Immune Activation and the Role of TLRs and TLR Agonists in the Pathogenesis of HIV-1 Infection in the Humanized Mouse Model

J. Judy Chang and Marcus Altfeld

Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology and Harvard, Harvard Medical School, Boston

Immune activation plays a critical role in HIV-1 pathogenesis, but the pathways that are responsible for HIV-1-associated immune activation are not well understood. Recent advances in the development of a humanized mouse model for HIV-1 infection might provide new approaches to study the mechanisms of HIV-1 associated immune activation, and to test interventions aimed at reducing HIV-1 pathogenesis.

Keywords. TLR; innate immunity; HIV-1; humanized mouse; pathogenesis.

IMMUNE ACTIVATION IN HIV-1 INFECTION

Chronic HIV-1 infection is characterized by high immune activation levels that are driven directly by HIV-1 replication and indirectly through changes in the microbiome, microbial translocation, co-infections, immune deregulation, and lymphoid tissue fibrosis [1–4]. Importantly, the level of activation of CD8⁺ T cells in chronic infection has been shown to be a better predictor of HIV-1 disease progression than HIV-1 viral load alone [5]. Furthermore T cell activation has 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 [6, 7]. Dissecting the mechanisms that drive chronic immune activation and development of interventions that can reduce immune activation will provide important avenues to improve disease management in HIV-1-infected individuals.

Although immune activation is often measured as the increase in expression of activation molecules on

T cells, in particular CD38 and HLA-DR, an increasing body of data suggests that activation of T cells is not simply a consequence of antigen-specific T cell activation, but rather a reflection of bystander activation resulting from the activation of innate immune responses. HIV-1 encodes for multiple TLR7/8 ligands and potentially additional components recognized by innate immune cells, which can mediate direct activation of the immune system in vitro [8, 9]. In particular activation of plasmacytoid dendritic cells (pDCs) by HIV-1 is associated with increased IFN α production, a pro-inflammatory cytokine that recruits and activates adaptive T cell responses [10–12] and induces CD4⁺ T cell apoptosis [13]. IFN α has been suggested to be a key mediator in SIV pathogenesis in infected non-human primates. pDCs in Rhesus Macaques (RM), the pathogenic host of SIV, produce large quantities of IFN α in response to SIV [14, 15], while pDCs in Sooty Mangabeys, the natural host with reduced pathogenesis [16], have been suggested to produce lower IFN α levels in chronic SIV infection [14, 17, 18]. The elevated IFN α levels are also reflected in an increase in the expression levels of interferon stimulated genes (ISGs) in chronically SIV-infected macaques [19, 20]. Indeed using mouse models, 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 [21, 22]. Additionally, pDC activation by HIV-1 has also been shown to induce an increase in

Correspondence: Marcus Altfeld, MD, PhD, Professor of Medicine, Ragon Institute of MGH, MIT and Harvard, Massachusetts General Hospital, 9th Floor 400 Technology Square, Cambridge, MA 02139 (maltfeld@partners.org).

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indoleamine 2,3-dioxygenase expression which, through modulating regulatory T cells, can suppress T cell responses and proliferation [10, 23–25]. However, human studies of chloroquine treatment, an inhibitor of TLR signaling, provided mixed results with some studies showing a reduction in HIV-1 replication [26, 27] and immune activation [28–30], while others demonstrated an increase in HIV-1 infectivity [31, 32]. The repercussions of suppressing specific arms of the innate immune response is therefore still unpredictable, requiring further studies in adequate in vivo models.

Stimulation of innate immune cells is not limited to HIV-1-encoded ligands. HIV-1-mediated depletion of gut-associated CD4⁺ T cells in early in HIV-1-infection contributes to an increase in microbial translocation through a compromised mucosal barrier. The resulting elevated systemic levels of microbial products such as lipopolysaccharide (LPS) can stimulate a cascade of cytokine production via TLR4 [1, 4]. Elevated plasma LPS levels are associated with a general increase in immune activation and also an abridged CD4⁺ T cell recovery [1, 4] and can suppress dendritic cell function [33]. Furthermore, HIV-1 infection also modulates the TLR responses by altering levels of TLR expression and deregulating responses of innate immune cells to TLR stimulation [20, 34–38]. As an example, pre-stimulation with HIV-1-encoded TLR8 ligands can enhance the subsequent pro-inflammatory cytokine response to TLR4 stimulation [38], suggesting that different TLR pathways can amplify each-other during HIV-1 infection. The role of innate immunity in HIV-1 pathogenesis is therefore multifactorial, and better in vivo models are needed to understand and modulate the individual pathways that contribute to progressive HIV-1 disease. The humanized mouse model might provide a small animal model of HIV-1 infection that will allow for the investigation of some of these path ways in vivo, as discussed below.

RECONSTITUTION OF INNATE IMMUNITY IN HUMANIZED MOUSE MODELS

Humanized mouse models have been used to examine the role of innate immune cells in various diseases models, including autoimmunity and viral infections [39–42], during vaccinations, and to assess the interplay between various immune cells through blocking and cell-depletion experiments [43–45]. The use of the BLT mouse model, in which immuno deficient NOD/SCID or NOD/SCID/IL2r $\gamma_c^{-/-}$ mice are reconstituted with human liver, thymus and hematopoietic CD34⁺ cells, provides an intriguing model to study immune responses against viral infections as this model allows for the de-novo induction of antigen-specific T cell responses [42, 46]. Using a BLT humanized mouse model with a NOD/SCID mouse background, Melkus et al demonstrated that dendritic cells can be activated by EBV infection and induce subsequent T cell responses against the virus [42]. In addition to EBV, several studies have demonstrated that

humanized mouse models can respond to various viral infections and have already been utilized to examine HIV-1, HBV, HCV, HSV-2 and influenza infection [39–41, 47–51]. This highlighted the possibility of using the BLT humanized mouse model to examine not only the role of innate immunity in human viral infections but also its consequences on the development of the adaptive immunity and subsequent disease outcome.

To date, substantial efforts have been made to reduce murine innate immune cells in humanized mouse models. However, the functionality of the human innate immune populations still needs to be more comprehensively characterized and improved in the various humanized mouse models, and the consequences of interactions with mouse stroma cells and mouse cytokines represent important confounding factors. Early examination of the NOD/SCID mouse model that was reconstituted with CD34⁺ cord blood or liver cells showed that it resulted in poor reconstitution of both dendritic and NK cells [52, 53]. More recent optimizations of humanized mouse models have shown significant improvements on the development of human innate immunity. Cytokines and chemokines have been used to increase both myeloid and NK cell populations (reviewed in [54]). The use of GM-CSF and IL-4 has enhanced the reconstitution of dendritic cell populations in NOD/SCID/IL2r $\gamma_c^{-/-}$ mice transplanted with human CD34⁺ cells [48]. mDCs and monocytes in this humanized NOD/SCID/IL2r $\gamma_c^{-/-}$ mouse model also demonstrated functional capabilities including cytokine production and phagocytosis [55, 56]. Similarly, using alternative humanized mouse models, the administration of CSF-1 [57] and IL-3/GM-CSF [58] knock-ins has also allowed for the development of macrophages. Cytokine treatment using hydrodynamic delivery of plasmids expressing human IL-15 in NOD/SCID/IL2r $\gamma_c^{-/-}$ mice transplanted with human CD34⁺ cells has furthermore resulted in successful development of NK cells in this model [59]. Changing the backbone in different humanized mouse models has also allowed for various innate immune cells to reconstitute better in the blood and tissue. As an example, NOD/SCID/IL2r γ^{tm1Wjl}/SzJ mice transplanted with liver CD34⁺ hematopoietic progenitor cells have been shown to have pDCs and both CD1c⁺ and CD141⁺mDCs in blood, spleen and bone marrow [60]. This approach allowed for mDCs and pDCs to be detected in the spleen, blood and to a lesser extent in the bone marrow, with mDCs having similar expression of surface markers as those observed on mDCs in humans [60, 61]. Likewise, Rag2^{-/-}IL2r $\gamma_c^{-/-}$ mice transplanted with CD34⁺ cord blood have dendritic cell reconstitution with functional pDCs identified in the bone marrow, spleen, lymph node and thymus of the humanized mouse [62]. Lastly, the quality, genetics and gender of the human tissue transplanted can also alter the resulting human immune cell reconstitution and function. The NOD/SCID BLT mice, which not only received CD34⁺ hematopoietic cells but also transplant of fetal liver and thymus, have even better development of dendritic

cells and macrophages [63], but still had poor NK cell reconstitution due to the lack of recognition of murine IL-15 by human cells. These and other developments [64, 65] have allowed for the improvement of human innate immunity in humanized mouse models but further work is required to determine the functionality of the innate cells that have been reconstituted, and their trafficking to different tissues, such as mucosa sites.

In addition to the degree and types of innate immune cell reconstitution, the expression patterns and functionality of innate immune receptors on human cells in the different humanized mouse models need additional investigation. In NOD/SCID/IL2 $\gamma_c^{-/-}$ mice transplanted with CD34 $^{+}$ human cord blood, TLR3 has been shown to be expressed on mDCs from bone marrow and spleen, and to respond to TLR3 ligand stimulation [43]. In NOD/SCID/ $\beta_2m^{-/-}$ mice transplanted with CD34 $^{+}$ cord blood, stimulation with specific TLR7/8 ligands demonstrated that they were functionally expressed on pDCs, resulting in cytokine production [66]. More recently NOD/SCID/IL2 γ_c^{tm1Wjl}/SzJ mice transplanted with liver-derived CD34 $^{+}$ hematopoietic progenitor cells were examined for their ability to respond to TLR3, 4, 7, 8, and 9 stimulations. These studies showed adequate responses to TLR3, 4, 7, and 8 ligands by the respective pDC and mDC populations, but TLR9 responses by pDCs were less prominent [60]. The examination of innate immune receptors has been largely limited to TLRs with only recent work starting to address the potential role of other host restriction factors such as APOBEC3 [67]. Overall, initial data on innate immune responses in humanized mouse models are encouraging, but more comprehensive examination of TLR and other innate immune receptor expression profiles, tissue distribution, trafficking patterns, maturation status and function of innate immune cells in humanized mice is still needed.

ASSESSMENT OF INNATE IMMUNE RESPONSES IN HUMANIZED MOUSE MODELS FOLLOWING HIV-1 INFECTION

Although a better understanding of innate immunity in humanized mice is still required, many studies have already begun to utilize these *in vivo* models to examine and modulate innate immune responses and innate-adaptive cross-talk during viral infections. Early infection events are often difficult to examine in human HIV-1 infection as infected individuals are rarely identified during this early window, and precise infection dates often remain unknown. Studies of SIV infection in RM allow for more controlled experiments, but are limited by costs, differences in MHC class I genotypes, and the use of SIV instead of HIV-1. A small animal model such as the humanized mouse model might be able to overcome some of these limitations and provide new insights into the early *in vivo* effects of HIV-1 infection. Using the Rag2 $^{-/-}$ IL2 $\gamma_c^{-/-}$ CD34 $^{+}$ cord blood transplanted mouse model, Zhang et al demonstrated that early in

HIV-1 infection CD4 $^{+}$ T cells but not pDCs are depleted. The pDCs, which also contained HIV-1, were activated and initially produced large quantities of IFN α [62]. However, as the CD4 $^{+}$ T cell population declined and infection progressed, pDCs from the bone marrow developed an impairment in responding to TLR7 and TLR9 stimulation, similar to what has been suggested in chronic HIV-1 infection in humans [68, 69]. The role of microbial translocation in contributing to HIV-1 disease has recently been assessed in humanized mouse models of HIV-1 infection. Hofer et al have demonstrated that similar to human HIV-1 infection, HIV-1 infection of Rag2 $^{-/-}$ IL2 $\gamma_c^{-/-}$ CD34 $^{+}$ cord blood transplanted mice resulted in an increase in microbial translocation [70]. This was determined by the detection of elevated levels of LPS, sCD14 and LPS binding protein in the plasma. The use of the humanized mouse model furthermore allowed for controlled comparison of HIV-1-induced microbial translocation and dextran sodium sulfate induced disruption of the intestinal barrier. This comparison demonstrated that microbial translocation levels were higher following HIV-1 infection than following dextran sodium sulfate treatment, suggesting that elevated levels of microbial products in the plasma of HIV-1-infected animals might be the result of not only the disruption of gut wall integrity but also an impairment in the clearance of these products [70]. Although the authors demonstrated that the presence of microbial translocation was associated with T cell activation, it remains to be determined whether this was through TLR4 recognition of LPS alone, stimulation of additional innate pathways by other microbial products, direct microbial cytotoxic activity, or a combination of these and other yet unknown factors.

An additional important application of humanized mouse models of HIV-1 infection is the investigation on effects of *in vivo* modulation/depletion of specific cell subsets or treatments with immune-modulatory agents on HIV-1 pathogenesis. Using NOD/SCID/IL2 $\gamma_c^{-/-}$ BLT mice, Long et al compared immune activation induced by HIV-1 infection against that induced by treating BLT mice with recombinant IFN α 2 and observed similar levels of T cell activation [71]. The authors further observed that activated T cells were detectable both in secondary lymphoid organs and the peripheral blood of humanized mice, adding to the growing evidence that IFN α contributes to immune activation in HIV-1 infection [10–13]. Despite the potential downside of chronic IFN α stimulation and resulting immune activation, some of the ISGs also possess well characterized antiviral activities [72–74]. One such gene product, cholesterol-25-hydrolase (25CH), has been shown to have antiviral activity against a range of viruses including HIV-1. In addition to using *in vitro* cell cultures to demonstrate that CH25H inhibits HIV-1 entry, it was recently shown that this inhibition can also lead to reduced HIV-1 viral replication *in vivo* when HIV-1-infected NOD/Rag2 $^{-/-}$ IL2 $\gamma_c^{-/-}$ humanized mice were treated with CH25H [75]. These early studies using

the humanized mouse model of HIV-1 infection demonstrate the versatility of this model and its potential to be used to evaluate inhibitors of individual innate pathways, such as TLR antagonists, 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.

Taken together these early studies of innate immunity in HIV-1 infection using the humanized mouse model suggest that this model might provide an important future tool to examine events of early HIV-1 infection, to understand the role of innate immunity in viral infections for both viral control and virus-mediated pathogenesis, and to perform in vivo manipulations of innate immune pathways in a small animal model.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (<http://jid.oxfordjournals.org/>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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