Chapter 17
Humanized Mouse Versus Non-human Primate Models of HIV-1 Infection

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17.1 Introduction

Animal models are critical for biomedical research, in particular for human immunodeficiency virus type one (HIV-1), since HIV-1 infection still remains a major burden to global public health. As for 2011, it is estimated that 34 million people are living with HIV-1, 2.5 million people are newly infected, and only a small portion (8 million) of infected people are currently receiving the combined antiretroviral therapy, which is expensive and has to be lifelong [1] (https://www.unaids.org/en/resources/publications/2013/). This dire reality further highlights the importance of animal models in developing vaccine to prevent HIV infection and testing new approaches to purge latently infected reservoir in order to cure HIV infection. An ideal animal model should be able to model HIV-1 transmission, pathogenesis, evaluate the efficacy of antiretroviral agents, immune modulators, and vaccines in preventing, treating, and curing HIV-1 infection in humans. Unfortunately, the universal and ideal model does not exist. Instead, different animal models are often used independently or in combination, of which non-human primates (NHPs) and humanized mice (hu-mice) are the two available models.

In this chapter, we: (1) compare and contrast the pros and cons of NHP and humouse models of HIV-1 infection of humans in general; (2) discuss in detail which model is more relevant in studying HIV-1 transmission and vaccine; and (3) discuss what aspects of these models need to be further improved in order to meet the HIV-1 research need. Since there are many different variables in both models, such as different types of macaques and hu-mice, different types of simian immunodeficiency

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viruses (SIVs) and HIV-1 viruses, different routes and dose of virus infection, we can only compare the best available representatives of NHP and hu-mouse models.

17.2 The Current Status of NHP Models of HIV-1 Infection

More than 30 African NHP species are naturally infected with more than 40 different strains of SIVs [2, 3]; African NHPs have coexisted with SIVs for more than 32,000 years [4] and host the immediate ancestral virus of HIV-1 [5–7] and HIV-2 [8], but infected animals generally do not develop the AIDS-like disease even in the face of a chronic infection with high level of replicating virus [9].

The common chimpanzees in West Central Africa (*Pan troglodytes troglodytes*) are endemically infected with SIVcpzPtt and are the zoonotic source of pandemic HIV-1 group M and non-pandemic group N; Eastern chimpanzees in East Africa (*Pan troglodytes schweinfurthii*) are infected with SIVcpzPts, but this virus has not yet been found in humans [5, 7, 10–13]. Gorillas (*Gorilla gorilla gorilla*) are infected with gorilla SIV (SIVgor) and are the zoonotic source of HIV-1 group P [14, 15]. The simian zoonotic source of HIV-1 group O remains to be identified [15]. Although new data indicate that SIVcpz infections of chimpanzees had negative effects on their health, reproduction, and lifespan [16], the clinical course is still different from HIV-1 infection of humans. For ethical reasons and none/low pathogenic infection of SIV, the endangered species of chimpanzees are not feasible to be used as a model for HIV-1 research [17].

Sooty Mangabeys (*Cercocebus atys*) of African origin are the primate reservoir for HIV-2 [8] and the immediately ancestral virus of SIVmac transmitted to Asian macaques in captivity [18]. Sooty Mangabeys and African green monkeys (genus *Chlorocebus*) do not develop disease with high levels of SIV replication and are mainly used to study the mechanisms of non-pathogenic SIV infection [9, 19–22].

Asian NHPs of macaques, including rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*M. fascicularis*), and pigtailed macaques (*M. nemestrina*) are non-natural hosts to SIVs and develop AIDS-like diseases after infection, of which rhesus macaque has been most widely used in HIV-1 research. Asian NHPs of macaques are regarded as a good model of HIV-1 infection of humans because of the following characteristics: (1) the proximity of macaques to humans, genetically, anatomically, and physiologically [23]; (2) the clinical manifestations and pathogenesis of macaques infected with SIV are similar to humans' infection by HIV-1 [18, 22, 24]; (3) the innate and adaptive immune responses ($CD8^+$ T [25, 26–28] and B cells [29–31]) of macaques to SIV infection are similar to humans' responses to HIV-1. Hence, this model has been widely used for transmission, immunopathogenesis, immune correlates of protection and vaccine efficacy studies, and has gained tremendous insights into the mechanisms of transmission and pathogenesis, and immune correlates of protection. However, this model also has several limitations: (1) macaques are not susceptible to HIV-1 infection; instead only to

SIV or related chimeric virus, expressing HIV-1 envelope (Env-SHIV or SHIV) or reverse-transcriptase (RT-SHIV) in SIV backbone. Although recently, it was reported that pig-tail macaques can support simian-tropic HIV-1 strains that encode only SIV vif protein (stHIV-1) replication [32], however, the virus replication lasted only for several months and its biological relevance to HIV-1 infection remained to be determined; (2) SIV viruses are naturally resistant to many FDA approved anti-HIV-1 drugs, including non-nucleoside reverse transcriptase inhibitors (NNRTIs), some entry inhibitors, and some proteinase inhibitors [33–35]. Although RT-SHIV and Env-SHIV can partly offset this drawback, many preventive/therapeutic regimens used in clinic cannot be studied in this model and vice versa; (3) SIV differs from HIV-1 genotypically and phenotypically, the vaccines designed and tested in this model using SIV or SHIV cannot be directly applied into human clinical trial; (4) only a limited number of SIV viruses are available for macaque studies. Which SIV challenge virus should be used in vaccine protective studies is still being debated [36], since many commonly used SIV challenge viruses in vaccine protective studies have different sensitivity to antibody neutralization and cytotoxic T lymphocytes (CTL)-mediated control. For example, SIVmnE660 can be neutralized more easily than SIVmac251 or SIVmac239 [30, 37], and SHIV89.6P can be controlled by CTL more easily [38]. Thus, the results with uncertain challenge viruses could be either underestimating or exaggerating the protective effect [38], and there are renewed efforts generating better challenge viruses [39, 40]; and (5) macaques and humans are genetically different, especially in major histocompatibility complex (MHC) and T cell receptors (TCR) which are more complex in the macaque species [41–43]. Thus, alternative models are sought to overcome the limitations of the macaque/SIV model. The hu-mice , especially the new generation of hu-mice, has emerged as a good alternative system to study HIV in addition to NHP (Fig. 17.1).

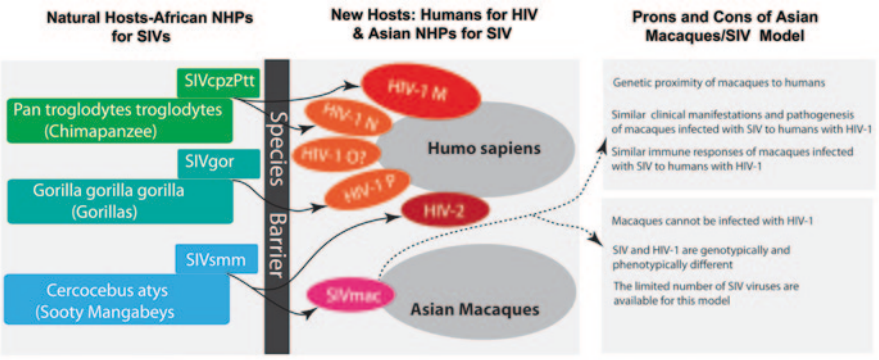

Fig. 17.1 The pros and cons of SIV/macaque model of HIV-1 infection. African NHPs are the natural host of SIVs and generally do not develop AIDS-like disease. The pandemic HIV-1 group M (HIV-1 M) and non-pandemic group N (HIV-1 N) are originated from SIVcpzPtt in Chimpanzees, and non-pandemic HIV-1 P is originated from SIVgov in Gorillas. HIV-2 and SIVmac originated from SIVsmm in sooty Mangabeys. SIVmac infects Asian macaques and cause simian AIDS

17.3 The Current Status of hu-mouse Models of HIV-1 Infection

The major driving force for developing hu-mice is to interpose an in vivo model between in vitro and clinical trials for studying human diseases, since the findings of in vitro experiments cannot be directly tested in human clinical trials due to ethical reasons. Furthermore, macaques are not susceptible to HIV-1 infection, therefore, SIV/macaque always requires a two-stage design and testing in order to move into clinical trials. For example, vectors and immunogen of SIV vaccines tested in the macaque model have to be redesigned into the human version for clinical trials. The hu-mouse model has a potential to serve as an alternative to complement the SIV/macaque model for vaccine studies.

The hu-mice are a heterochimera of the human immune system in the murine body in a delicate balance to avoid human graft versus murine host disease (GVHD) and murine host versus human graft disease (HVGD) while reconstituting the human system. In the past 25 years, this model has gone through several rounds of revolution primarily through two approaches. First, by genetically modifying the mouse to further eliminate murine immune cells and their functions in order to prevent HVGD; and second, by refining the procedures of implantation of human tissues and/or hematopoietic stem cells (HSC) in order to prevent GVHD, to attain a new level of human immune reconstitution in the lymphatic and non-lymphatic tissues, including mucosa. The new generation of hu-mice has drastically expanded its utility and has great potential in studying HIV mucosal transmission, pathogenesis, latency, pre-exposure prophylaxis (PrEP), treatment, and vaccine.

The history of hu-mouse model has been extensively reviewed elsewhere [44–46]; here, we will only highlight the major events in order to better compare NHP and hu-mouse models. The initial two independent groups conducted proof-of-concept experiments in 1988 generating hu-mice by two different approaches [47, 48] based on SCID mice [49]. The seminal paper by McCune [47] laid the conceptual and technical foundation for subsequent stable long-term reconstitution of multilineage human immune cells through implanting human fetal thymus and liver tissue fragments under mouse renal capsule (Thy/Liv SCID-hu mice) [50, 51]. Using this first generation of hu-mouse (thy/Liv SCID-hu), some key HIV pathogenesis and treatment questions were studied [52–55]. Meanwhile, Mosier group generated hu-PBL-SCID mice by transferring peripheral blood leukocyte (PBL) to SCID mice [48]. Although the human immune reconstitution is limited and unstable [50, 56, 57] in the hu-PBL-SCID mice, subsequent replacement of PBL with HSC implantation improved the human immune reconstitution [58, 59]

To further eliminate murine NK cells and reduce the “leakiness” of murine functional lymphocytes in some SCID mice, NOD/SCID mice were generated in 1995 [60] by backcrossing SCID and NOD mice, since NOD mice have defects in NK cells, myeloid development and function, and complement pathways [61, 62]. The engraftment of human $CD45^+$ cells in NOD/SCID mice has dramatically increased as compared to the SCID mouse recipients [60, 63]. In addition, to further improve the SCID mouse, RAG-1 [64] and RAG-2 (recombination-activating proteins) [65] deficient mice with

no mature T and B cells were generated in 1992. Additionally, the mice with homozygous cytokine common receptor gamma chain mutant, a component of receptors for cytokine IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 [66–70], were generated (cγ−/−) [68, 69] in 1995. These mice have defects in T and B cells and absence of natural killer cell (NK) activity. With the crossbreeding of different immune deficiency mice above, more severely combined immune deficiency (current generation) mice were generated. These include the BRG (BALB/c_ RAG2−/− cγ−/−) [71, 72] and B6RG (C57BL6_ RAG2−/− cγ−/−) [73], NOG (NOD/Shi-scid *I* cγ−/−) [74], NSG (NOD/LtSzscid *I* cγ−/−) [75], and NOD-RAG1−/− cγ−/− mice [76].

Based on the current generation of immune deficient mice, two general types of hu-mice are commonly generated for biomedical research. First is hu-BLT (bone marrow, liver, and thymus) mice [77] generated through sub-lethal irradiation, implantation of human fetal liver and thymus pieces into the adult mouse left renal capsule, and injection of autologous $CD34^+$ HSC intravenously [77, 78]. Hu-BLT mice are a new generation of hu-mice with a long-term and multi-lineage reconstitution of human hematopoietic system (T, B, NK, DC, and Macrophages) in both lymphatic and mucosal tissues, and can elicit antigen-specific T cell and humoral responses [77, 79–83]. The hu-BLT mouse became the best hu-mouse model for studying HIV-1 mucosal transmission and its prevention, because there is a good immune reconstitution in mucosa, and the T cells can be educated in autologous human thymic tissues [77, 79–81]. Second is the hu-HSC mice generated by sub-lethal irradiation and injection of human $CD34^+$ HSC isolated from fetal liver, umbilical cord blood, or mobilized peripheral blood leukocytes with granulocyte colony stimulating factor (G-CSF) into the new generation of immune deficiency mice [74, 75, 84–87]. It is apparent that injection (intra-hepatic or intra-cardiac) of $CD34^+$ HSC into neonates of the current generation of immune deficient mice leads to much better de novo development of adaptive immune system (B, T, DC, and structured lymphatic organ) as compared with adult recipients [72, 85].

To further improve the human immune responses of the current generation of hu-mice, the human cytokines and growth factors cytokines (GM-CSF [88–90], IL-3 [88, 90], IL4 [88, 89], and IL-15 [91]) and MHC class I (HLA-A*0201 [92–94], HLA-B*51:01 [95]) and II (HLA-DRA and HLA-DRB1:0405 [96], HLA-DR4 [97], HLA-DR1 [93]) or in combination [90] were provided by transgenic, knock-in, vector expression, or hydrodynamic injection (Fig. 17.2). The current generation of hu-mice has increasingly been used in HIV-1 research, because of the following reasons: (1) besides chimpanzees, it is the only model that can directly study HIV-1 infection; (2) it reconstitutes most of human immune system functionally and structurally, thus it can recapitulate many aspects of HIV–host interaction, including $CD4^+$ T-cell depletion, increased $CD4^+$ and $CD8^+$ T-cell turnover, and immune activation [82, 98]; (3) it can be used to study HIV-1 mucosal transmission [79, 99], pathogenesis [88, 98, 100–102], prevention [103, 104], treatment [105–107], and latency [108]; and (4) it is much cheaper than NHP macaque. However, there are several limitations as well: (1) hu-mice are a chimera of human and mouse cells and tissues. Although human immune system is partly reconstituted, the non-lymphatic cells and tissues remain as murine; (2) there is a delay of humoral (3 month PI) and

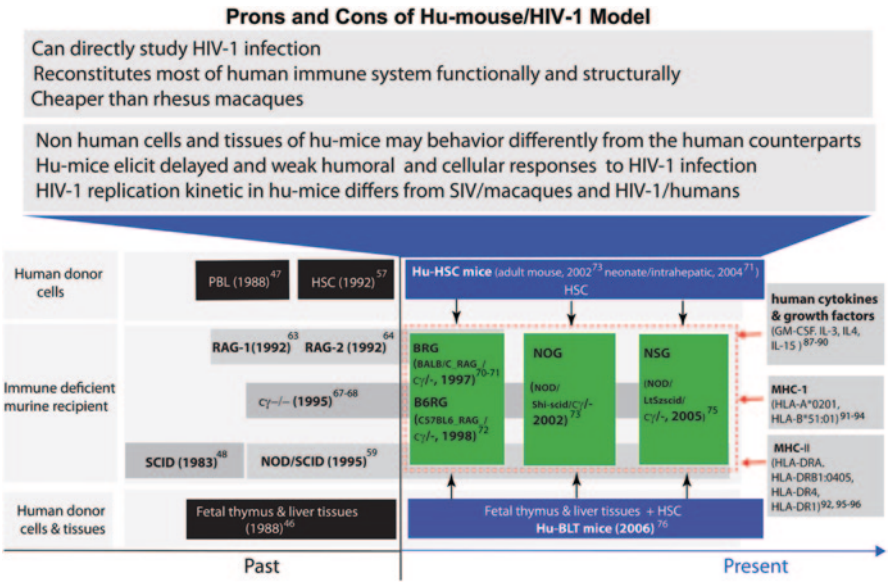

Fig. 17.2 The pros and cons of current hu-mouse model of HIV-1 infection. The two types of new generation of hu-mice: hu-BLT and hu-HSC are developed by improving the implantation method of human cell and tissues and genetically refining the immune deficiency of recipient mouse. Recently, human cytokines, growth factors, and MHC class I and II transgenic mice have further improved the human immune function of current hu-mice

cellular responses (9 weeks PI) in HIV-1-infected hu-BLT mice as compared with SIV/macaque and humans (2 weeks PI) [82]. The adaptive immune responses in hu-mice, even hu-BLT mice, therefore need to be further improved, especially IgG response [82, 87, 109]; (3) the HIV-1 replication kinetics in hu-mice is different from SIV/macaques and HIV-1/humans. In the hu-mice, the virus peaks around 2–3 weeks post infection, but is maintained for several weeks before declining [82], reflecting that there is a delay of the host control of HIV-1 replication.

17.4 Mucosal Transmission of HIV-1 and Its Prevention

HIV-1 is mainly transmitted through mucosal surfaces, such as cervicovagina, foreskin, and anorectum. Better understanding of the early events of HIV-1 mucosal transmission and their underlying mechanisms holds the keys to the better designed microbicide and vaccine. The key body of knowledge on the early events in mucosal transmission of HIV-1 was mainly acquired from the macaque/SIV model, of which atraumatic high-dose or repeated low-dose inoculations of cell-free viruses are often used. For example, in the early vaginal transmission, there is a small infected

founder cell population at the portal of entry before systemic virus dissemination [110, 111]; there is a genetic bottleneck as revealed by using single genome amplification in vaginal [112], rectal [113, 114], and penile [115] transmission. Only recently, the infections of macaques, vaginally [116] and rectally, [117] with cell-associated SIV were reported; surprisingly, cell-associated virus that transmits infection across the mucosa was found to be more efficient than cell-free virus [117]. Of cervicovaginal, foreskin, and anorectal routes in SIV/macaque model, anorectal mucosa is the easiest route for transmission, followed by vaginal and penile [115, 118]. Although macaque penile transmission was reported previously, this route of transmission model has been used only very recently [115, 119–121].

In contrast to the long history of the use of macaque/SIV as a model for studying mucosal transmission of HIV-1, hu-mice have been used only recently, since the current generation hu-mice were available, specifically after the hu-BLT mice were developed. However, due to their advantages in being susceptible to HIV-1 infection, cheaper, and easier to manipulate than macaque, the current generation hu-mice are increasingly used in mucosal transmission and prevention studies. This model is especially useful to test microbicide in preventing mucosal transmission of HIV-1 [80, 104, 122]. Except for penile transmission, vaginal [80, 123, 124] and rectal [79, 122, 124] transmission of HIV-1 have both been reported.

17.5 Vaccine

The goal of vaccine development is to elicit protective memory immunity against infection, disease, and death [125, 126]. Macaque-SIV/SHIV model is still the best available model to identify the immune correlates of protection and evaluate vaccine efficacy, since hu-mice have delayed adaptive immune responses, especially very limited IgG response [82, 87, 109]. Currently, human cytokines, growth factors, and MHC class I and II transgenic NSG or NOG mice are generated which may improve this model for vaccine study. Conversely, the current generation of hu-mice is exceptionally useful in testing new preventive and therapeutic strategies, such as human broadly neutralizing antibodies [105], antibody-expressing vector [127], and engineering HIV-1 resistant cells [106, 128]. Its usefulness as a model for testing of HIV-1 vaccines remains to be determined.

17.6 Summary and Prospective

SIV/macaques model has been widely used for HIV-1 research since the middle 1980s and has provided critical insights into the HIV-1 transmission, pathogenesis, treatment, latency, microbicide, and vaccine. However, macaques are genetically distinct from humans, especially in MHC class I and TCR, and are not susceptible to HIV-1 infection. Thus, results derived from this model may not be directly

translatable into human clinical trials; for example, vaccines designed and tested in this model using SIV or SHIV have to be redesigned in order to be tested in human clinical trial. The new generation of hu-HSC and hu-BLT mice, especially the hu-BLT mice with transgenic expression of human cytokines, growth factors, and MHC class I and II, offers a new opportunity to study HIV-1 infection using HIV-1 directly. Although there is still room to improve the humoral and cellular immune responses of hu-mice to HIV-1 infection [44, 46, 100, 129, 130], this model already recapitulates many key aspects of mucosal transmission [79, 99], prevention [103, 104], immunopathogenesis [88, 98, 100–102], treatment [105–107], and latency [108]. The new generation of hu-mouse and SIV/macaque models are complementary and together they will overcome the idea that "mice lie and monkeys exaggerate" [131].

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