Animal models to study *Mycobacterium tuberculosis* and HIV co-infection

Ming GUO, Wen-Zhe HO*

Center for Animal Experiment / Animal Biosafety Level ⅢLaboratory, *Wuhan University*, *Wuhan* 430071, *China*

Abstract: *Mycobacterium tuberculosis* (*M.tb*) and human immunodeficiency virus (HIV) co-infection has become a public health issue worldwide. Up to now, there have been many unresolved issues either in the clinical diagnosis and treatment of *M.tb*/HIV co-infection or in the basic understanding of the mechanisms for the impairments to the immune system by interactions of these two pathogens. One important reason for these unsolved issues is the lack of appropriate animal models for the study of *M.tb*/HIV co-infection. This paper reviews the recent development of research on the animal models of *M.tb*/HIV co-infection, with a focus on the non-human primate models.

Keywords: HIV; *Mycobacterium tuberculosis*; Co-infection; Animal model

Tuberculosis (TB) and HIV/AIDS remain two major global health problems. An estimated 12 (11−13) million people worldwide suffered from active TB, and 35.3 (32.2−38.8) million people were living with HIV in 2012 (UNAIDS, 2013; WHO, 2013). In 2012, 2.3 (1.9−2.7) million people were newly infected with HIV, and 1.6 (1.4−1.9) million people died from AIDS-related causes globally (UNAIDS, 2013). In 2012, there were an estimated 8.6 million new cases of TB (13% co-infected with HIV), and 1.3 million people died from TB, including 320 000 among people who were HIV-positive (UNAIDS, 2013; WHO, 2013).

Starting in the mid-1980s, the HIV epidemic led to a major upsurge in TB cases (Ye & Lu, 2004). TB is the most common opportunistic infection affecting 20%−50% of HIV patients. About one-quarter of deaths among people with HIV are due to TB (WHO, 2013). HIV is the most powerful known risk factor for reactivation of latent TB infection, which is a leading killer of people living with HIV. Co-infection with TB and HIV leads to challenges in both the diagnosis and treatment of TB. The mechanisms of *M.tb*/HIV co-infection/interaction and the breakdown of the immune defense of the co-infected individual are not well known. The research in the biology of concurrent *M.tb* and HIV infection is urgently needed, as it will help to reduce the risk of morbidity and mortality and the socioeconomic burden associated with these two diseases.

One of the most important challenges in studies of *M.tb*/HIV co-infection is to establish an appropriate animal model. Although small animals such as mice can be infected by *M.tb*, they are not hosts of HIV and are not suitable for the *M.tb*/HIV co-infection modelling. But some complementary mouse models, such as humanized mouse, can be used to reproduce the relevant features of *M.tb* and HIV infections. By contrast, some nonhuman primates, such as *Macaca* (macaques: rhesus, cynomolgus), can be infected by simian immunodeficiency virus (SIV), a retrovirus similar to HIV causing immunodeficiency in macaques (Lei et al, 2013; Xia et al, 2010; Zhou et al, 2013). These SIV macaque models have been widely used in HIV/AIDS research (Zhang et al, 2007a). Macaques can also be infected by *M.tb* and develop TB that is closely resemble humans. Addition-

Received: 12 August, 2013; Accepted: 20 February 2014
Foundation items: This work was supported by grants from the National Natural Sciences Foundation of China (81201261, 81301428), the National Science Foundation for Post-doctoral Scientists of China (2013M5317456) and the National Science and Technology Major Project of the Ministry of Science and Technology of China (2012ZX-10004501-001-004)
* Corresponding author, E-mail: wenzheho@whu.edu.cn

ally, similar to humans, macaques can maintain TB latency for years and only a small proportion progress to active disease (Gormus et al, 2004). Therefore, macaques may be ideal for establishing future *M.tb*/HIV co-infection animal models.

M.tb/HIV co-infection mouse model

To circumvent the limitation that the mice cannot be infected by HIV, researchers developed two complementary mouse models. The first model is called the humanized mouse model. In this paradigm, the human immune system is reconstituted in immunodeficient mice by transplanting human hematopoietic progenitor cells ($CD34^+$) from human cord blood (Traggiai et al, 2004). The second model is the BLT (bone marrow, liver, thymic) mice model. NOD/SCID mice are co-transplated with live human fetal thymus tissues along with autologous $CD34^+$ hematopoietic stem cells (Gorantla et al, 2010). These BLT mice gain human immunity as a functional human thymus can produce more proper humanized T cells. Therefore, these mice could be infected by HIV, causing $CD4^+$ T cells depletion and prolonged viremia (Baenziger et al, 2006; Denton et al, 2010; Gorantla et al, 2010; Sun et al, 2007; Zhang et al, 2007b). In addition, as the transplanted human cells can be maintained in the mucosal surface, the mice can be infected with HIV by the intravaginal and intrarectal routes (Joseph et al, 2010). This model has been used extensively to study the prevention or treatment of HIV infection with human-neutralizing antibodies, antiretroviral drugs and T cell-specific siRNA (Denton et al, 2010; Joseph et al, 2010; Kumar et al, 2008). Recently, the humanized mouse model has also been used in the TB infection research. Heuts et al (2013) reported that humanized mice were infected with either Bacillus Calmette-Guerin (BCG) by intravenous injection or *M.tb* by aerosol. In contrast to the nonhumanized BCG-infected control, the core of the granulomas in humanized mice contained giant cells, human $CD68^+$ macrophages, and high bacilli numbers surrounded by a layer of human $CD3^+$ T cells and a fibrotic response encapsulating the lesion in the liver and lungs (Heuts et al, 2013). Paradoxically, humanized mice contained higher mycobacterial numbers in organs than nonhumanized controls (Heuts et al, 2013). The higher mycobacterial loads was mediated by the human $CD4^+$ T cells, as BCG loads in the lungs or liver of anti-human CD4-treated humanized mice were reduced compared with nontreated humanized mice (Heuts et al, 2013). These humanized mice can be a good model in pathogenic infection and in the study of the formation and maintenance of human granulomas in TB.

Calderon et al (2013) recently developed an improved humanized mouse model using NOD-SCID mice, which were engrafted with human fetal liver and thymus tissue, and supplemented with $CD34^+$ fetal liver cells. The peripheral blood in well-reconstituted mice expressed high levels of human CD45 pan-leukocyte marker. Human T cells (CD3, CD4 and CD8), natural killer and monocyte/macrophages were observed within the human leukocyte population 12 weeks after engraftment. Human T cells in humanized mice were functionally competent as determined by proliferative capacity and effector molecule expression in response to positive stimuli. Once intranasally infected with *M.tb*, these humanized mice had progressive *M.tb* infection in the lung, which disseminated to the spleens and livers 2−8 weeks post-infection (Calderon et al, 2013). In addition to the organized granulomatous lesions, caseous necrosis, bronchial obstruction and crystallization of cholesterol deposits could be found in the sites of infected lungs. Importantly, human T cells were found in the lungs, liver and spleen at the site of inflammation and *M.tb* growth (Calderon et al, 2013). These findings clearly demonstrated the feasibility of using this mouse model for *M.tb* infection. However, there has been no report about using this model for *M.tb*/HIV co-infection studies.

HIV transgenic mice incorporating the entire vial genome have also been used in *M.tb* infection (Scanga et al, 2007). *M.tb*-infected mice showed an increase in HIV expression at the site of bacterial replication and associated with the areas of inflammation. *M.tb* induces HIV transgene expression by both TNF-dependent and independent mechanisms, the former playing a more substantial role at a later stage of infection. Antibiotic treatment of *M.tb* can markedly reduce HIV transgene expression. These data suggest that this animal model is useful in testing therapeutic regimens for reducing the disease burden in patients with HIV-associated TB (Scanga et al, 2007).

MAC/SIV co-infection macaque model

Macaques provide a valuable model for SIV and *Mycobacterium avium* complex (MAC) co-infection.

MAC is known to cause a severe opportunistic infection for HIV-infected people. Although the murine model of MAC infection is well established, this model is not suitable for investigating the interaction among the host, MAC and HIV. MAC in SIV-infected macaques is a frequent opportunistic infection that shares many features with humans infected with AIDS.

Through a retrospective analysis, Mansfield et al (1995) found that 17% (23/135) of SIV-induced simian AIDS macaques which received neither antiretroviral nor antimicrobial therapy were infected with MAC, and 31.3% (21/67) of the SIVmac251-infected macaques were MAC-positive. MAC positivity specifically for SIVmac29 and SIVmac239/316EM was detected in 1.9% (1/53) and 6.7% (1/15), respectively. In addition, compared to the SIV mono-infected macaques, animals with MAC had a longer mean survival after SIV primary infection and lower CD4 cell counts at death. However, the mechanism of the longer survival time in co-infected macaques remains unclear. Mansfield et al (2001) also developed an experimental system to co-inoculate rhesus macaques with SIV and a clinical MAC strain. By using this animal model, they found that the development of disseminated MAC is dependent on the SIV strain. The rhesus monkeys co-infected with SIVmac251 and MAC developed progressive disease, whereas the control animals infected with only MAC and animals co-infected with SIVmac239 or SIVmac239MER and MAC developed self-limiting infection. The ability of self-limiting infection which can eliminate mycobacterial disease was independent of the CD4 T cell counts and the viral load but associated with the size and composition of micro-granulomas.

Whether infection with MAC among HIV patients is a result of recent exposure to virulent strains or reactivation of latent MAC infection is unclear at present. To address this question, Maslow et al (2003) cultured the tissue samples from SIV-infected as well as uninfected rhesus macaques, showing that 68.1% (32/47) SIV-infected and 22.2% (14/63) SIV-uninfected macaques were MAC-positive. Twenty-five SIV-infected animals and one uninfected animal were infected with MAC strain K128A, which is highly virulent for rhesus macaques (Newman et al, 1999). These data demonstrate that disseminated MAC disease appears to be from reactivation of latent infection, as well as from recent infection with virulent MAC strains.

BCG/SIV co-infection macaque model

The safety of BCG use in people infected with HIV has been controversial (Nuttall & Eley, 2011). To examine the impact of BCG inoculation on SIV replication, Cheynier et al (1998) repeatedly inoculated BCG to three SIVmac251-infected macaques by intravenous injection and showed that the recruitment of BCG-specific T cells facilitated SIV replication and dissemination. This finding was supported by another study (Zhou et al, 1999), showing that BCG-mediated T cell activation correlated with a marked increase in viral loads in SIV-infected macaques. Moreover, the prolonged T cell activation coincided with the enhanced depletion of CD4 T cells and the accelerated progression to clinical AIDS in the co-infected monkeys. Within 2 to 7 months after BCG co-infection, all chronically SIV infected monkeys died from AIDS, including TB-like disease. These results suggested that BCG-driven T cell activation may be an important mechanism that enhances the pathogenicity of HIV (Cheynier et al, 1998; Zhou et al, 1999). Surprisingly, in this study (Zhou et al, 1999), 2 weeks after simultaneous SIV and BCG inoculation, naïve monkeys manifested a T cell activation-related toxic shock syndrome and a profound depletion of $CD4^+$ T cells. These co-infected naive monkeys all died of AIDS within 2 months after co-infection. In contrast, the control SIV mono-infected naïve monkeys showed only a natural course of chronic SIV infection, and the BCG mono-infected naïve monkeys survived BCG infection. Thus, this SIV/BCG co-infection model supports the hypothesis that HIV and *M.tb* co-infection can remarkably impact AIDS virus-induced disease.

In another study (Croix et al, 2000), rhesus macaques were first infected with SIV/DeltaB670 and then inoculated with BCG. All animals had a dramatic transient increase in plasma viral loads and CCR5 expression on T lymphocytes after BCG inoculation. Two of the four SIV-infected animals had strong proliferative responses to PPD; the other two with poor responses developed disseminated BCG during the course of the experiment. The interaction of BCG with SIV was also examined in SIV-infected long-term non-progressor (LTNP) monkeys (Croix et al, 2000). Similar to the acutely infected monkeys, two of three LTNPs had an increase in plasma viral loads and CCR5 expression, but none had accelerated progression to AIDS (Croix et al, 2000).

Newly acquired *M.tb* infection in HIV-infected patients can spread readily and progress to active TB disease (Daley et al, 1992). Following BCG co-infection, the SIV-infected macaques with high viral loads developed a SIV-related TB-like disease (Shen et al, 2004b; Shen et al, 2002a). The clinical symptoms included diarrhea, anorexia, weight loss and altered levels of consciousness, and pathological studies revealed the presence of disseminated granulomas. In contrast, co-infected macaques with low viral loads either showed no evidence of BCG-induced disease or developed focal granulomatous lesions (Shen et al, 2002a). The interaction between SIV and BCG may play a critical role in triggering the development of SIV-related TB-like disease. BCG infection can enhance the destruction of $CD4^+$ T cells in macaques with high SIV loads. The progression of SIV disease led to marked suppression of BCG-specific T cell responses, causing the persistence of BCG infection and development of SIV-related TB-like disease. The naïve macaques simultaneously infected with SIV and BCG also developed the SIV-related TB-like disease (Shen et al, 2002a).

In order to examine antiretroviral therapy-induced restoration of memory anti-mycobacterial immunity, macaques were inoculated intravenously with BCG, then SIVmac251 and finally BCG again in 2-month intervals (Shen et al, 2001). The co-infected macaques received antiretroviral treatment, which controlled the SIV replication and the SIV-related BCG-induced disease. The resolution of this disease coincided with the restoration of the PPD-specific T cell response. By contrast, the co-infected macaques which did not receive antiretroviral treatment had a depressed PPD-specific response and they all died from TB-like disease (Shen et al, 2001). The results of this study suggested that antiretroviral agents can improve the outcome of AIDS virus-related TB-like disease by restoring the *M.tb* specific immune response. $V\gamma2V\delta2^+$ T cells may contribute to adaptive immunity to mycobacterial infection (Shen et al, 2002b). Zhou et al (2003) investigated the $V\gamma2V\delta2^+$ T cells in SIV and BCG co-infected macaques. The primary and recall expansions of phosphoantigen-specific $V\gamma2V\delta2^+$ T cells can be found after BCG infection and BCG reinfection in control SIVmac negative macaques (Shen et al, 2002b; Zhou et al, 2003). Conversely, SIV infected macaques showed only subtle expansions of $V\gamma2V\delta2^+$ T cells in both peripheral and bronchoalveolar lavage fluid (Zhou et al, 2003). But this adaptive $V\gamma2V\delta2^+$ T cell responses during SIV/*M.tb* co-infection can be generated by effective antiretroviral treatment (Shen et al, 2004a).

M.tb/SIV co-infection macaque model

Safi et al (2003) was among the first to characterize the manifestations of *M.tb* and SIV co-infection, using Indian rhesus monkeys. In this study, 15 animals were vaginally infected with SIV/Delta B670; three animals died within 6 months after infection (Safi et al, 2003). The surviving 12 animals were divided into two groups at about 400 days post SIV infection. Six animals were infected intrabronchially with *M.tb* H37Rv (200 cfu/animal). Two of three co-infected animals with high levels of plasma viral loads had significant body weight loss, and died within 15 weeks after *M.tb* infection. All three animals with high levels of plasma viremia yielded *M.tb* from multiple organs with extensive inflammation and caseous necrosis. The other three animals with moderate levels of plasma viremia survived for 6 months after the *M.tb* challenge and showed no loss in body weight. Necropsy showed pulmonary granulomata and acid-fast organisms. Four of the six SIV mono-infected monkeys were alive without weight loss at the end of the study; the other two died of pneumonia and *M. avium* complex enteritis. In this study, the clinical, immunologic and pathologic findings in survived macaques were similar compared to humans with latent tuberculosis infection (LTBI). These observations suggested that an animal model of LTBI in SIV-infected macaques can be developed. Such a model can be used in investigating the immunologic and microbial factors in HIV and *M.tb* co-infection.

The cellular and molecular mechanisms of TB reactivation by HIV infection are unclear. HIV-infected patients with latent *M.tb* infection have a significantly greater risk of TB reactivation than HIV-negative individuals with latent TB, even if the CD4 T cells are well-preserved (Hanson et al, 1995; Mukadi et al, 1993; Post et al, 1995). To study the reactivation of TB, the latent *M.tb*-infected macaque models are needed. It appears that cynomolgus macaques are more suitable for latent *M.tb* infection than rhesus macaques. Diedrich et al (2010) showed that out of 10 cynomolgus macaques infected with *M.tb* Erdman strain, six were classified as latently infected, as these animals only showed TST positive but no signs of other clinical manifestation. However, when these animals were infected with

SIVmac251, they developed activated TB. Reactivation was independent of viral load but with early depletion of peripheral T cells during acute SIV infection (Diedrich et al, 2010). Comparing with SIV-mono-infected animals, co-infected animals had fewer CD4 T cells in involved lungs. The granulomas from the co-infected animals had histopathologic characteristics that are consistent with a chronically active disease process. These results suggested that initial T cell depletion may contribute reactivation of TB in HIV-*M.tb* co-infection. They also found the T cell cytokines responses in SIV/*M.tb* co-infected macaques are associated with timing of reactivation (Mattila et al, 2011). The *M.tb*-specific Th1 T cells responses were increased 3−5 weeks post SIV infection; more multi-functional $CD4^+$ T cells 3−5 weeks post SIV infection and more Th2-polarized and fewer Th0, Th1-polarized $CD8^+$ T cells 1-10 weeks post SIV infection were founded in animals reactivating <17 weeks post SIV infection than animals reactivating >26 weeks post SIV infection (Mattila et al, 2011). The distortions in pro-inflammatory and anti-inflammatory T cell responses have significant effects on the reactivation of latent TB.

Besides the TB reactivation cynomolgus model, Mehra et al (2011) also developed a similar model by using rhesus macaques at the same time. First, twelve Indian adult rhesus macaques were infected with 500 cfu *M.tb* CDC1551 via a head-only aerosol method. Eight weeks later, six animals with latent tuberculosis and four *M.tb*-negative animals were infected with SIVmac239 via intravenous injection (200 TCID50/animal). The co-infected group had significantly higher body temperature, CRP levels and body weight loss than the *M.tb* mono-infected group (Mehra et al, 2011). SIV not only reactive TB but also increase *M.tb* dissemination in latently infected monkeys as the co-infected group had shorter survival time and higher *M.tb* loads in multi-organs (Mehra et al, 2011). By using confocal microscopy, they also found *M.tb* and SIV can be in the same cells; numerous SIV-positive cells were located in the vicinity of *M.tb* infected cells (Mehra et al, 2011). This study suggests that rhesus macaques serve as an Excellent model to study the phenomena of *M.tb* latency and reactivation.

Although HIV patients are highly susceptible to *M.tb,* even with well-preserved CD4 T cell levels, the risk of *M.tb* infection increases as CD4 T cell levels decrease (Lawn et al, 2006; Mtei et al, 2005). CD4 T cells are still important in the protection against *M.tb*. CD4-depleted monkeys significantly had more severe gross pathology, bacterial burden, and dissemination of bacteria than the control animals (Lin et al, 2012). Out of six latently infected macaques treated with neutralizing antibody to CD4, three had clinical signs of reactivation, although *M.tb*-specific production of IFN-γ was similar to the latently infected control between CD4-depleted reactivators and nonreactivators (Lin et al, 2012). In contrast, SIV infection of latently-infected macaques led to reactivation in all the co-infected animals (Diedrich et al, 2010). CD4 T cells are important for the control of *M.tb* infection, but it is not the only immune factor impaired by HIV. HIV still has other ways to contribute to enhanced susceptibility of the host to *M.tb* infection.

Conclusion

The global HIV epidemic is driving the re-emergence of *M.tb* infection, resulting in an increasingly prevalent TB epidemic across the globe. The significant impact of both HIV and TB on socioeconomic and public health underscore the need for the development of new preventives and therapeutics, which require appropriate animal models for the evaluation. Fortunately, macaques have been recognized as a suitable animal model for both SIV and *M.tb* infection. The data generated from the literature clearly indicate that macaques are appropriate for the study of *M.tb*/HIV co-infection. However, more investigations on testing newly-developed TB or HIV vaccines in co-infected macaque model are very much needed. Such future studies should further prove that macaque model is a valuable animal model for *M.tb* and/or HIV research.

Acknowledgement: We would like to thank Dr. Juliet Peña for her editorial support during the preparation of this manuscript.

References

Baenziger S, Tussiwand R, Schlaepfer E, Mazzucchelli L, Heikenwalder M, Kurrer MO, Behnke S, Frey J, Oxenius A, Joller H, Aguzzi A, Speck RF. 2006. Disseminated and sustained HIV infection in $CD34^+$ cord blood cell-transplanted $Rag2^{-/-}\gamma c^{-/-}$ mice. *Proceedings of the National Academy of Sciences of the United States of America,* **103**(43): 15951-15956.

Calderon VE, Valbuena G, Goez Y, Judy BM, Huante MB, Sutjita P, Johnston RK, Estes DM, Hunter RL, Actor JK, Cirillo JD, Endsley JJ. 2013. A humanized mouse model of tuberculosis. *PLoS One,* **8**(5): e63331.

Cheynier R, Gratton S, Halloran M, Stahmer I, Letvin NL, Wain-Hobson S. 1998. Antigenic stimulation by BCG vaccine as an in vivo driving force for SIV replication and dissemination. *Nature Medicine,* **4**(4): 421-427.

Croix DA, Capuano S, Simpson L, Fallert BA, Fuller CL, Klein EC, Reinhart TA, Murphey-Corb M, Flynn JL. 2000. Effect of mycobacterial infection on virus loads and disease progression in simian immunodeficiency virus-infected rhesus monkeys. *AIDS Research and Human Retroviruses,* **16**(17): 1895-1908.

Daley CL, Small PM, Schecter GF, Schoolnik GK, Mcadam RA, Jacobs Jr WR, Hopewell PC. 1992. An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus-an analysis using restriction-fragment-length polymorphisms. *New England Journal of Medicine,* **326**(4): 231-235.

Denton PW, Krisko JF, Powell DA, Mathias M, Kwak YT, Martinez-Torres F, Zou W, Payne DA, Estes JD, Garcia JV. 2010. Systemic administration of antiretrovirals prior to exposure prevents rectal and intravenous HIV-1 transmission in humanized BLT mice. *PLoS One,* **5**(1): e8829.

Diedrich CR, Mattila JT, Klein E, Janssen C, Phuah J, Sturgeon TJ, Montelaro RC, Lin PL, Flynn JL. 2010. Reactivation of latent tuberculosis in cynomolgus macaques infected with SIV is associated with early peripheral T cell depletion and not virus load. *PLoS One,* **5**(3): e9611.

Gorantla S, Makarov E, Finke-Dwyer J, Gebhart CL, Domm W, Dewhurst S, Gendelman HE, Poluektova LY. 2010. $CD8^+$ cell depletion accelerates HIV-1 immunopathology in humanized mice. *The Journal of Immunology,* **184**(12): 7082-7091.

Gormus BJ, Blanchard JL, Alvarez XH, Didier PJ. 2004. Evidence for a rhesus monkey model of asymptomatic tuberculosis. *Journal of Medical Primatology,* **33**(3): 134-145.

Hanson DL, Chu SY, Farizo KM, Ward JW. 1995. Distribution of $CD4^+$ T lymphocytes at diagnosis of acquired immunodeficiency syndrome-defining and other human immunodeficiency virus-related illnesses. *Archives of Internal Medicine,* **155**(14): 1537.

Heuts F, Gavier-Widén D, Carow B, Juarez J, Wigzell H, Rottenberg ME. 2013. $CD4^+$ cell-dependent granuloma formation in humanized mice infected with mycobacteria. *Proceedings of the National Academy of Sciences of the United State of America,* **110**(16): 6482-6487.

Joseph A, Zheng JH, Chen K, Dutta M, Chen C, Stiegler G, Kunert R, Follenzi A, Goldstein H. 2010. Inhibition of in vivo HIV infection in humanized mice by gene therapy of human hematopoietic stem cells with a lentiviral vector encoding a broadly neutralizing anti-HIV antibody. *Journal of Virology,* **84**(13): 6645-6653.

Kumar P, Ban HS, Kim SS, Wu H, Pearson T, Greiner DL, Laouar A, Yao J, Haridas V, Habiro K, Yang YG, Jeong JH, Lee KY, Kim YH, Kim SW, Peipp M, Fey GH, Manjunath N, Shultz LD, Lee SK, Shankar P. 2008. T cell-specific siRNA delivery suppresses HIV-1 infection in humanized mice. *Cell,* **134**(4): 577-586.

Lawn SD, Bekker L-G, Middelkoop K, Myer L, Wood R. 2006. Impact of HIV infection on the epidemiology of tuberculosis in a peri-urban community in South Africa: The need for age-specific interventions. *Clinical Infectious Diseases,* **42**(7): 1040-1047.

Lei AH, Pang W, Zhang GH, Zheng YT. 2013. Use and research of pigtailed macaque in nonhuman primate HIV/AIDS models. *Zoological Research,* **34**(2): 77-88.

Lin PL, Rutledge T, Green AM, Bigbee M, Fuhrman C, Klein E, Flynn JL. 2012. CD4 T cell depletion exacerbates acute *Mycobacterium tuberculosis* while reactivation of latent infection is dependent on severity of tissue depletion in cynomolgus macaques. *AIDS Research and Human Retroviruses,* **28**(12): 1693-1702.

Mansfield KG, Pauley D, Young HL, Lackner AA. 1995. *Mycobacterium avium complex* in macaques with AIDS is associated with a specific strain of simian immunodeficiency virus and prolonged survival after primary infection. *Journal of Infectious Diseases,* **172**(4): 1149-1152.

Mansfield KG, Veazey RS, Hancock A, Carville A, Elliott M, Lin KC, Lackner AA. 2001. Induction of disseminated *Mycobacterium avium* in simian AIDS is dependent upon simian immunodeficiency virus strain and defective granuloma formation. *The American Journal of Pathology,* **159**(2): 693-702.

Maslow JN, Brar I, Smith G, Newman GW, Mehta R, Thornton C, Didier P. 2003. Latent infection as a source of disseminated disease caused by organisms of the *Mycobacterium avium* complex in simian immunodeficiency virus-infected rhesus macaques. *Journal of Infectious Diseases,* **187**(11): 1748-1755.

Mattila JT, Diedrich CR, Lin PL, Phuah J, Flynn JL. 2011. Simian immunodeficiency virus-induced changes in T cell cytokine responses in cynomolgus macaques with latent *Mycobacterium tuberculosis* infection are associated with timing of reactivation. *Journal of Immunology*, **186**(6): 3527-3537.

Mehra S, Golden NA, Dutta NK, Midkiff CC, Alvarez X, Doyle LA, Asher M, Russell-Lodrigue K, Monjure C, Roy CJ, Blanchard JL, Didier PJ, Veazey RS, Lackner AA, Kaushal D. 2011. Reactivation of latent tuberculosis in rhesus macaques by coinfection with simian immunodeficiency virus. *Journal of Medical Primatology,* **40**(4): 233-243.

Mtei L, Matee M, Herfort O, Bakari M, Horsburgh CR, Waddell R, Cole BF, Vuola JM, Tvaroha S, Kreiswirth B, Pallangyo K, Von Reyn CF. 2005. High rates of clinical and subclinical tuberculosis among HIV-infected ambulatory subjects in Tanzania. *Clinical Infectious Diseases,* **40**(10): 1500-1507.

Mukadi Y, Perriens J, St Louis M, Brown C, Ryder R, Portaels F, Piot P, Willame J, Prignot J, Pouthier F, Kaboto M. 1993. Spectrum of immunodeficiency in HIV-1-infected patients with pulmonary

tuberculosis in Zaire. *Lancet,* **342**(8864): 143-146.

Newman GW, Adams DL, Maslow JN, Murphy-Corb M, Didier PJ. 1999. Differential tumor necrosis factor α production in simian immunodeficiency virus-infected rhesus macaques coinfected with *Mycobacterium avium*. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America,* **28**(3): 514-519.

Nuttall JJC, Eley BS. 2011. BCG vaccination in HIV-infected children. *Tuberculosis Research and Treatment,* 2011: Article ID 712736, 10.1155/2011/712736.

Post FA, Wood R, Pillay GP. 1995. Pulmonary tuberculosis in HIV infection: Radiographic appearance is related to $CD4^+$ T-lymphocyte count. *Tubercle and Lung Disease,* **76**(6): 518-521.

Safi H, Gormus BJ, Didier PJ, Blanchard JL, Lakey DL, Martin LN, Murphey-Corb M, Vankayalapati R, Barnes PF. 2003. Spectrum of manifestations of *Mycobacterium tuberculosis* infection in primates infected with SIV. *AIDS Res Hum Retroviruses,* **19**(7): 585-595.

Scanga CA, Bafica A, Sher A. 2007. Viral gene expression in HIV transgenic mice is activated by *Mycobacterium tuberculosis* and suppressed after antimycobacterial chemotherapy. *Journal of Infectious Diseases,* **195**(2): 246-254.

Shen L, Shen Y, Huang D, Qiu L, Sehgal P, Du GZ, Miller MD, Letvin NL, Chen ZW. 2004a. Development of $V\gamma2V\delta2^+$ T cell responses during active mycobacterial coinfection of simian immunodeficiency virus-infected macaques requires control of viral infection and immune competence of $CD4^+$ T cells. *Journal of Infectious Diseases,* **190**(8): 1438-1447.

Shen Y, Shen L, Sehgal P, Huang D, Qiu L, Du G, Letvin NL, Chen ZW. 2004b. Clinical latency and reactivation of AIDS-related mycobacterial infections. *Journal of Virology,* **78**(24): 14023-14032.

Shen Y, Shen L, Sehgal P, Zhou DJ, Simon M, Miller M, Enimi EA, Henckler B, Chalifoux L, Sehgal N, Gastron M, Letvin NL, Chen ZW. 2001. Antiretroviral agents restore Mycobacterium-specific T-cell immune responses and facilitate controlling a fatal tuberculosis-like disease in macaques coinfected with simian immunodeficiency virus and *Mycobacterium bovis* BCG. *Journal of Virology,* **75**(18): 8690-8696.

Shen Y, Zhou DJ, Chalifoux L, Shen L, Simon M, Zeng XJ, Lai XM, Li YY, Sehgal P, Letvin NL, Chen ZW. 2002a. Induction of an AIDS virus-related tuberculosis-like disease in macaques: a model of simian immunodeficiency virus- mycobacterium coinfection. *Infection and Immunity*, **70**(2): 869-877.

Shen Y, Zhou DJ, Qiu L, Lai XM, Simon M, Shen L, Kou Z, Wang Q, Jiang L, Estep J, Hunt R, Clagett M, Sehgal PK, Li YY, Zeng XJ, Morita CT, Brenner MB, Letvin NL, Chen ZW. 2002b. Adaptive immune response of $V\gamma2V\delta2^+$ T cells during mycobacterial infections. *Science,* **295**(5563): 2255-2258.

Sun ZF, Denton PW, Estes JD, Othieno FA, Wei BL, Wege AK, Melkus MW, Padgett-Thomas A, Zupancic M, Haase AT. 2007. Intrarectal transmission, systemic infection, and $CD4^+$ T cell depletion in humanized mice infected with HIV-1. *The Journal of Experimental Medicine*, **204**(4): 705-714.

Traggiai E, Chicha L, Mazzucchelli L, Bronz L, Piffaretti JC, Lanzavecchia A, Manz MG. 2004. Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science,* **304**(5667): 104-107.

UNAIDS. 2013. Global report: UNAIDS Report on the Global AIDS Epidemic 2013. Geneva: WHO.

WHO. 2013. Global Tuberculosis Report 2013. WHO, Geneva, Switzerland. WHO/HTM/TB.

Xia HJ, Zhang GH, Zheng YT. 2010. Roles of dendritic cell in disease progression of AIDS primate models. *Zoological Research,* **31**(1)**:** 57-65.

Ye RG, Lu ZY. 2004. Internal Medicine. 6th ed. Beijing: Peoples Medical Publishing House.

Zhang GH, Li MH, Zheng YT. 2007a. Application of AIDS macaques animal model in HIV vaccine research. *Zoological Research,* **28**(5): 556-562.

Zhang LG, Kovalev GI, Su LS. 2007b. HIV-1 infection and pathogenesis in a novel humanized mouse model. *Blood,* **109**(7): 2978-2981.

Zhou DJ, Lai XM, Shen Y, Sehgal P, Shen L, Simon M, Qiu LY, Huang D, Du GZ, Wang QF, Letvin NL, Chen ZW. 2003. Inhibition of a $V\gamma2V\delta2^+$ T-cell responses during active mycobacterial coinfection of simian immunodeficiency virus SIVmac-infected monkeys. *Journal of Virology,* **77**(5): 2998-3006.

Zhou DJ, Shen Y, Chalifoux L, Lee-Parritz D, Simon M, Sehgal PK, Zheng L, Halloran M, Chen ZW. 1999. *Mycobacterium bovis* bacille Calmette-Guérin enhances pathogenicity of simian immunodeficiency virus infection and accelerates progression to AIDS in macaques: A role of persistent T cell activation in AIDS pathogenesis. *The Journal of Immunology,* **162**(4): 2204-2216.

Zhou Y, Bao R, Haigwood N, Persidsky Y, Ho W Z. 2013. SIV infection of rhesus macaques of Chinese origin: A suitable model for HIV infection in humans. *Retrovirology,* **10**(1): 89.