

## REVIEW

# HIV-1 immunopathogenesis in humanized mouse models

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In recent years, the technology of constructing chimeric mice with humanized immune systems has markedly improved. Multiple lineages of human immune cells develop in immunodeficient mice that have been transplanted with human hematopoietic stem cells. More importantly, these mice mount functional humoral and cellular immune responses upon immunization or microbial infection. Human immunodeficiency virus type I (HIV-1) can establish an infection in humanized mice, resulting in CD4<sup>+</sup> T-cell depletion and an accompanying nonspecific immune activation, which mimics the immunopathology in HIV-1-infected human patients. This makes humanized mice an optimal model for studying the mechanisms of HIV-1 immunopathogenesis and for developing novel immune-based therapies.

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## INTRODUCTION

Human immunodeficiency virus type I (HIV-1) infection is characterized by progressive CD4<sup>+</sup> T-cell depletion and acquired immunodeficiency syndrome (AIDS). Approximately 60 million people have been infected with HIV-1, and half of them have died from AIDS-related diseases.<sup>1</sup> After more than 30 years of extensive research, the precise mechanism by which HIV-1 infection leads to immunodeficiency is still poorly understood, mainly as a result of the lack of robust small animal models. The recent development of humanized mice with functional humanized immune systems may help to improve our understanding of HIV-1 pathogenesis and lead to new treatments.

## A BRIEF HISTORY OF THE HUMANIZED MOUSE MODEL

In this review, humanized mice are defined as immunodeficient mice that have been transplanted with human hematopoietic stem cells (HSCs), lymphoid tissue or peripheral blood cells. Early attempts to reconstitute the human immune system in nude mice (which lack T cells) were unsuccessful because of the significant rejection mediated by the remaining mouse B and natural killer (NK) cells.<sup>2</sup> The first breakthrough in this field came with the development of CB17-SCID (SCID) mice,<sup>3</sup> which lack both T and B lymphocytes. Human peripheral blood leukocytes (SCID-hu PBL)<sup>4</sup> and human fetal liver and thymus tissue (SCID-hu Thy/Liv)<sup>5</sup> were successfully reconstituted in SCID mice. Non-obese diabetic (NOD)/SCID mice exhibit additional defects in T, B, NK cell and macrophage function<sup>6</sup> and thus are superior to SCID mice at accommodating human peripheral mononuclear cells (PBMCs)<sup>7</sup> and HSCs.<sup>8</sup> However, these early models have limitations. The SCID-hu PBL mice lack human lymphoid organs and

develop severe graft-versus-host disease mediated by xeno-reactive donor T cells. In contrast, the SCID-hu Thy/Liv mice have very low levels of human cells in the blood and peripheral organs. Collectively, the lack of human cells in the peripheral lymphoid organs and the inability to mount functional immune responses limit the applicability of these early humanized models.

## RECENT PROGRESS IN HUMANIZED MOUSE MODELS

It was reported that depletion of NK cells by antibody treatment significantly increases human HSC engraftment efficiency in NOD/SCID mice.<sup>9</sup> This finding encouraged the generation of mice that are completely devoid of T, B and NK cells (reviewed by Ito *et al.*<sup>10</sup> and Shultz *et al.*<sup>11</sup>). These newly developed immunodeficient mice allowed much better human HSC reconstitution and significant improvements in human immune function. In addition to the development of novel immunodeficient mouse strains, more efforts have been made to enhance engraftment, such as by introducing human cytokines,<sup>12–14</sup> by using human leukocyte antigen (HLA) transgenics<sup>15</sup> and by inhibiting mouse macrophage function.<sup>16</sup>

## Mice lacking T, B and NK cells

The interleukin-2 (IL-2) receptor gamma chain (IL2R $\gamma$ ) is a common signaling component of IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 signaling. The absence of IL2R $\gamma$  blocks NK cell development as a result of the ablation of IL-7 and IL-15 signaling. Efficient multilineage hematopoiesis was first reported in NOD/Shi-SCID *Il2rg*<sup>null</sup> (NOG) mice after human HSC transplantation (NOG-hu HSC),<sup>17</sup> and a subsequent study showed similar human immune cell differentiation in *rag2*<sup>-/-</sup>

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*Il2rg*<sup>null</sup> mice (DKO-hu HSC). More importantly, functional human immune responses were observed in DKO-hu HSC mice, including antigen-specific T cells and antibody production in response to immunization and microbial infection.<sup>18,19</sup> Several other mutant mouse strains with an *Il2rg* gene knockout have been successfully developed, such as NOD/LtSZ-SCID *Il2rg*<sup>null</sup> (NSG),<sup>19,20</sup> NOD-*rag1*<sup>-/-</sup> *Il2rg*<sup>null</sup> (NRG)<sup>21</sup> and *rag1*<sup>-/-</sup> *Il2rg*<sup>null</sup> mice.<sup>22</sup> It is worth noting that NSG mice have been shown to support increased human cell engraftment over the other strains.<sup>22–24</sup>

### Inhibition of mouse macrophages

In addition to T, B and NK cells, macrophages also contribute to xenograft rejection. Signal regulatory protein alpha (SIRP $\alpha$ ) is an inhibitory receptor that is highly expressed on myeloid cells, whereas its ligand CD47 is expressed on all cell types. Ligation of SIRP $\alpha$  by CD47 inhibits macrophage phagocytosis, which contributes to the recognition of self and non-self by innate immunity.<sup>25</sup> Additionally, this CD47–SIRP $\alpha$  interaction also plays an important role in macrophage-mediated xenograft rejection in humanized mice. The SIRP $\alpha$  of NOD mice shows enhanced binding to human CD47, which results in reduced rejection and improved human cell reconstitution.<sup>26</sup> These polymorphisms of the *sirpa* gene may at least partially explain why NSG mice are more efficient than DKO mice in supporting human HSC transplant.<sup>22,27</sup> It was recently reported that HSC transduction with mouse CD47 by a lentiviral vector led to increased engraftment in humanized mice.<sup>28</sup> Meanwhile, human *sirpa* gene-transgenic DKO mice support improved human cell reconstitution and a stronger antigen-specific immune response.<sup>16</sup>

### Improvement of graft efficiency by introducing human cytokines

Many mouse cytokines are poorly crossreactive with their human receptors, so supplementing human cytokines *in trans* can improve the development and differentiation of certain cell lineages in humanized mice: such cytokines include IL-7 for T cells,<sup>29</sup> IL-15 for NK cells,<sup>12,30</sup> erythropoietin for erythrocytes and granulocyte-macrophage colony-stimulating factor (GM-CSF)/IL-4/macrophage colony-stimulating factor (M-CSF) for monocytes/macrophages.<sup>12,31</sup>

Recently, progress has been made by knock-in replacement of mouse cytokines with their human counterparts.<sup>32</sup> Because transcription of the knock-in genes is controlled by mouse regulatory elements, the genes are expressed at the correct time, in the correct location and at physiological levels. Moreover, the replacements lead to defects in the targeted mouse cells, thus providing a competitive advantage to human cells. Three mouse strains have been developed with this technology to produce human thrombopoietin,<sup>14</sup> human IL-3/GM-CSF<sup>13</sup> and M-CSF.<sup>33</sup> The thrombopoietin replacement results in better maintenance of human HSC and higher levels of human cell engraftment.<sup>14</sup> The human IL-3/GM-CSF<sup>13</sup> and M-CSF<sup>33</sup> knock-in genes dramatically improve myeloid cell differentiation and function.

### Human HLA transgenic mice

In humanized mice, human T cells are educated in the mouse thymus by both mouse thymic epithelial cells and human bone marrow-derived cells.<sup>18,19</sup> The T-cell receptor affinity and specificity may be different from those in humans with matched MHC types.<sup>34</sup> Transgenic expression of human HLA-A2 (MHC I) significantly improves human CD8<sup>+</sup> T-cell responses to both Epstein–Barr virus (EBV)<sup>34,35</sup> and dengue virus<sup>36</sup> in infected mice. Interestingly, EBV-infected humanized mice with the HLA-A2 transgene generate antigen-specific T cells to lytic EBV antigens that predominate over T cells

specific to latent antigens, which is similar to the T-cell response in human EBV carriers.<sup>34</sup> Significantly increased human cell reconstitution and better immune responses, including immunoglobulin class switching and elevated human IgG responses, were also observed in HLA-DR4 (MHC II) transgenic mice.<sup>37,38</sup>

### Other factors affecting human cell engraftment

In addition to the mouse genetic background, there are other factors that may affect human cell reconstitution. First, co-transplant of human fetal thymus with autologous HSC will significantly increase human immune reconstitution and function in NOD/SCID mice.<sup>39,40</sup> Mice transplanted with human fetal thymus and liver tissue in addition to HSC are called BLT mice.<sup>39,40</sup> BLT mice have been constructed on both NOD/SCID and NSG backgrounds, and the reconstitution of NSG-BLT has proved to be higher than NOD/SCID-BLT.<sup>24</sup> It has also been demonstrated that newborn mice (less than 3 days) support higher transplant efficiency.<sup>18,19,27,41</sup> Mouse gender was found to play a role in accommodating human HSC grafts because engraftment of human hematopoietic stem cells was more efficient in female NSG recipient mice than in male mice.<sup>23,42</sup>

### HIV-1 INFECTION IN HUMANIZED MICE

Early generations of humanized mice were developed to study HIV-1 infection,<sup>43,44</sup> and the SCID-hu Thy/Liv model is still being used to test antiviral drugs (Table 1).<sup>45–47</sup> However, these models are limited in the modeling of HIV-1 immunopathogenesis owing to the lack of a functional immune system. In the improved humanized mice, several HIV-1 strains have been successfully used for infection. These include CCR5-tropic (JR-CSF,<sup>48,49</sup> Yu-2,<sup>50</sup> BAL,<sup>51,52</sup> ADA<sup>53</sup> and NFN-SX<sup>52,53</sup>), CXCR4-tropic (NL4-3)<sup>50,51</sup> and dual-tropic (NL4-R3A) viruses.<sup>48,54</sup> HIV-1 infection can be established by inoculation through intraperitoneal,<sup>50,51,53,55</sup> intravenous<sup>48,49</sup> or mucosal routes.<sup>56</sup> Sustained viral replication and CD4<sup>+</sup> T-cell depletion were observed by all routes of infection. As is the case for HIV-1 infected patients, CXCR4-tropic HIV-1 quickly depletes both CD45RA<sup>+</sup> naive and CD45RA<sup>-</sup> effector/memory CD4<sup>+</sup> T lymphocytes, whereas CCR5-tropic HIV-1 preferentially depletes CD45RA<sup>-</sup>CD4<sup>+</sup> T lymphocytes.<sup>57</sup>

Humanized mice have been used to study various aspects of HIV-1 infection (Table 1): the roles of regulatory T cells (Tregs)<sup>54</sup> and plasmacytoid dendritic cells (pDCs)<sup>73</sup> in HIV-1 infection, the immunopathogenesis of HIV-1, viral evolution *in vivo*,<sup>58,59</sup> new antiviral treatments,<sup>79–81,84,86</sup> gene therapy,<sup>83,88</sup> mucosal transmission<sup>56</sup> and microbicide development.<sup>68,70</sup> In the presence of antiviral drugs, latent infection can be established, making humanized mice a valuable model to study HIV-1 latency.<sup>61–63</sup>

Most importantly, the anti-HIV-1 immune responses were observed in the infected mice. These include anti-HIV-1 antibodies<sup>49,50,52,56</sup> and HIV-1-specific T-cell responses.<sup>52</sup> HIV-1 infection resulted in increased CD8<sup>+</sup> T cells in the blood, which were derived from CD45RA effector/memory T cells, not CD45RA<sup>+</sup> naive T cells.<sup>60</sup> The depletion of CD8<sup>+</sup> T cells by antibody treatment resulted in increased viral load, robust immune cell activation and cytopathology in lymphoid tissues.<sup>89</sup> These improvements make the new generation of humanized mice superior to the early models for studying HIV-1 immune responses and immunopathogenesis.

### IMMUNE ACTIVATION AND HIV-1 PATHOGENESIS

Although HIV-1 infection kills target cells, the majority of CD4<sup>+</sup> T-cell loss is not due to productive infection.<sup>90,91</sup> It is widely accepted that chronic, generalized immune activation induced by HIV-1 infection is

**Table 1 HIV infection in current humanized mouse models**

Research areas		Models	References
HIV-1 evolution		DKO-hu HSC	Ince <i>et al.</i> , <sup>58</sup> 2010
		NOG-hu HSC	Sato <i>et al.</i> , <sup>59</sup> 2010
Immune response		NOG-hu HSC	Nie <i>et al.</i> , <sup>57</sup> 2009
		NOG-hu HSC	Sato <i>et al.</i> , <sup>60</sup> 2010
		NSG-BLT	Brainard <i>et al.</i> , <sup>52</sup> 2009
		NOD/SCID-BLT	Brainard <i>et al.</i> , <sup>52</sup> 2009
Latency		DKO-HSC	Choudhary <i>et al.</i> , <sup>61</sup> 2012
		NSG-BLT	Denton <i>et al.</i> , <sup>62</sup> 2012
			Marsden <i>et al.</i> , <sup>63</sup> 2012
Mucosal transmission and prevention		DKO-hu HSC	Berges <i>et al.</i> , <sup>64</sup> 2008
			Hofer <i>et al.</i> , <sup>65</sup> 2008
			Neff <i>et al.</i> , <sup>66</sup> 2010
		Rag1 <sup>-/-</sup> γC <sup>-/-</sup> -hu HSC	Akkina <i>et al.</i> , <sup>67</sup> 2011
		NSG-BLT	Denton <i>et al.</i> , <sup>68</sup> 2011
			Stoddart <i>et al.</i> , <sup>24</sup> 2011
			Wheeler <i>et al.</i> , <sup>69</sup> 2011
		NOD/SCID-BLT	Sun <i>et al.</i> , <sup>56</sup> 2007
			Denton <i>et al.</i> , <sup>70</sup> 2008
			Denton <i>et al.</i> , <sup>71</sup> 2010
			Denton <i>et al.</i> , <sup>68</sup> 2011
			Stoddart <i>et al.</i> , <sup>24</sup> 2011
Immune activation and pathogenesis	Tregs	DKO-hu HSC	Jiang <i>et al.</i> , <sup>54</sup> 2008
	GALT and mucosal microbes	DKO-hu HSC	Hofer <i>et al.</i> , <sup>72</sup> 2010
	pDCs	DKO-hu HSC	Zhang <i>et al.</i> , <sup>73</sup> 2011
	Interferon-α	NSG-BLT	Long <i>et al.</i> , <sup>74</sup> 2012
	Interferon-α	SCID-hu Thy/Liv	Stoddart <i>et al.</i> , <sup>75</sup> 2010
	Neuropathology	NSG-hu HSC	Dash <i>et al.</i> , <sup>76</sup> 2011
			Gong <i>et al.</i> , <sup>77</sup> 2011
			Gorantla <i>et al.</i> , <sup>78</sup> 2010
Antiviral drug	siRNA	DKO-hu HSC	Neff <i>et al.</i> , <sup>79</sup> 2011
			Zhou <i>et al.</i> , <sup>80</sup> 2011
			Ter Brake <i>et al.</i> , <sup>81</sup> 2009
		NSG-hu HSC	Kumar <i>et al.</i> , <sup>82</sup> 2008
			Kim <i>et al.</i> , <sup>83</sup> 2010
		NSG-BLT	Wheeler <i>et al.</i> , <sup>69</sup> 2011
	Small molecules	DKO-hu HSC	Choudhary <i>et al.</i> , <sup>84</sup> 2009
			Sango <i>et al.</i> , <sup>85</sup> 2010
		SCID-hu Thy/Liv	Stoddart <i>et al.</i> , <sup>46</sup> 2007
			Stoddart <i>et al.</i> , <sup>47</sup> 2007
	Peptides	DKO-hu HSC	van Duyn <i>et al.</i> , <sup>86</sup> 2008
		SCID-hu Thy/Liv	Stoddart <i>et al.</i> , <sup>45</sup> 2012
Gene Therapy	shRNA	NSG-BLT	Shimizu <i>et al.</i> , <sup>87</sup> 2010
	HIV-1 neutralizing antibody	NSG-hu HSC	Joseph <i>et al.</i> , <sup>88</sup> 2010

Abbreviations: BLT, human thymus and liver tissues and HSC; DKO, *rag2*<sup>-/-</sup> *Il2rg*<sup>null</sup>; hu HSC, human CD34<sup>+</sup> hematopoietic stem/progenitor cells; hu Thy/Liv, human thymus and liver tissues; NOG, NOD/Shi-SCID *Il2rg*<sup>null</sup>; NSG, NOD/LtSZ-SCID *Il2rg*<sup>null</sup>; pDCs, plasmacytoid dendritic cells; Tregs, regulatory T cells; shRNA, small hairpin RNA; siRNA, small interfering RNA.

the major driving force of immunodeficiency.<sup>92-94</sup> The level of T-cell activation (the percentage HLA-DR<sup>+</sup>CD38<sup>+</sup> T cells out of all the CD8<sup>+</sup> T cells) predicts disease progression independent of and more accurately than CD4<sup>+</sup> T cell count.<sup>95</sup> Additionally, it was recently reported that anti-malarial drugs such as chloroquine<sup>96</sup> and hydroxychloroquine<sup>97</sup> inhibit immune activation in HIV-1-infected patients when used as a monotherapy<sup>96</sup> or in combination with antiviral treatment.<sup>97</sup> The reduction in immune activation correlates with an increase in CD4<sup>+</sup> T cells.<sup>97</sup>

Additional data supporting the hypothesis that immune activation drives AIDS development come from simian immunodeficiency virus (SIV)-infected monkeys. SIV-infected Asian monkeys (e.g., rhesus macaques, cynomolgus macaques and pigtail macaques) experience a dramatic increase in immune activation, rapid CD4<sup>+</sup> T-cell loss and

progression to AIDS. Conversely, infected natural African hosts (e.g., green monkeys, sooty mangabeys and mandrills) exhibit minimal T-cell activation and rarely progress to immunodeficiency despite a viral load comparable to pathogenic SIV infections.<sup>98-100</sup> Moreover, experimental induction of immune activation by lipopolysaccharide (LPS) in SIV-infected African green monkeys has been shown to result in CD4<sup>+</sup> T-cell loss.<sup>101</sup> Interestingly, the transcriptomes of patients with preserved CD4<sup>+</sup> T cell numbers in the presence of constant, high HIV-1 viral loads are very similar to the transcriptomes of SIV-infected sooty mangabeys.<sup>102</sup>

Long-term immune activation can cause damage even in the absence of viral infection. For example, transgenic mice expressing CD70 develop chronic immune activation and lethal immunodeficiency.<sup>103</sup> Moreover, treatment with Toll-like receptor (TLR) 9<sup>104</sup> or

TLR7<sup>105</sup> ligands in mice induces immune activation, lymphoid organ distraction and immune suppression.

The exact mechanism by which HIV infection leads to immune activation is not fully understood. It has been proposed that HIV-1 viral proteins, whole viral particles, infected cells and infection-induced cytokines contribute to immune cell activation.<sup>93</sup> Other factors have also been proposed as the cause of immune activation, such as loss of tissue integrity during acute phase infection of gut-associated lymphoid tissue (GALT) and microbial products translocation,<sup>106</sup> loss of Tregs,<sup>107,108</sup> activation of pDCs,<sup>112</sup> and production of type I interferons (IFN-I).<sup>109,110</sup>

#### GALT infection and intestinal bacteria translocation

HIV-1 infection causes massive depletion of T cells in GALT and breaks down the mucosal barrier, resulting in translocation of intestinal bacterial products (including LPS) and immune activation.<sup>106</sup> Injection of LPS into SIV-infected African green monkeys resulted in increased immune activation and viral replication.<sup>101</sup> It was recently reported that circulating LPS in the first years of chronic HIV-1 infection is a strong predictor of disease progression independent of CD4<sup>+</sup> T-cell counts and HIV-1 viral load, so plasma LPS may serve as a candidate biomarker for HIV-1 monitoring and evaluation of treatments.<sup>111</sup>

#### IFN-I and pDC activation

IFN-I is a group of multifunctional cytokines that plays an essential role in antiviral immunity. pDCs constitute 0.2%–0.5% of human PBMCs, but they are capable of producing 100 times more IFN-I than other cell types. They preferentially express TLR7 and TLR9, sensing viral RNA and DNA, respectively, during infection. Upon viral infection or other stimulation, pDCs produce large amounts of IFN-I and other inflammatory cytokines.<sup>112</sup> IFN-I play important roles in immune cell development and normal immune responses. However, persistent expression of IFN-I induces immune dysfunction and may lead to autoimmune disease.<sup>113</sup>

Elevated expression of IFN-I has been documented in HIV-1-infected patients.<sup>114–116</sup> HIV-1 infection also stimulates IFN-I production in cultured human PBMCs or purified pDCs.<sup>117–119</sup> As would be expected, both IFN-I<sup>120,121</sup> and pDCs<sup>122</sup> show the capacity to inhibit HIV-1 replication *in vitro*. pDCs are numerically decreased<sup>123–125</sup> and functionally impaired in the peripheral blood of HIV-1-infected individuals. The decreased capacity of pDCs to produce IFN-I correlates with opportunistic infection independent of CD4<sup>+</sup> T-cell counts.<sup>126–128</sup> These observations suggest that pDCs and IFN-I are protective during HIV-1 infection, which is similar to their role in other viral infections.

Paradoxically, the high levels of IFN-I in HIV-1-infected patients do not correlate with viral control; rather, they are predictive of HIV-1 disease progression and AIDS development.<sup>115,129,130</sup> Additionally, IFN-I is induced during the acute phase of SIV infection in both pathogenic and non-pathogenic hosts, but is rapidly controlled during non-pathogenic SIV infection. Only pathogenic SIV infection is characterized by sustained IFN-I production during a chronic infection, which correlates with immune activation and AIDS development.<sup>131–134</sup> However, it is still not clear if pDCs are the major source of IFN-I during chronic HIV-1 infection because the IFN-I-producing cells in the spleens of HIV-1 infected patients do not seem to express pDC-specific markers.<sup>135</sup> The mechanisms of IFN-I production and pDC activation in HIV-1 pathogenesis are poorly understood. HIV-1 infection can stimulate pDCs to express TNF-related apoptosis-inducing

ligand, which may contribute to CD4<sup>+</sup> T-cell depletion.<sup>136–138</sup> However, the induction of CD4<sup>+</sup> T-cell death by TNF-related apoptosis-inducing ligand-expressing pDCs remains controversial.<sup>139</sup> These conflicting reports highlight that IFN-I and pDCs may play multiple roles in HIV-1 infection and immunopathogenesis.

#### Tregs

Human CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> Tregs are central players in balancing the induction and suppression of immune activation.<sup>140,141</sup> During HIV infection, Tregs could be either beneficial, by inhibiting immune activation, or detrimental, by suppressing virus-specific T-cell responses.<sup>107,142</sup> It has been reported that, during HIV infection, the absolute Treg count decreases and that Treg loss correlates with immune activation and disease progression.<sup>143,144</sup> However, other studies have shown that Treg numbers are elevated in both the PBMCs<sup>145,146</sup> and the GALT<sup>147</sup> of HIV-1-infected patients, independently of CD4<sup>+</sup> T-cell count and viral load.<sup>145,146</sup> One study in SIV-infected rhesus macaques demonstrated that Tregs are depleted from the GALT but accumulate in PBMCs and lymphoid organs.<sup>148</sup> These conflicting reports underscore the complex role of Tregs in HIV infection and immune activation.

### STUDYING THE MECHANISMS OF HIV-1 PATHOGENESIS IN HUMANIZED MOUSE MODELS

HIV-1 infection in humanized mice results in sustained viral replication and significant CD4<sup>+</sup> T-cell depletion in the peripheral blood and lymphoid organs.<sup>48,50,51,53,55,56</sup> Viral antigens have been observed in T cells, CD68<sup>+</sup> macrophages<sup>50,56</sup> and pDCs.<sup>73</sup> Importantly, HIV-1 infection results in T-cell activation in the humanized mice, and the immune activation correlates with viral load<sup>74</sup> and T-cell depletion.<sup>73</sup> Several experiments to delineate the mechanisms of HIV-1 immunopathogenesis have been carried out in humanized mice and will be summarized in this section.

#### GALT infection and gut bacteria translocation

NOD/SCID-BLT or NSG-BLT mice support human cell reconstitution in the gut and vaginal tissues through mucosal inoculation.<sup>24,56</sup> These mice have been used to study microbicides and the prevention of HIV-1 mucosal transmission.<sup>24,68,70,71</sup> DKO-hu HSC mice show very limited levels of human cells in the gut mucosa<sup>65</sup> and whether these mice can support mucosal infection remains controversial.<sup>64,65</sup> Application of dextran sodium sulfate induces bacterial endotoxin translocation in DKO-hu HSC mice but does not result in elevated plasma LPS levels unless phagocytic cells are depleted with clodronate liposomes or impaired by HIV-1 infection.<sup>72</sup> This finding highlights the role of macrophages in modulating microbial translocation and immune activation.

#### pDCs and IFN-I in HIV-1 pathogenesis

Human pDCs in these chimeric mice phenotypically resemble their counterparts from human PBMCs in their expression of specific surface markers such as blood dendritic cell antigen 2, CD123, HLA-DR and CD4.<sup>18,52,73</sup> Moreover, they function similarly to human pDCs by producing IFN-I and other inflammatory cytokines upon influenza virus or herpes simplex virus infection.<sup>18,52,73</sup> HIV-1 infection in humanized mice can also activate pDCs to produce IFN-I and other cytokines. Importantly, the activation of pDCs positively correlates with immune activation and CD4<sup>+</sup> T-cell depletion in infected mice.<sup>73</sup> It has also been shown that IFN-I application to NSG-BLT mice causes immune activation similar to that induced by HIV-1 infection.<sup>74</sup>

It was recently reported that chloroquine<sup>118</sup> and rapamycin<sup>151,152</sup> inhibit IFN-I production by pDCs *in vitro*. Meanwhile, clinical studies show that chloroquine,<sup>96</sup> hydroxychloroquine<sup>97</sup> and rapamycin<sup>149,150</sup> could reduce immune activation and inhibit pathogenesis in HIV-1-infected patients. Whether these drugs function through inhibiting pDCs *in vivo* needs to be examined further. Humanized mice provide a robust *in vivo* model for these studies and other hypothesis-driven experiments that test the roles of pDCs and IFN-I in HIV-1 pathogenesis.

### Roles of Tregs in HIV-1 infection and pathogenesis

Tregs were observed in different organs of humanized mice, and purified Tregs have suppressive functions that are similar to those of their human PBMC-derived equivalents.<sup>54,153</sup> During the acute phase of infection, CD4<sup>+</sup>FoxP3<sup>+</sup> Tregs are preferentially infected and depleted by a pathogenic HIV-1 isolate in infected DKO-hu HSC mice. When Tregs are depleted with an IL-2-toxin fusion protein (denileukin diftitox, trade name Ontak), HIV-1 replication is significantly impaired in infected mice. This is observed in the reduced number of infected cells in lymphoid organs and lower plasma viremia.<sup>54</sup> Notably, Ontak does not efficiently deplete Tregs in monkeys,<sup>101</sup> which highlights the advantage of humanized mouse models.

### AIDS-related neurological disorders

Neurocognitive disorders are common causes of morbidity in HIV-1-infected patients.<sup>154</sup> SIV-infected rhesus macaques have been developed to study HIV-1-related neurological disorders.<sup>155</sup> However, species specificity and high costs preclude their widespread usage. Recently, it was reported that HIV-1 infection in humanized mice induces neuroinflammatory responses, including leukocyte infiltration, microglial activation, meningitis and encephalitis.<sup>78</sup> Structural changes in mouse cortical gray matter were also observed, evidenced by the loss of microtubule-associated protein 2, synaptophysin and neurofilament antigens.<sup>76</sup> These reports suggest that humanized mice would be a valuable system for modeling AIDS-related neurodegeneration.

### FUTURE DIRECTIONS

Substantial advances have been made in developing mice with humanized immune systems since the first report more than 20 years ago,<sup>5</sup> although the functions of the human cells in these chimeric mice are still in need of further improvements.<sup>156,157</sup> These mice have been shown to be invaluable for several aspects of HIV-1 research, especially for studying immune responses and immunopathogenesis.<sup>54,72,73</sup> All of the human immune cell types that have been implicated in HIV-induced immune pathogenesis can be studied in humanized mice. Additionally, humanized mice can be genetically modified to test different hypotheses about immune activation and the underlying mechanisms. More importantly, data collected from humanized mice are readily translatable to clinical studies because the same agents can be used. In summary, humanized mouse models will increase our understanding of how HIV infection leads to AIDS and accelerate the development of therapeutic strategies.

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