained with hematoxylin and eosin (HE) and Silver methenamine for light microscopic examination.

Flow Cytometric Analyses: The fluorochrome-conjugated antibodies for surface staining rat cells (Anti-Rat CD3 FITC eBioG4.18, Anti-Rat CD45RA APC clone OX33, Anti-Rat CD4 PE Clone OX35, Anti-Rat CD8a PerCP-eFluor 70 clone OX8), together with appropriate isotype control antibodies, were purchased from eBioscience (San Diego, CA). The cells were incubated with a mixture of fluorochrome conjugated antibodies on ice for 45 min, followed by two washes in sterile phosphate buffered saline (PBS) supplemented with 1% fetal bovine serum. Flow cytometric analysis was conducted at the Institute of Human Virology using the FACSAria II cell sorter (BD Bioscience) and FlowJo software (Tree Star, Inc Ashland, OR USA).

PCR for HIV Tat gene: Total RNA was isolated by homogenizing tissue in TRIzol reagent (Invitrogen, Carlsbad, CA) and processed according to the manufacturer's instructions. RNA was reversed transcribed into cDNA with the Retroscript kit (Ambion, TX). Polymerase chain reactions (PCR) for HIV-1 genes were performed using the following primers:

GAPDH:
SN 5'-TGG AGA AGG CCG GGG CCC ACT T-3'
AS 5'-TCA GAT CCA CGA CGG ACA CAT TGG -3'
HIV-1 Tat:
SN 5'-GGA GCC AGT ATA TCC TAG GAT TAG-3'
AS 5'-AAT CGC ACG GAT CTG CCT CTG TCT-3'

PCR was terminated near the end of the linear-expansion phase (26 cycles for GAPDH and 28 cycles for HIV-1 Tat). Taq polymerase was from Takara Bio USA (now a subsidiary of Clontech, Mountain View, CA). PCR conditions: Denaturation at 92 °C×3 min, Main cycle at 94 °C×45 s, annealing at 55 °C×45 s, and extension at 72 °C× 30 sec, Final Extension at 72°C for 3 min. PCR products were resolved in 1.5% agarose gels containing ethidium bromide.

Statistical Analysis: Results were expressed as the arithmetic mean ± standard error of the mean. Two-tailed Student's t-tests (for comparison of unpaired samples) were performed. P values. 0.05 were considered to be significant. The Kaplan Meier survival curve was used to analyze the survival of the three groups of rats under study using the Log-rank (Mantel-cox) test.

Results and Discussion

We detected a2-kb multiply spliced HIV-1tat gene, and cataracts in the HIV-1 transgenic nude rat similar to what was reported in the HIV-1 Tg rat figure 2[12]. Furthermore these HIV-1 transgenic nude rats were hairless. Reverse-transcriptase PCR demonstrated the expression of spliced HIV transcript that encodes Tat in splenocytes. Splenic cells were harvested from the spleens of the HIV-1 Tg rat, HIV-1 Tg nude rat and F344. Total RNA was extracted and cDNA was synthesized using dT as the primer. PCR was conducted to use a set of primers that only gives rise to PCR product if a spliced HIV-1 transcript encoding for the Tat gene was expressed (lane b). As a control, cDNA from the spleen of a normal F344 rat was tested (lane a). To validate the quality of the cDNA, a house-keeping GAPDH fragment was amplified (Bottom).

T/B cells analysis: To confirm that the immunologic phenotype of the athymic nude rat was maintained in the HIV-1 Tg nude rat,after backcrossing with the HIV-1 Tg rat we did a flow cytometric analysis of splenic B & Tcells. There is a general consensus that T cells in athymic rats are mostly CD4 or CD8 single positive cells in the ratio of 3:1 [20,21]. As shown in figure 3, the HIV-1 transgenic nude rat had a depleted T cell compartment, meanwhile those of the HIV-1 transgenic rat and Fischer F344 are comparable. The ratios of the CD4/CD8 are also comparable in the F344 and HIV-1 Tg rat as against the HIV-1 Tg nude rat that is skewed. This confirms that the HIV-1 Tg nude rat maintained the athymic nature of the nude rat. We had hypothesized that a compromised immunity could be represented by skewed lymphocyte subsets in these HIV-Tg nude rats and that this mimics the athymic nude rat. This should render this model more susceptible to infection.

Pneumocystis in HIV-1 transgenic nude rats: Littermate HIV-1 Tg nude rats with cataracts, developed a wasting disease by 4 months characterized by severe wasting and loss of body weight. The clinical course was usually chronic and progressive, with wasting, dyspnea, and cyanosis with marked pale ear pinnae. We performed necropsy and observed severe lung lesions described below. We then decided to house these rats in cages with F344(n=10) and HIV-1 transgenic rats (n=10). By 4 months the HIV-1 Tg nude rats weighed significantly less (p=.0008) compared to the F344. The weight curvein figure 5 shows that the HIV-1 Tg nude growth rate and maximum weight at each time point was less than the others. The Kaplan-Meier curve in Figure 4, showed a statistically significant (p=.0001) survivability of the HIV-1 Tg nudes as compare to the HIV-1 Tg rat and the F344.The three risk groups have different observed survival probability (*P*<.0001). The observed median survival time were 44 weeks (95% CI, 44 to 44weeks), 42.8 weeks (95% CI, 41.2 to 44.3 weeks), 16.4 weeks (95% CI, 12.2 to 20.6 weeks), for the F344, the HIV-1 Tg and the HIV-1 Tg nude rats. The corresponding median predicted survival time for the HIV-1 Tg nude was 14 weeks (95%Cl, 12.0 to 24.0 weeks).

Post mortem lesions: Gross Lesions: Gross pulmonary lesions included 4-6 mm greyish white, flat to raised foci randomly distributed throughout the lung lobesin HIV-1 Tg nude rats (n=8), 100%. The lungs appeared rubbery, gray-purple, heavy, and consolidated (did not collapse) (figure 1C). All other organs appeared normal. Two out of 8 HIV-1 Tg rats (0.25%) had a few raised foci on their lungs, while there were no observable lesions on the lungs of the F344 rats.

Microscopic lesions: Demonstration of typical lesions and organisms is sufficient for diagnosis. Three sections of lung were examined from the HIV-1 transgenic nude rats. In all sections, the airspaces were multifocally to diffusely consolidated, (figure 1D) and occasionally expanded with eosinophilic foamy material (figure 1E). The foamy material was acellular and infrequently contained nuclear debris or red blood cells. In the expanded regions irregular basophilic concretions interpreted to be mineral deposits were within the foamy material. Alveoli free from the foamy eosinophilic material were frequently filled with activated macrophages with abundant eosinophilic cytoplasm and aground glass or finely vacuolated appearance. Alveolar septae were thickened with infiltrates of neutrophils and lymphocytes and with interstitial eosinophilic fibrillar material refractive on polarized light exam and interpreted to be collagen. Septae were lined with plump to

cuboidal alveolar pneumocytes. Bronchioles in the consolidated regions have a mild accumulation of the eosinophilic foamy material with few activated alveolar macrophages in the lumens. There was mild to moderate periarterial edema and mild to moderate infiltrates of lymphocytes with few brown pigment-laden macrophages that surrounded pulmonary arteries, veins and bronchioles. Sections of lungs stained with Silver methenamine, a special stain for pneumocystis were also examined. Argyrophilic cysts, 4 to 5 microns in diameter, were scattered throughout the parenchyma (figure 1F). The staining characteristic and size were consistent with the cysts of Pneumocystis. Some of these lesions were seen in the HIV-1 transgenic ratwhose lungs had some raised foci. None of the F344 rats had any of these lesions and none had clinical signs/symptoms of disease. PCR demonstrated Pc DNA in the lungs of all of the HIV-1 Tg nude rats (100% incidence), and in 40% of HIV Tg rats but in only 2 of the F344 rats (20%).

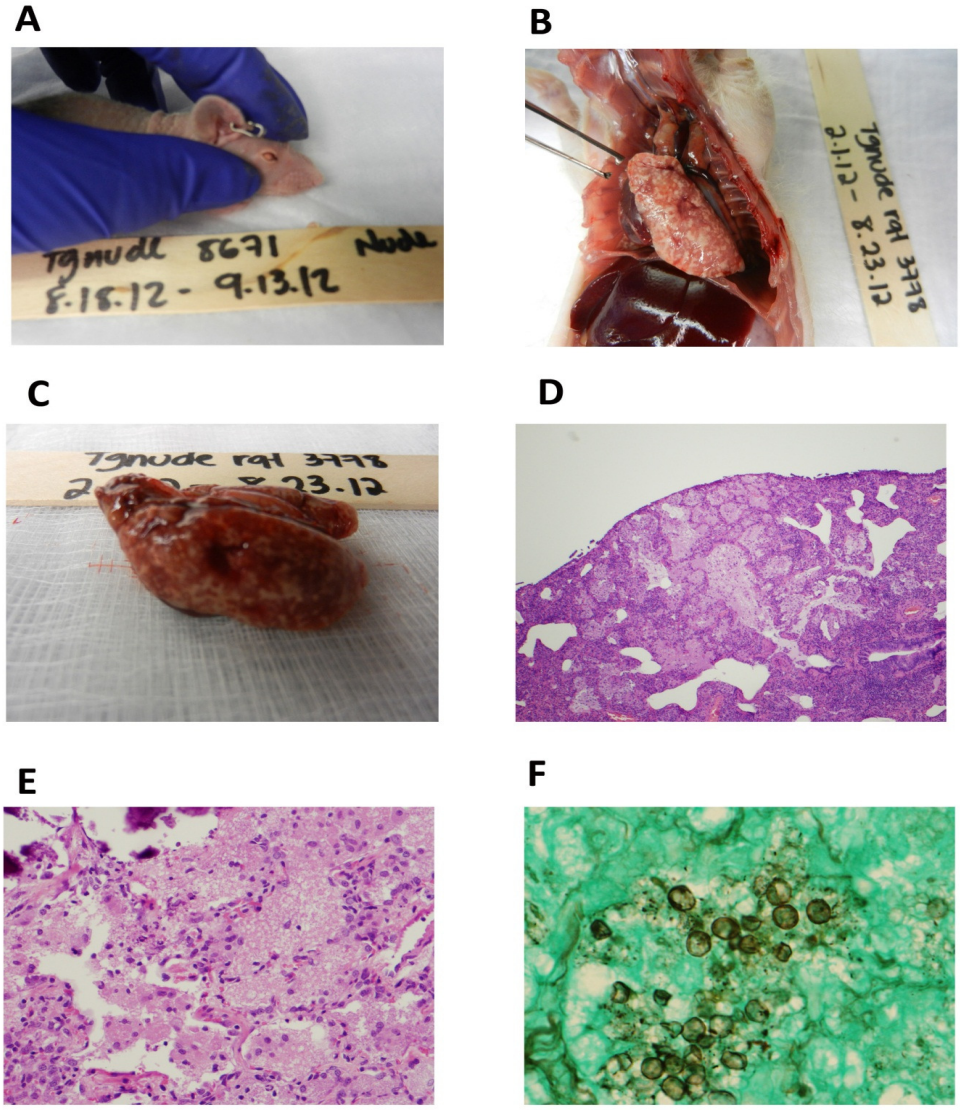

Figure-1

A. HIV-1 Tg Nude with Cataract. Heterozygote rats bearing the HIV transgene were selected by noting the presence of cataracts. B. Gross lung lesions: IV-1 Tg nude mice showed characteristic gross lung lesions of Pc: the lungs are rubbery, gray-purple, with 2-6mm grey, flat to raised randomly distributed foci throughout all lung lobes, heavy, and do not collapse. C. The lungs from HIV-1 Tg nude rats with *Pneumocystis carinii* are consolidated and do not collapse. D. The airspaces are multifocally consolidated with eosinophilic foamy material. The foamy material is acellular and infrequently contain nuclear debris and irregular basophilic concretions interpreted to be mineral deposits. There is mild to moderate periarterial edema and mild to moderate infiltrates of lymphocytes with few brown pigment-laden macrophages and lipid-laden macrophages that surround pulmonary arteries, veins and bronchioles.(H&E stain x100). E One-third of the parenchyma is diffusely consolidated with aggregates of activated macrophages, many of which form multinucleated cells. Alveoli are frequently filled with eosinophilic foamy material that is often mixed with activated macrophages. Bronchioles are surrounded by a moderate lymphohistiocytic infiltrate H&E). F. Argyrophilic cysts, 4 to 5 microns in diameter, are scattered throughout the parenchyma. The staining characteristic and size are consistent with the cysts of Pneumocystis (Silver methenamine stain 100x).

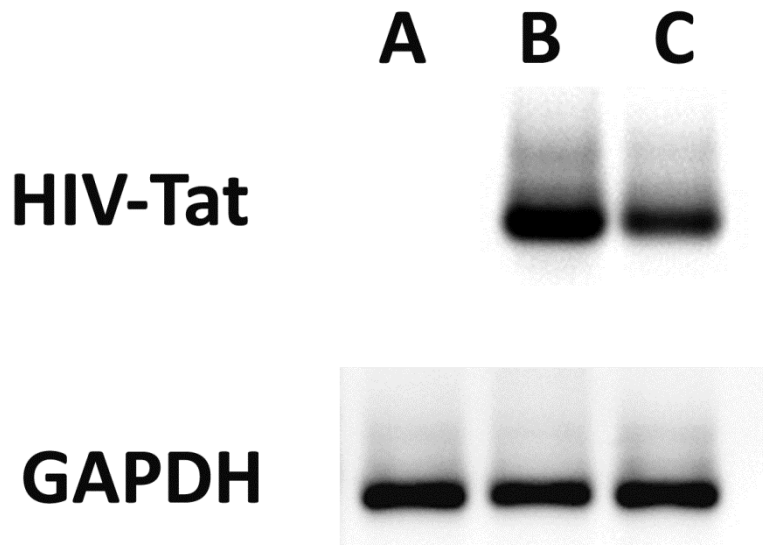

Figure-2

Reverse-transcriptase PCR to demonstrate the expression of spliced HIV transcript that encodes Tat in the HIV-1 Tg rat. Splenocytes were extracted from the spleens of the Fischer F344, HIV-1 Tg rat and the HIV-1 Tg nude rat. Total RNA was extracted and cDNA was synthesized using dT as the pirmer. PCR was conducted to use a set of primers that only gives rise to PCR product if a spliced HIV-1 transcript encoding for the Tat gene was expressed. To validate the quality of the cDNA, a house-keeping GAPDH fragment was amplified (Bottom)

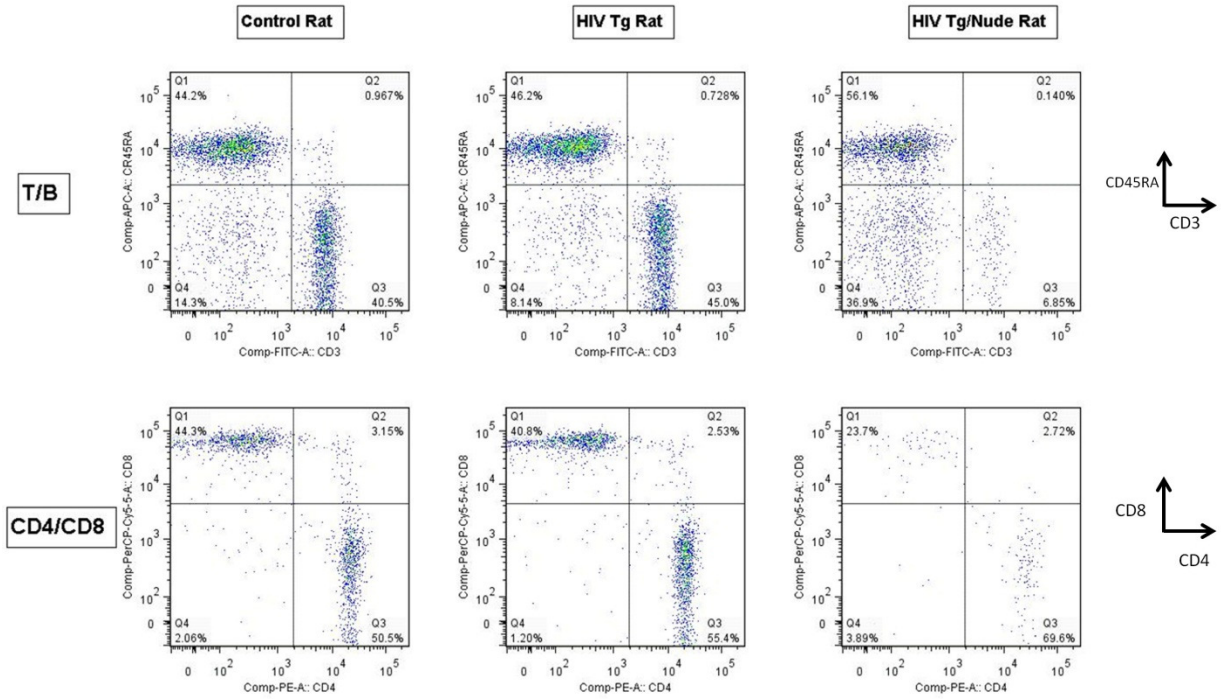

Figure-3

The HIV-1 Tg nude rat had a depleted T cell compartment meanwhile those of the HIV-1 transgenic rat and Fischer F344 are comparable. Also the rations of the CD4/CD8 are also comparable in the F344 and HIV-1 transgenic rat as against the HIV-1 transgenic nude rat that is skewed. This confirms that backcrossing the nude onto the HIV-transgenic rat did not change the athymic nature of the nude rat. Cells were analyzed using the FACSAria II cell sorter and FlowJo software

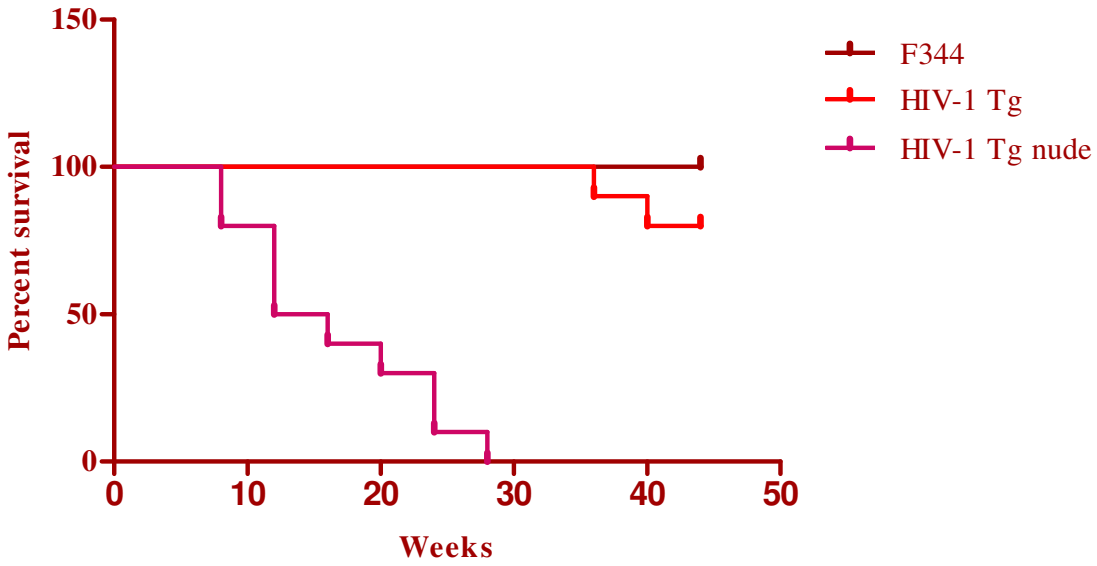

Figure-4
Kaplan-Meier Survival curve: Survival curve showing probability of survival of the control rat F344, the HIV-1 Tg rat and the HIV-1 Tg nude rat co-housed. While the control F344 rats survived to beyond the 40 weeks, the HIV-1 Tg nude rats all died from Pc by 30 weeks

Discussions: The athymic nude rat resembles the athymic nude mouse in that they are largely deficient in mature T cells and have severe defects in T-cell-mediated immune responses[22]. T lymphocytes are critically important in host defense to *P. carinii* [23]. The athymic nude rat has been a useful tool for the study of mechanisms of tumor growth, allograft rejection and also some autoimmune-like disorders in immunodeficient animals [22]. The HIV-1 transgenic rat after its development[12] in 2001has been used to study the pathogenesis of HIV AIDS diseases affecting various organs including the lungs[1,24-26], kidneys[12,27,28], skeletal muscle [29,30]brain[31-34] and the heart[35,36]. The advantage of this rat model, whencompared with the mouse model, is that the HIV-1–transgenicrat has a functional Tat and efficient viral gene expression indifferent organs. These rats have circulating gp120 in their blood, and tissue mRNA expression of gp120, Nef, Tat, and Rev.Furthermore, they develop muscle wasting, cataracts, nephropathy,and immune deficiencies that are all consistent with anAIDS-like phenotype[12].

In this study, we have shown that clinical disease of pneumocystis occurs in the HIV-1 Tg nude rat while F344 and HIV-1 Tg rats are only carriers. It is possible that an interaction between the expression of HIV-1 gene products and the lack of mature T cells contributed to the lung colonization and subsequent disease by *Pneumocystis carinii*. Previous studies had determined that alveolar macrophages from HIV-1–transgenic rats have decreased intracellular expression of the GM-CSFRb subunit and decreased bacterial phagocytic function[25,26] and we know that alveolar macrophages play important roles in both innate and adaptive immune responses. These studiessuggests that pulmonary zinc status may be important in HIV-1 infection, and that pulmonary zinc deficiency could be one of the mechanisms by which HIV-1infection impairs alveolar macrophage immune function. Other studies have shown, that the direct treatment of alveolar macrophages with the HIV-1 envelope protein, gp120, inhibited antifungal activity and reduced phagocytosis[37]. The interactions of *P. carinii* organisms with alveolar macrophages represent a significant component of host recognition and defense against *P. carinii* infection[38]. Both innate and adaptive immunity influence alveolar host defense against *P. carinii*, and yet it is the alveolar macrophage that is ultimately responsible for recognition, phagocytosis, and ultimate destruction of the organism[37].

Recent findings show that *Pneumocystis* is widespread in commercial rat colonies[39], and this has implications in understanding the life cycle of *Pneumocystis*. Different studies have suggested that immunocompetent hosts may be reservoirs of *Pneumocystis* infection[40,41]. The immunocompetent F344 and the HIV-1 Tg rat, have shown to be latently infected with Pc as evidenced by a fatal Pc pneumonia occurring in the HIV-1 Tg nude following their cohousing in the same cage.

The athymic nude rat was been characterized and proposed as a model for Pc because of the ease and consistency of horizontal transmission of spontaneous Pcpneumonia [11]. The relevance of the HIV-1 Tg nude rat is that, it is a model that can be used to study Pc in the context of HIV infection and without the confounding effects of immunosuppressive drugs that are at times used to create relevant rat models used to study Pc.

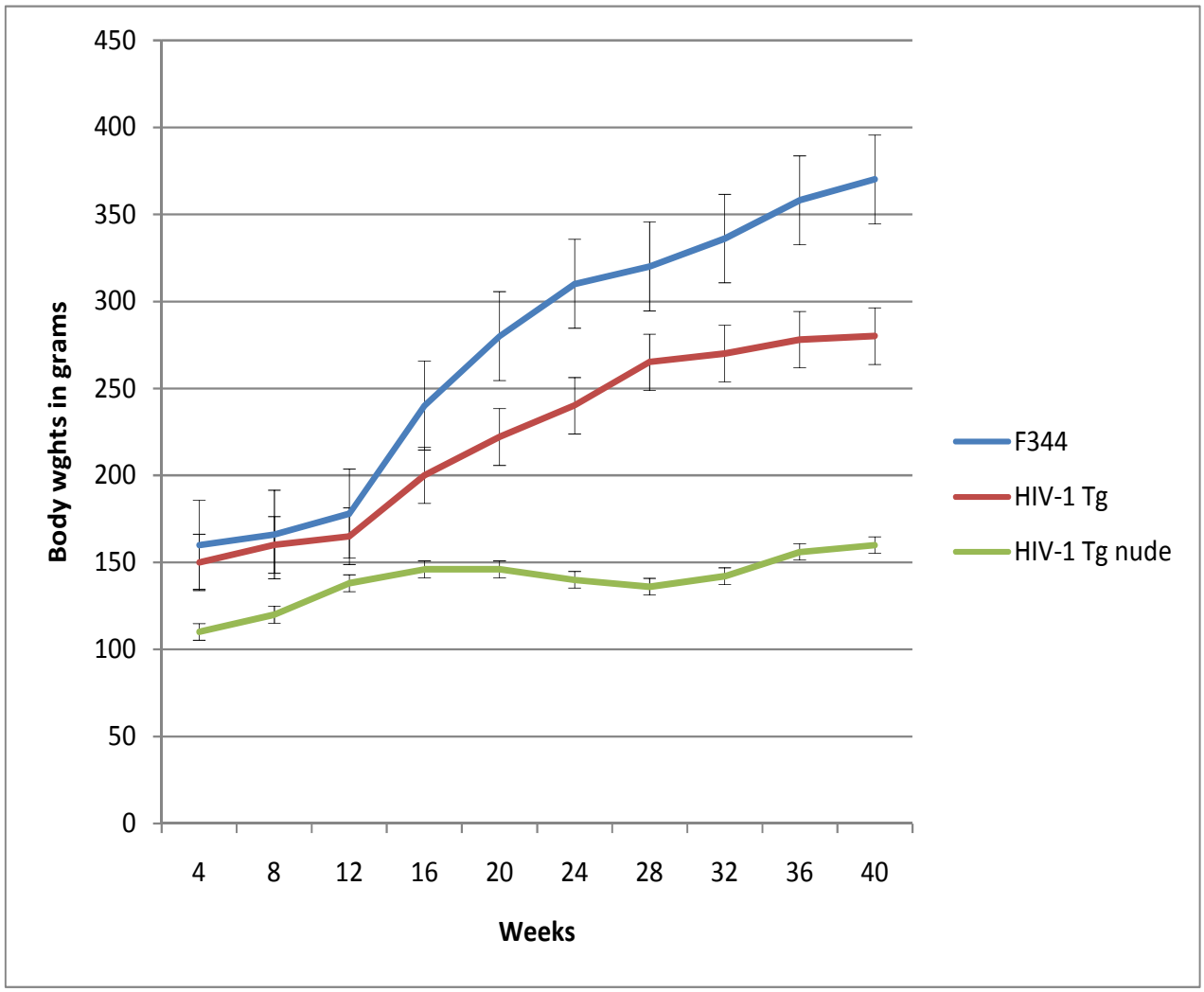

Figure-5
Body Weights of rats
Comparison of Bodyweight of F344, HIV-1 Tg rat and HIV-1 Tg nude rats measure from 4 – 40 weeks. Ordinate is body weight in grams while the abscissa is timein weeks. Note the smaller size of the HIV-1 Tg nude rat ascompared with the controls and the HIV-1 Tg rat. As seen the HIV-1 Tg nude rats had a low body weights from 6 weeks and by 40 weeks or prior to death had a significant low body weight.

The lung lesions described in the HIV-1 Tg rat and the increased susceptibility of the HIV-1 Tg nude rat to Pc are important and consistent with the increased susceptibility tolung infections that causes so much morbidity and mortality in HIV infected patients. It is considered that although anti-retroviral therapy can control viral replication in most cases, the HIV related proteins still circulate[42] and can still continue to have adverse effects on cellular, tissue and organ functions long after viral replication has been effectively curtailed in patients.

Pneumocystis pneumonia was thought to occur only in immunodeficient rats caused by genetic (for example, nude rats) or artificial (for example, immunosuppressive doses of glucocorticoids) means[11,19]. There is currently no animal model to study the effects of pneumocystis in the context of HIV proteins. Although this transgenic model clearly differs from human disease in certain respects, including the lack of viral infection and/or viral replication, it nonetheless would be relevant to study the pathophysiological effects of HIV-1-related proteins on target tissues such as the alveolar epithelium that are not infected directly by the virus. The HIV-1 Tg nude model is relevant in the studyof potential treatment options for *Pneumocystis carinii* infection without the confounding effects of corticosteroids. Over the past 30 years, major advances have

been made in our understanding of HIV/AIDS and *Pneumocystis* pneumonia [6], but significant gaps remain and this model may accelerate and further our understanding of this major cause of HIV associated morbidity and mortality.

Conclusion

Pneumocystis pneumonia is a serious opportunistic infection among immunocompromised patients and in the context of HIV the pathogenesis is poorly understood. Although this HIV-1 Tg nude model, lack viral infection or viral replication, it is a relevant model to study not only the pathophysiological effects of HIV-1 related proteins on target tissues but also the potential treatment options for Pc infection. This model will be particularly relevant to evaluate the *in vivo*naturally occurring pathogenesis of Pc in the setting of HIV infection without the complicating effects of corticosteroids on the immune functions of the rats. An improved understanding of the mechanisms underlying the infection of the alveolar epithelium by Pc in this model may provide clues to the predisposition to Pc infection in HIV-infected subjects.

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References

1. Lassiter C. *et al.,* HIV-1 transgene expression in rats causes oxidant stress and alveolar epithelial barrier dysfunction, *AIDS Res Ther,* **6**, 1 **(2009)**

2. Afessa B., Green W., Chiao J. and Frederick W., Pulmonary complications of HIV infection: autopsy findings, *Chest,* **113**, 1225-9 **(1998)**

3. Crothers K. *et al.,* Increased COPD among HIV-positive compared to HIV-negative veterans, *Chest,* **130**, 1326-33 **(2006)**

4. Crothers K., Chronic obstructive pulmonary disease in patients who have HIV infection, *Clin Chest Med,* **28**, 575-587 **(2007)**

5. Thomas C.F., Jr. and Limper A.H., Pneumocystis pneumonia, *N Engl J Med.,* **350**, 8 **(2004)**

6. Huang L. *et al.,* HIV-associated Pneumocystis pneumonia, *Proc Am Thorac Soc,* **8**, 294-300 **(2011)**

7. Walzer P.D., Schultz M.G., Western K.A. and Robbins J.F., Pneumocystis carinii pneumonia and primary immune deficiency diseases, *Natl Cancer Inst Monogr,* **43**, 65-74 **(1976)**

8. Redhead S.A., Cushion M.T., Frenkel J.K. and Stringer J.R., Pneumocystis and Trypanosoma cruzi: nomenclature and typifications, *J Eukaryot Microbiol,* **53**, 2-11 **(2006)**

9. Cushion M.T., Pneumocystis: unraveling the cloak of obscurity, *Trends Microbiol,* **12**, 243-249 **(2004)**

10. Henderson K.S. *et al.,* Pneumocystis carinii causes a distinctive interstitial pneumonia in immunocompetent laboratory rats that had been attributed to "rat respiratory virus", *Vet Pathol,* **49**, 440-452 **(2012)**

11. Pohlmeyer G. and Deerberg F., Nude rats as a model of natural Pneumocystis carinii pneumonia: sequential morphological study of lung lesions, *J Comp Pathol,* **109**, 217-230 **(1993)**

12. Reid W. *et al.,* An HIV-1 transgenic rat that develops HIV-related pathology and immunologic dysfunction, *Proc Natl Acad Sci U S A,* **98**, 9271-9276 **(2001)**

13. Koziel H. *et al.,* Reduced binding and phagocytosis of Pneumocystis carinii by alveolar macrophages from persons infected with HIV-1 correlates with mannose receptor downregulation, *J Clin Invest,* **102**, 1332-44 **(1998)**

14. Petitpretz P. *et al.,* Pulmonary hypertension in patients with human immunodeficiency virus infection. Comparison with primary pulmonary hypertension, *Circulation,* **89**, 2722-2727 **(1994)**

15. Barnett C.F. and Hsue P.Y., Human immunodeficiency virus-associated pulmonary arterial hypertension, *Clin Chest Med,* **34**, 283-292 **(2013)**

16. Porter K.M. *et al.,* Human immunodeficiency virus-1 transgene expression increases pulmonary vascular resistance and exacerbates hypoxia-induced pulmonary hypertension development, *Pulm Circ,* **3**, 58-67 **(2013)**

17. Shrivastav S. *et al.,* Role of T lymphocytes in renal disease in HIV-transgenic mice, *Am J Kidney Dis,* **35**, 408-417 **(2000)**

18. Dei-Cas E., Brun-Pascaud M., Bille-Hansen V., Allaert A. and Aliouat E.M., Animal models of pneumocystosis, *FEMS Immunol Med Microbiol,* **22**, 163-168 **(1998)**

19. Aliouat E.M. *et al.,* Development of pneumocystosis animal models: corticosteroid-treated Wistar rat; SCID mouse and nude rat, *J Eukaryot Microbiol,* **44**, 41S-42S **(1997)**

20. Sarawar S.R., Yang C.P. and Bell E.B., T-cell receptor-bearing cells from athymic nude rats respond to alloantigen in vitro but are defective in vivo, *Immunology,* **73**, 334-341 **(1991)**

21. Schwinzer R., Hedrich H.J. and Wonigeit K.T cell differentiation in athymic nude rats (rnu/rnu): demonstration of a distorted T cell subset structure by flow cytometry analysis, *Eur J Immunol,* **19**, 1841-1847 **(1989)**

22. Festing M.F., May D., Connors T.A., Lovell D. and Sparrow S., An athymic nude mutation in the rat, *Nature,* **274**, 365-366 **(1978)**

23. Roths J.B. and Sidman C.L., Both immunity and hyperresponsiveness to Pneumocystis carinii result from transfer of CD4+ but not CD8+ T cells into severe combined immunodeficiency mice, *J Clin Invest,* **90**, 673-678 **(1992)**

24. Lund A.K., Lucero J., Herbert L., Liu Y. and Naik J.S., Human immunodeficiency virus transgenic rats exhibit pulmonary hypertension, *Am J Physiol Lung Cell Mol Physiol,* **301**, L315-326 **(2011)**

25. Fan X., Joshi P.C., Koval M. and Guidot D.M., Chronic alcohol ingestion exacerbates lung epithelial barrier dysfunction in HIV-1 transgenic rats, *Alcohol Clin Exp Res,* **35**, 1866-1875 **(2011)**

26. Joshi P.C., Raynor R., Fan X. and Guidot D.M., HIV-1-transgene expression in rats decreases alveolar macrophage zinc levels and phagocytosis, *Am J Respir Cell Mol Biol,* **39**, 218-226 (**2008)**

27. Avila-Casado C., Fortoul T.I. and Chugh S.S., HIV-associated nephropathy: experimental models, *Contrib Nephrol,* **169**, 270-285 **(2011)**

28. Ray P.E. *et al.* A novel HIV-1 transgenic rat model of childhood HIV-1-associated nephropathy, *Kidney Int,* **63**, 2242-2253 **(2003)**

29. Clary C.R., Guidot D.M., Bratina M.A. and Otis J.S., Chronic alcohol ingestion exacerbates skeletal muscle myopathy in HIV-1 transgenic rats, *AIDS Res Ther,* **8**, 30 **(2011)**

30. Vikulina T. *et al.,* Alterations in the immuno-skeletal interface drive bone destruction in HIV-1 transgenic rats, *Proc Natl Acad Sci U S A,* **107**, 13848-13853 **(2010)**

31. Basselin M. *et al.,* Imaging upregulated brain arachidonic acid metabolism in HIV-1 transgenic rats, *J Cereb Blood Flow Metab,* **31**, 486-493 **(2011)**

32. Kass M.D., Liu X., Vigorito M., Chang L. and Chang S.L., Methamphetamine-induced behavioral and physiological effects in adolescent and adult HIV-1 transgenic rats, *J Neuroimmune Pharmacol,* **5**, 566-573 **(2010)**

33. Sultana S. *et al.,* Quantitation of parvalbumin+ neurons and human immunodeficiency virus type 1 (HIV-1) regulatory gene expression in the HIV-1 transgenic rat: effects of vitamin A deficiency and morphine, *J Neurovirol,* **16**, 33-40 **(2010)**

34. June H.L., Tzeng Yang A.R., Bryant J.L., Jones O. and Royal W., 3rd. Vitamin A deficiency and behavioral and motor deficits in the human immunodeficiency virus type 1 transgenic rat, *J Neurovirol,* **15**, 380-389 **(2009)**

35. Pruznak A.M. *et al.,* Skeletal and cardiac myopathy in HIV-1 transgenic rats, *Am J Physiol Endocrinol Metab,* **295**, E964-973, **(2008)**

36. Otis J.S., Ashikhmin Y.I., Brown L.A. and Guidot D.M., Effect of HIV-1-related protein expression on cardiac and skeletal muscles from transgenic rats, *AIDS Res Ther***5**, 8, **(2008)**

37. Martin W.J., 2nd & Pasula R., Role of alveolar macrophages in host defense against Pneumocystis carinii. *Am J Respir Cell Mol Biol***23**, 434-435 **(2000)**

38. Limper A.H., Hoyte J.S. and Standing J.E., The role of alveolar macrophages in Pneumocystis carinii degradation and clearance from the lung, *J Clin Invest,* **99**, 2110-2117 **(1997)**

39. Icenhour C.R., Rebholz S.L., Collins M.S. and Cushion M.T., Widespread occurrence of Pneumocystis carinii in commercial rat colonies detected using targeted PCR and oral swabs, *J Clin Microbiol,* **39**, 3437-3441 **(2001)**

40. Dumoulin A. *et al.* Transmission of Pneumocystis carinii disease from immunocompetent contacts of infected hosts to susceptible hosts, *Eur J Clin Microbiol Infect Dis,* **19**, 671-678 **(2000)**

41. Vargas S.L. *et al.* Transmission of Pneumocystis carinii DNA from a patient with P. carinii pneumonia to immunocompetent contact health care workers, *J Clin Microbiol,* **38**, 1536-1538 **(2000)**

42. Popovic M. *et al.,* Persistence of HIV-1 structural proteins and glycoproteins in lymph nodes of patients under highly active antiretroviral therapy, *Proc Natl Acad Sci U S A,* **102**, 14807-14812 **(2005)**