Chapter 23
Role of Toll-Like Receptor (TLR) Signaling in HIV-1-Induced Adaptive Immune Activation

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Abbreviations

DC	Dendritic cells
EBV	Epstein Barr virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
huCD34$^+$	Human CD34$^+$ hematopoietic cells
mDC	Myeloid dendritic cells
NK	Natural killer
NSG	NOD/SCID/IL-2rγ$^{-/-}$
pDC	Plasmacytoid dendritic cells
TLR	Toll-like receptor

23.1 Immune Activation in HIV-1 Infection—Pathogenesis and Priming

Human immunodeficiency virus (HIV)-1 infection induces activation of the innate and adaptive immune responses, which can mediate both beneficial viral control and detrimental immune pathology. At first encounter with the virus, innate immunity

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can contribute to the initial restriction of viral replication and plays an important role in the activation of the adaptive immune response. Despite these initial benefits of activating the immune response, the level of ongoing $CD8^+$ T cell activation during chronic HIV-1 infection has been revealed to be a better predictor of HIV-1 disease progression than HIV-1 viral load alone [1]. Furthermore, T cell activation has also been associated with slower $CD4^+$ T cell recovery and enhanced $CD4^+$ T cell apoptosis even when HIV-1 viral load is suppressed by antiretroviral therapy [2, 3]. This elevated immune activation in chronic HIV-1 infection can be driven directly by HIV-1 replication and indirectly through changes in the microbiome, microbial translocation, coinfections, immune deregulation, and lymphoid tissue fibrosis [4–7]. The mechanisms that drive chronic immune activation in HIV-1, therefore, need to be examined and targeted to improve disease management in HIV-1-infected individuals. Examination of HIV-1 has largely been limited to in vitro work with human samples or to studies in nonhuman primate models. The development of a small animal model will allow for easier dissection of the individual mechanisms contributing to HIV-1-associated immune activation, in part through the possibility to perform interventional studies blocking and enhancing individual pathways.

23.2 Innate Immune System in Humanized Mouse Model

The use of humanized mouse model in examining human immune responses has been carried out in cancer, autoimmunity, viral infections, and vaccinations [8, 9]. The advantage of this model is that it allows for the in vivo manipulation of human immune response through the depletion of specific cell populations [10], administration of inhibiting and activating compounds [10–14] and inoculation with infectious agents [11, 12, 15]. Over 20+ years, the humanized mouse model itself has been modified and adapted, and several different models are now available. The dominant mouse strains recently used for the humanized mouse model can include NOD/SCID[16, 18], NOD/SCID/$\beta 2m^{-/-}$ [18], and NOD/SCID/IL-$2r\gamma^{-/-}$ (NSG) mice [19, 20], as well as other mouse strains deficient in both Rag2 and IL-2rγ [21, 22]. Furthermore, the method of "humanizing" the mice with a human immune system can also vary and include adoptive transfer of human PBMC (Hu-PBL mice) [23], human $CD34^+$ hematopoietic cells ($huCD34^+$) isolated from cord blood [21] or fetal liver tissue (Hu-HSC mice) [22], co-engraftment with human fetal thymus and liver (SCID-Hu mice) [24], and a combination of human fetal thymus and liver engraftment with injection of $huCD34^+$ cells (BLT mice) [11, 25]. The reconstituted human immune system can be further enhanced by the administration of cytokines or cytokine-expressing plasmids [26].

These variations of the mouse models result in differing human immune system developments. Those mice with the NOD/SCID-based backgrounds and $Rag2^{-/-}$/IL-$2r\gamma^{-/-}$-based backgrounds have different reconstitution of immune cells at different tissue sights [27, 28]. However, further enhancements can be used to assist the development of the human immune system and overcome some of the defects

in each model. In both the NOD/SCID/β2m$^{-/-}$ and NSG models, human T cells, B cells and antibodies can successfully develop [29]. Human fetal thymus and liver engraftment allowed for development of the major human T cell subsets and can be improved by the administration of huCD34^{+} cells (BLT mouse model), which also have better development of B cells and monocytes [30, 31]. The presence of dendritic cells (DC)s in different humanized mouse models has been demonstrated in bone marrow, blood, and spleen [10]. HuCD34^{+}-transplanted NSG and NOD/SCID/β2m$^{-/-}$ mice develop myeloid dendritic cells (mDCs) [10, 13], plasmacytoid dendritic cells (pDCs) [10, 32], and natural killer (NK) cells [13]. DCs and monocyte development in NSG mice reconstituted with huCD34^{+} cells can further be enhanced by the administration of GM-CSF and IL-4 [33]. Presence of GM-CSF and IL-4 can also help the development of human T cells, B cells, as well as IgG antibody production [33]. Using mice with the NOD/SCID/β2m$^{-/-}$ or NSG backgrounds also helps to reduce murine NK cell development as compared to NOD/SCID models but unfortunately also result in poor human NK cell reconstitution [34–36]. Administration of human IL-15 and Flt-3 via DNA vectors or recombinant proteins assists in the development of human NK cells in both NOD/SCID, NSG and Balb/c/Rag2$^{-/-}$/IL-2rγ$^{-/-}$ mouse models reconstituted with huCD34^{+} cells [26, 34, 37]. Furthermore, the manipulation of the genetic background of the mouse can also be used to enhance development of specific human cell subsets. Knock-ins of human IL-3 and GM-CSF in Rag2$^{-/-}$/IL-2rγ$^{-/-}$ mice and CSF1 in Balb/c/Rag2$^{-/-}$/IL-2rγ$^{-/-}$ mice allowed for better macrophage reconstitution following administration with huCD34^{+} cells [38, 39]. Overall, the genetic background of mice, tissue used for human reconstitution, and any subsequent cytokine treatment can all vary between different humanized mouse models described, each with its strengths and weaknesses. The specific humanized mouse model to be used should, therefore, be carefully chosen and depends on the experimental question and the specific immune cell population of interest.

The various improvements to the humanized mouse model allow for the examination of the interplay between the innate and adaptive immune system during viral infections or vaccinations, and provide avenues of intervention. BLT, Hu-HSC, and Hu-PBL mice can be successfully infected with Epstein Barr virus (EBV), which activates and matures human DCs, and in turn, induces both a virus-specific T cell response as well as NK cell response [11, 15, 28, 40]. Utilizing the ability to deplete specific cell populations, depletion of pDCs demonstrated their importance in delaying EBV-induced mortality [15] . Rag2$^{-/-}$IL-2rγ$^{-/-}$ and NSG mice reconstituted with huCD34^{+} cells can be infected with influenza A virus and produce inflammatory cytokines and neutralizing antibodies in response to the virus. This demonstrates the ability of the humanized mouse model to imitate the natural multifaceted immune responses that is observed in vivo as compared to in vitro models [33, 38]. HuCD34^{+}-transplanted Balb/c/Rag2$^{-/-}$/IL-2rγ$^{-/-}$ mouse model can be infected with Hepatitis C virus (HCV), which not only induced HCV-specific T cell responses but infiltration of the mouse liver with human innate and adaptive immune cells [41]. The same mouse model can also be infected with HSV-2, and when the virus is used in a vaginal challenge model, T cells and NK cells were detected at the mucosa [27].

Both of these viral infection models demonstrated the ability to use these mice to examine local immune responses at specific sites of infection. In addition, the use of attenuated HSV-2 in humanized mice demonstrated protection against subsequent challenge infections [27]. These examples of viral infections in different humanized mouse models demonstrate the possibilities of using these models to examine interactions between human immune cell subsets with each other and the virus, tissue and mucosa specific immunity, and the effects of vaccination. Similar to other viruses, HIV-1 can also successfully infect human immune cells in the humanized mice, thus providing a small animal model alternative to nonhuman primates. Like several other viruses, HIV-1 can infect a range of humanized mouse models including huCD34^{+}-transplanted Rag2$^{-/-}$/IL-2r$\gamma^{-/-}$ [42], NSG BLT mice [30, 31], and NOD/SCID BLT mice [31]. Following HIV-1 infection, a decline of CD4^{+} T cell is observed [30, 31, 42], CD4^{+} and CD8^{+} T cell activation develops [30, 31], and antigen-specific T cell and B cell responses are induced [31, 43]. Furthermore, the viral reservoir can be assessed, and initial studies have tested approaches to eliminate the HIV-1 reservoir using the humanized mouse model [44]. This highlighted the possibility of using the BLT humanized mouse model to examine not only the role of innate immunity in HIV-1 infections but also its consequences on the development of the adaptive immunity and subsequent disease outcome.

23.3 Direct Activation of TLR7/8 by HIV-1 and IFNα-Mediated Immune Activation

HIV-1 ssRNA encodes for multiple TLR7/8 ligands which have been shown to activate pDCs, mDCs, and other immune cells in the in vitro system [45–47]. In pDCs, activation of the TLR7 pathway by HIV-1 leads to the production of IFNα [45–48] and induction of the adaptive T cell response [46, 49]. This has potentially beneficial outcomes as the stimulation of TLRs have demonstrated importance not only in the priming of CD4^{+} and CD8^{+} T cells but also in directly modulating the type of adaptive immune response induced [50, 51]. However, the persistent production of IFNα might be detrimental in the setting of a chronic persistent infection [52, 53]. In SIV infection of nonhuman primates, pDCs in rhesus macaques, the pathogenic host of SIV, produce large quantities of IFNα [54], while in sooty mangabeys, the natural host with reduced pathogenesis [55], lower IFNα levels are observed in chronic infection [54, 56, 57]. This is also observed in the expression of interferon-stimulated genes (ISGs), which are elevated in SIV-infected rhesus macaques [58]. Likewise, in human HIV-1 infections, pDCs also express high levels of IFNα, resulting in the upregulation of ISGs, which may contribute to the overall systemic immune activation [48, 59, 60]. Consistent expression of IFNα can also contribute to the apoptosis of CD4^{+} T cells and hence, exacerbate the immune deficiency observed in HIV-1-infected individuals [61]. In addition, pDC activation by HIV-1 has also been shown to induce an increase in indoleamine 2,3-dioxygenase expression which, through modulating regulatory T cells, can suppress T cell responses and

Table 23.1 TLRs in humanized mouse model—Determined by expression and function

Mouse model	Humanization	TLR	References
NOD/SCID/IL-2rγ$^{-/-}$	huCD34^{+}	TLR2 on monocytes	[13]
		on mDCs	[13]
		TLR3 on mDCs	[10]
		TLR4 on monocytes	[13]
		on mDCs	[10, 13]
		TLR7 on pDCs	[10]
		TLR8 on mDCs	[10]
NOD/SCID/β2m$^{-/-}$	huCD34^{+}	TLR7 on pDCs	[32]
NOD/SCID	huCD34^{+}	TLR3 on mDCs	[36]
		TLR4	[89]
		TLR4 on mDCs	[89]
Rag2$^{-/-}$IL2rγ$_c^{-/-}$	huCD34^{+}	TLR4[a]	[38]
		TLR7 on pDCs	[42]
		TLR9 on pDCs	[42]

[a] Mice also have human IL-3 and GM-CSF knock-in

proliferation [49, 62–64]. Therapeutic manipulation to suppress the pDC/TLR7/IFNα responses have led to conflicting results. Treatments with chloroquine, which can inhibit endosomal acidification and therefore inhibit signaling through TLR3, 7, 8 and 9, have shown to help reduce HIV-1 replication [65, 66] and immune activation [67–69]. However, other studies have also demonstrated that chloroquine can increase HIV-1 infectivity [70, 71]. Further studies in adequate in vivo models are needed to assess the repercussions of suppressing specific arms of the innate immune response for HIV-1 pathogenesis and immune activation, and also to examine other venues that may more specifically target isolated pathways of innate immunity. These studies will also provide important insights into the contribution of individual pathways to the overall immune activation associated with HIV-1 infection.

The humanized mouse model is one such model that could be utilized to examine responses by specific innate immune pathways and to manipulate these pathways to alter the resulting immune response. NSG BLT mice and Hu-HSC mice all have demonstrated reconstitution of human pDCs, which can respond to TLR7 stimulation (Table 23.1) [10, 42]. Even sex-based differences observed in human studies of TLR7-associated IFNα production [46] have been duplicated in huCD34^{+}-transplanted NOD/SCID/β2m$^{-/-}$ mouse models [32]. Furthermore, TLR7 stimulation of pDCs and its contribution to immune activation and deregulation can be mimicked in mice, where chronic stimulation of TLR7 and TLR9 by synthetic ligands has been shown to result in immune activation and lymphoid follicle destruction similar to that observed in chronic HIV-1 infection in humans [30, 72, 73]. Humanized mice can be infected with HIV-1 and following such infection in huCD34^{+}-transplanted Rag2$^{-/-}$IL2rγ$_c^{-/-}$ and NSG BLT mice, pDC activation and production of IFNα can be observed, demonstrating that the humanized mouse model can be used to exam-

ine the roles of pDCs, TLR7, and IFNα in HIV-1 infection [30, 42]. Using the NSG BLT mouse model, the administration of recombinant IFNα2b not only induced immune activation levels comparable to that induced by HIV-1 infection, but also induced infiltration of activated T cells in secondary lymphoid organs, adding to the growing evidence that IFNα contributes to immune activation in HIV-1 infection [30]. Although IFNα has potentially detrimental properties in inducing chronic immune activation, it also stimulates the expression of ISGs which possess antiviral activities [74–76]. Cholesterol-25-hydrolase (CH25H), an ISG which can inhibit HIV-1 entry in in vitro cell cultures, can also lead to reduced HIV-1 viral replication in vivo when used to treat HIV-1-infected NOD/Rag2$^{-/-}$IL2r$\gamma_c^{-/-}$ humanized mice [77]. Overall, these early studies using different humanized mouse models of HIV-1 infection suggest that they allow for a reasonable assessment of TLR pathways, the type I IFN axis, and DC function. Future studies will determine the validity of this model to study pathways leading to HIV-1-associated immune activation and to assess interventions aimed at reducing HIV-1-associated immune activation.

23.4 Activation of TLRs by Microbe and Microbial Products

Although HIV-1 directly encodes for TLR7/8 ligands, it can also stimulate directly and indirectly through the other TLR pathways. It has been well established that HIV-1 infection leads to depletion of gut-associated CD4$^+$ T cells and therefore compromises the gut mucosal barrier allowing for microbial translocation [4]. One microbial product, lipopolysaccharide (LPS), which can stimulate through the TLR4 pathway has been associated with HIV-1 associated T cell activation [4, 7, 78], monocyte activation [79], lower CD4$^+$ T cell count [80], and CD4$^+$ T cell recovery [7]. In addition to stimulation of TLR4, stimulation of other TLR pathways including TLR3, 5, and 9 also activates T cells and leads to CD4$^+$ T cell apoptosis [78]. Furthermore, gram-positive bacterial stimulation via the TLR2 pathway has been described to result in increased HIV-1 susceptibility of skin-resident DCs [81] as well as transmission of HIV-1 from DCs to CD4$^+$ T cells [82]. Presence of microbial products resulting from microbial translocation or coinfection can not only cause deregulation of the immune system by themselves, but the activating signal might be further amplified by the presence of HIV-1 [83]. Preexposure of monocytes with HIV-1 and HIV-1-encoded TLR8 ligands enhanced subsequent TLR4 stimulation, exacerbating the microbial-induced immune activation [83], and the stimulation of TLR2, 4, and 9 can increase HIV-1 replication in chronically infected cells [84]. In addition, HIV-1 can also lead to increase in TLR2, 3, 4, 6, 7, and 8 expression, all of which can amplify the activation of these immune cells [85–87]. All these factors can contribute to and amplify HIV-1-associated immune activation, which suggest that suppression of HIV-1 or microbial stimulation of TLRs might therefore be beneficial in reducing HIV-1 pathogenesis. These studies have demonstrated the complexities of the various immune cell populations and TLR pathways that play a

role in HIV-1 infection and pathogenesis. The question of how each pathway contributes to progressive HIV-1 disease and methods of therapeutically modulating these pathways still needs to be examined.

The role of microbial translocation and the stimulation of the other TLR pathways have also been examined in humanized mice (Table 23.1). Although limited, the expression and function of TLRs in different humanized mouse models have now been started to be studied. TLR2 and TLR4 expressions have been observed on monocytes in huCD34$^+$-transplanted NSG mice [13], and TLR3 expression on mDCs in huCD34$^+$-transplanted NOD/SCID and both huCD34$^+$ and human PBMC-transplanted NSG mice [10, 36, 88]. Stimulation through TLRs can lead to DC activation and maturation [10, 33, 36, 88, 89], production of cytokines [10, 38, 39], and recruitment and priming of T cells [10, 36, 88, 89]. HIV-1 infection of huCD34$^+$-transplanted Rag2$^{-/-}$IL2$\gamma_c^{-/-}$ mice also results in depletion of gut CD4$^+$ T cells and subsequent microbial translocation [90]. The use of humanized mice also allowed for the administration of dextran sodium sulfate, which can cause gut barrier dysfunction in the absence of HIV-1 infection. Treatment in huCD34$^+$-transplanted Rag2$^{-/-}$IL2$\gamma_c^{-/-}$ mice demonstrates that dextran sodium sulfate disruption only induces microbial translocation while HIV-1 additionally inhibits microbial clearance potentially reflecting the in vivo consequences of HIV-1 disruption of TLR responses highlighted above [90]. Although the presence of murine immune cells has been reduced in these different humanized mouse models, it is still possible that they may play a role in responding to microbial products. Examination of sepsis in the BLT mouse model, however, suggests that any remnant mouse innate or adaptive immunity that may respond to bacterial products are not enough to induce an inflammatory response on their own and that the reconstituted human immune cells are essential [12]. The humanized mouse models therefore can be utilized to examine not only TLR pathways directly stimulated by HIV-1 but also those induced by microbial translocation or coinfections. However, the expression and function of the various TLR pathways still needs to be more comprehensively studied, particularly the TLR expression patterns and function on the reconstituted human immune cells at various tissue sites.

23.5 Future Direction and Development of the Model

Significant progress has been made in the development of humanized mouse models to more accurately replicate the in vivo human immune system. The establishment of stable human T cell populations has allowed it to be used as a small animal model for HIV-1 infection. Although there have only been a very limited number of studies to date, initial studies assessing immune responses in different humanized mouse models have demonstrated that the TLRs respond to HIV-1 and other microbial pathogens in humanized mice similar to that observed in humans. Furthermore, studies employing in vivo stimulation and blocking of TLRs in humanized mice have already started to dissect how various TLR pathways can contribute to HIV-

1-associated immune activation. The expression and functionality of TLRs in the various humanized mouse models still needs to be fully examined in addition to the role of residual mouse TLR responses, which might represent a confounding factor in these models. Although these early studies are limited, they provide a potential avenue to evaluate activators and inhibitors of individual innate pathways for both dissecting the contribution of these pathways to HIV-1-induced immune activation as well as their therapeutic effect in reducing HIV-1 pathogenesis.

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