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Highlights:

- Mice with a human immune system (HIS mice) support lymphotropic virus infection.
- Viral pathologies, including oncogenesis, can be recapitulated in HIS mice.
- Primarily cell-mediated immune responses can be observed in HIS mice.

Keywords: HIV, EBV, dengue virus, NK cells, T cells,

Abstract

Pathogenic viruses are often difficult to study due to their exclusive tropism for humans. The development of mice with human immune system components opens the possibility to study those human pathogens with a tropism for the human hematopoietic lineage in vivo. These include HCMV, EBV, KSHV, HIV, HTLV-1, dengue virus and JC virus. Furthermore, some human pathogens, like HSV-2, adenovirus, HCV, HBV and influenza A virus, with an additional tropism for somatic mouse tissues or for additional transplanted human tissues, mainly liver, have been explored in these models. The cellular tropism of these viruses, their associated diseases and primarily cell-mediated immune responses to these viral infections will be discussed in this review. Already some exciting information has been gained from these novel chimeric in vivo models and future avenues to gain more insights into the pathology, but also potential therapies, will be outlined. Although the respective in vivo models of human immune responses can still be significantly improved, they already provide preclinical systems for in vivo studies of important viral pathogens of humans.

1. Mice with reconstituted human immune system components as models of human immune responses

Human immune responses can be studied in a correlative fashion in only a few tissues, which can be biopsied from patients or healthy individuals, including peripheral blood, some secondary lymphoid tissues, like tonsils, and the tumor microenvironment, assessing tumor infiltrating lymphocytes (TILs). For a more systematic analysis and in order to study the outcome of manipulations during immune responses, immunologists have primarily turned to the mouse as an in vivo animal model of mammalian immune responses. However, with a more and more detailed analysis of the mouse immune system it has become apparent that significant differences in immune responses of the two species have developed during the 60 to 70 million years of divergent evolution of mouse and man [1]. Indeed after completion of both mouse and man genome sequencing the immune system was identified after olfaction and reproduction as the third most divergent organ system between the two species [2]. Among the eight distinct domains containing protein families with the highest rate of non-synonymous mutations between mouse and man 6 represented immunity related molecules accounting for more than 800 genes with chemokines being among the most diverse. Furthermore, among the 25 gene clusters that had undergone differential expansions 5 were again immunity related genes, including the non-classical major histocompatibility complex (MHC) class Ib genes. These data indicate that the respective immune systems of mouse and man have been adjusted to their particular ecological niches and pathological challenges during evolution, and differ significantly in their immune responses.

One such group of inflammatory responses are burns, trauma and endotoxemia [3]. In comparing different mouse models for these diseases with patients, it was found that these conditions evoked immune responses with more similarities among patients than between patients and mouse models. Furthermore, the mouse models differed significantly in their gene expression among each other and were not predictive for the human responses. From these

studies the authors suggested to perform more clinical studies and develop new mouse models, which recapitulate human immune responses more closely.

One approach in this direction are mice with transferred or reconstituted human immune system components [4-7]. These are predominantly based on mouse strains, which lack murine lymphocyte development due to genetic lesions. The most popular strains are NOD-*scid* γ_c^{tm1Wjl} (NSG), NOD-*scid* γ_c^{tm1sug} (NOG), NOD Rag1$^{-/-}$ γ_c^{tm1Wjl} (NRG) and BALB/c Rag2$^{-/-}$ γ_c^{tm1sug} (BRG) mice. While NSG and NRG lack the common gamma chain (γ_c) for interleukin 2, 4, 7, 9, 15 and 21 entirely, NOG and BRG mice carry a truncated version that still allows cytokine receptor assembly. These assembled cytokine receptors might be able to serve as cytokine sinks in the respective mouse strains and thereby deprive the developing human cells of the affected cytokines. Human peripheral blood mononuclear cell (PBMC) transfer into these mice was initially used to study human immune system components in vivo. However, transplantation of fully differentiated human leucocytes leads to rapid graft-versus-host disease (GvHD), during which the incoming human immune cells attack the mouse host in a xenoreactive immune response. In contrast, for the reconstitution of human immune system components these mice receive human CD34$^+$ hematopoietic progenitor cells (HPCs) (Figure 1), which reconstitute at least in the NOD background around 60% of human hematopoietic cells in peripheral blood during three months. The reconstituted cells contain human lymphoid and myeloid compartments, including 50% B cells, 40% T cells, 5% natural killer (NK) cells, 3% monocytes and 2% dendritic cells. Within the T cell compartment 70-80% are CD4$^+$ helper T cells, while 20-30% are CD8$^+$ cytotoxic T cells. These average reconstitution frequencies can considerably vary from donor to donor and even within reconstituted mouse cohorts. Moreover, B cell reconstitution exceeds the frequency of human adult peripheral blood fivefold, while monocyte, T and NK cell development leads to three-, two- and twofold lower frequencies at three months after HPC transfer, respectively. In the BLT (bone marrow, liver and thymus) mice an organoid of human fetal liver and fetal thymus is in addition implanted to support human T cell education [8].

Furthermore, this human organoid even allows good reconstitution in NOD-scid mice without the innate lymphocyte compromising γ_c deletion and, thereby, allows for secondary lymphoid tissue anlage development, including mucosal reconstitution [9]. In contrast, BRG mice are usually less well reconstituted with human immune system components, often reaching only 10% of human hematopoietic cells in the peripheral blood after 3 months [10,11]. Xenoreactivity of the mouse myeloid compartment against the engrafting human cells was identified as the limiting factor. The inhibitory receptor SIRPα, engaging CD47 and cross-reacting with human CD47 only in the NOD background, was found to be responsible for this difference between the BALB/c and NOD mouse background [12]. Indeed transgenic or congenic expression of either the human or the NOD SIRPα [10,11], or mouse CD47 gene transfer into reconstituting human hematopoietic cells [11] allows for as efficient human immune system component reconstitution in the BALB/c as in the NOD mouse background. Furthermore, a recent study used human SIRPα bacterial artificial chromosome transgenic mice with additional knock-in of the four human cytokines M-CSF, IL-3, GM-CSF and TPO [13]. These MISTRG mice developed myeloid and NK cells more efficiently than NSG mice, but still a significant variation in human immune system component reconstitution could be observed. However, percentages of immune system components even vary between human individuals and the functionality of these is much more important for immunity against pathogens. Therefore, this review will concentrate on the immune responses that can be elicited in mice with reconstituted human immune system components (collectively called HIS mice) after infections with viruses.

2. DNA virus infections

2.1. Herpesvirus infections (HSV, HCMV, EBV, KSHV)

Herpesviruses are a group of persistent pathogens that can alternate between latency for persistence and periodic lytic replication for spreading within the infected host and transmission to other hosts. They have co-evolved with their hosts during evolution and therefore even closely

related human and rodent viruses are significantly different [14,15]. Herpesviruses can be divided into α-, β- and γ-subgroups, and viruses of all these have been explored in HIS mice.

Herpes simplex viruses (HSV) are α-herpesviruses and establish usually latency in peripheral neurons, from which they periodically reactivate and form genital and labial blisters. HSV-2 infection has been explored in HIS mice. Intravaginal infection with HSV2 was found to elicit lymphocyte infiltration at the site of infection in huBRG mice [16]. Moreover, HSV-2 specific T cells were detected at the infection site and in draining secondary lymphoid tissues. Furthermore, some IgG responses to HSV-2 were reported and, eliciting adaptive immunity by vaccination prior to HSV-2 infection, lowered viral titers after infection. These studies suggested that HIS mice can respond to HSV-2 infection by innate and adaptive immune responses, which in part are specific for this virus and seem to control infection. However, in these studies it remained unclear which murine and human cells were infected and how the human leucocytes were able to immune survey mouse tissues and controlled HSV2 within these.

Among the β-herpesviruses, human cytomegalovirus (HCMV) infection was explored in huNSG mice [17]. In a first study HCMV was found to infect the reconstituted human myeloid compartment and could be reactivated from it by G-CSF treatment. However, initial infection was performed in this study by adoptive transfer of HCMV infected fibroblasts into the peritoneal cavity of huNSG mice. Therefore, in a follow-up study NSG mice were reconstituted with mobilized human adult $CD34^+$ HPCs from either 3 HCMV positive or 1 negative donor [18]. HCMV infection could be transferred into huNSG mice with hematopoietic stem cells of HCMV positive, but not negative donors. From both studies the authors suggested that G-CSF mobilization of HPCs in adult patients for transplantation could mobilize HCMV and increase the risk of diseases that are associated with this virus in the respective immunosuppressed patient population.

Finally, both human γ-herpesviruses have been tested in HIS mice [19]. The γ1-lymphocryptovirus Epstein Barr virus (EBV) was the first pathogenic challenge that was used in

HIS mice [20]. EBV establishes latent infection in human B cells and is associated with B cell lymphomas as well as epithelial carcinomas in patients [21]. In order to do so the virus capitalizes on the physiological differentiation of B cells, while infection of epithelial cells in vivo is much less well understood. While the expression of all eight EBV latency gene products (latency III) can be found in naïve B cells in humans [22], germinal center B cells carry primarily the reduced latency II program with expression of EBV nuclear antigen 1 (EBNA1), latency membrane proteins (LMPs) 1 and 2, as well as the non-translated RNAs, including EBV encoded small RNAs (EBERs) and micro RNAs. It is thought that EBV uses these different latency programs in order to push infected B cells towards memory cell differentiation [23], which constitutes the site of long-term EBV persistence [24]. Upon cognate antigen recognition via their B cell receptor and plasma cell differentiation, EBV reactivates from infected B cells into the lytic program, which then expresses more than 80 gene products to form virus particles [25]. Of these EBV infection programs, mainly latency III and lytic replication, can be accessed in HIS mice, and B cells are the main, if not exclusive cell type that harbors EBV after infection [26]. Furthermore the tumors that develop at increased frequencies after T or NK cell depletion are primarily lymphoproliferative lesions of EBV latency III expressing B cells, which can be cultured as lymphoblastoid cell lines (LCLs) in vitro [26,27]. These tumors can develop in secondary lymphoid tissues like spleen and mesenteric lymph nodes or at extra-lymphoid sites like liver and kidneys. Irrespective of their location, they are usually heavily infiltrated with T cells, and polyclonal or oligoclonal in nature [27]. However, loss of immune control allows more frequently the outgrowth of more aggressive monoclonal tumor cells. These EBV latency III infected cells constitute the majority of the EBV infected B cells in mice after infection with the B95-8 EBV strain, which was originally isolated from an American patient with symptomatic primary infection. The lymphocyte infiltrates are significantly reduced during infection with a B95-8 strain that lacks the latency III gene product EBNA3B [28]. These tumors then histologically resemble diffuse large B cell lymphomas (DLBCL) and a subset of DLBCL in human patients harbor EBV

with loss-of-EBNA3B-expression mutations. Apart from EBNA3B, however, they express the rest of the EBV latency III program. The difference in lymphocyte infiltration is in part due to the failure of mutant EBV transformed B cells to express the chemokine CXCL10, which attracts lymphocytes to the tumor microenvironment [28]. In addition to EBV latency III cells, a smaller subset of infected cells spontaneously enters lytic infection, and the frequency of these cells is also elevated after T and NK cell depletion [26,27]. The frequency of these lytically EBV infected B cells also increases after infection with another EBV strain, called M81 and isolated from an Chinese patient with EBV associated nasopharyngeal carcinoma [29]. Both early and late lytic EBV antigen products are expressed in these reactivating B cells [27,29]. In addition, several groups have reported that lower EBV latencies can be also observed in HIS mice. Histologically, both latency I and II have been observed [30-32]. However, lack of EBV antigen expression can be sometimes difficult to ascertain by immunohistochemistry. For example LMP1 expression is cycling in LCLs and only one third of these EBV latency III expressing B cells are positive for LMP1 protein at any given time point [33]. In an attempt to search for these lower EBV latencies with a different approach Heuts, Nagy, Klein and colleagues have recently reported Q promotor usage for EBNA1 expression in infected HIS mice [34]. EBNA1 is transcribed into a long mRNA also encoding other EBNAs during latency III, but utilizes its Q promotor for transcription during latency I and II. This Q promotor usage was dependent on $CD4^+$ T cells, suggesting that follicular helper T cells might allow EBV infected B cells to access lower EBV latencies during low levels of germinal center reactions in HIS mice. Therefore, EBV infection of HIS mice might primarily cause latency III, but also allow low levels of lytic virus replication and maybe even EBV latencies I and II, which cannot be modelled by in vitro B cell infection with EBV. In addition to these EBV associated lymphoproliferations also immunopathologies have been observed after EBV infection in HIS mice. These include hemophagocytic lymphohistiocytosis and erosive arthritis [35,36]. In these studies, the Akata strain of EBV, which was originally isolated from a Japanese patient with Burkitt's lymphoma, was used. If these EBV associated disease

manifestations, however, resulted from this strain variation, higher initial infectious doses or prolonged infection periods requires additional investigations. Nevertheless, EBV associated malignancies seem to be not the only EBV associated diseases that can be modelled in HIS mice.

In contrast to the extensive literature on EBV infection, the human γ2-herpesvirus Kaposi Sarcoma associated herpesvirus (KSHV) has been much less explored in HIS mice. This virus is also associated with B cell lymphomas and the endothelial malignancy Kaposi sarcoma in humans [37]. An early report suggested that KSHV can establish persistent infection in NOD-*scid* mice [38]. This seemed independent of human immune component reconstitution and be supported by various murine leucocyte lineages. However, a more recent study suggested that KSHV infection without human lymphocyte reconstitution is minimal [39]. In this later study KSHV infection was primarily contained in human B cells, but could only be maintained by biweekly KSHV injection into BLT mice. Furthermore, no KSHV associated pathology was reported in infected HIS mice. Thus, persistent KSHV infection and virus associated malignancies are still difficult to achieve in HIS mice.

Nevertheless, HIS mice constitute an extremely valuable in vivo system to study human herpesvirus infections, which are so exquisitely adapted to their human host.

2.2. Adenovirus infections (Adenovirus)

In the above discussed studies only the HSV infection probably involves both human and mouse cells in HIS mice. Therefore, hepatotropic adenovirus infection was explored to investigate the extent, to which the reconstituted human immune system components can survey infections of mouse somatic tissues [40]. These non-enveloped viruses, of which seven species and more than fifty types exist in humans, cause usually upper respiratory tract infection. While the adenovirus tropism differs in mice, it was found that T cells contribute to the immune control of adenovirus in the liver in HLA transgenic mice. These data suggest that human immune

responses to pathogens with tropism for mouse tissues can also be studied in HIS mice to a certain degree.

2.3. Hepadnavirus infections (hepatitis B virus)

While the immune control of adenovirus infection in HIS mice relied on the cross-talk between the human hematopoietic lineage and mouse liver cells, hepatitis B virus (HBV) infection requires in addition reconstitution of human hepatocytes. In such a model of human immune system and liver reconstitution persistent HBV infection could recently be established [41]. In humans, HBV causes liver inflammation, fibrosis and due to chronic immune stimulation hepatocellular carcinoma. Some of these pathologies could be recapitulated in HIS mice. HBV infection elicited human immune responses, liver inflammation and liver fibrosis, which in part was driven by M2 polarized human macrophages. Thus HIS mice with human hepatocyte reconstitution can reproduce some features of HBV infection and immunopathology.

3. RNA virus infections

3.1. Retrovirus infections (HIV, HTLV-1)

So far HIS mice have been most often studied in the context of human immunodeficiency virus (HIV) infection, a lentivirus that depletes its main host cell, $CD4^+$ T cells, during chronic infection, resulting in the acquired immunodeficiency syndrome (AIDS). In fact the respective literature is so extensive that its discussion goes beyond the scope of this review. Moreover, it has been summarized recently in eight reviews in the Journal of Infectious Diseases [42]. Therefore, I will just concentrate on some high-lights from the studies of HIV infection in HIS mice. Both CCR5- and CXCR4-tropic HIV-1 strains have been found to replicate in HIS mice and cause $CD4^+$ T cell depletion [43,44]. To some extent, especially BLT mice on the NOD-*scid* background also allow rectal infection via mucosal transmission [9,45]. From the initial side of infection HIS mice were also used to investigated HIV dissemination [46]. Interestingly, it was found that primarily

infected T cells disseminate HIV and if their egress from secondary lymphoid tissues was blocked, systematic HIV infection was inhibited. During this systematic infection also the viral quasispecies formation by mutations could be monitored [47]. Furthermore, HIS mice establish a latent HIV reservoir in resting $CD4^+$ T cells [48-50]. Due to the recapitulation of these different aspects of HIV infection in humanized mice, therapeutic strategies against this retrovirus have been tested. These include anti-retroviral therapy (ART) [51,52], rectal microbicide treatment [53], anti-viral siRNA expression in T cells [54], CD4 and chemokine receptor knock-down or knock-out to reduce HIV infection [55,56] and adoptive transfer of HIV specific antibodies, either cocktails of broadly neutralizing, toxin conjugated or expressed from viral vectors [47,57,58]. Thus, depending on the particular mouse model various aspects of primarily T cell infection can be modelled in HIS mice and the efficacy of therapeutic interventions can be tested in this setting.

Apart from HIV, human T cell leukemia virus (HTLV) – 1 infection was explored in HIS mice. In all instances infection was transferred by HTLV-1 harboring cells, either with in vitro infected $CD34^+$ HPCs or with lethally irradiated infected T cells [59-61]. In all three studies expansions of HTLV-1 infected $CD25^+CD4^+$ T cells reminiscent of human adult T cell leukemia (ATL) were found. Therefore, HIS mice might serve as a valuable model to investigate HTLV-1 induced pathology and tumor formation, even so the infection has to be done in a somewhat artificial way by intraperitoneal or intrahepatic transfer of infected cells.

3.2. Flavivirus infections (dengue virus, HCV)

Two members of the Flavivirus family have been investigated in HIS mice, namely dengue virus and hepatitis C virus (HCV). Dengue virus infection was performed by intraperitoneal, subcutaneous of intravenous injection into HIS mice [62-66]. In most instances dengue serotype 2 viruses were used. Weight loss, skin rash, fever and thrombocytopenia were observed after infection. The most significant pathology associated with dengue virus infection in humans,

however, is dengue hemorrhagic fever after heterologous infection with another dengue virus serotype and it is thought that heterologous antibodies enhance infection by the second phenotype to cause this severe disease [67]. However, in HIS mice primarily IgM responses were so far detected after infection [64,66], and it remains unclear if these could mediate antibody mediated enhancement of heterologous dengue virus infection. Modelling of this disease would be the most valuable application of HIS mice to this viral infection.

While dengue virus infects leucocytes, possibly primarily myeloid cells [63], in HIS mice, establishing HCV infection in these models is much more difficult, because this virus infects primarily human hepatocytes. Moreover, it causes chronic liver inflammation that causes hepatocellular carcinoma in a subset of virus carriers. Due to the liver tropism of this virus both human immune system components and human hepatocytes need to be engrafted into mice in order to study their interaction. This was performed in BRG mice, in which liver damage was induced, by transfer of hepatocyte precursors and HPCs [68]. These mice were then infected with HCV intravenously [69]. Both HCV induced liver damage and induction of HCV specific immune responses could be observed. However, human hepatocyte engraftment was limited in this study, and, therefore, the observed liver pathology requires further clarification. While this is a very interesting model the requirement of autologous hepatocyte precursors and HPCs for its generation might limit its broader application.

3.3. Polyomavirus infections (JC)

The polyomavirus John Cunningham (JC) virus has lately moved into the spotlight of the biomedical research community, because it was identified as the etiological agents of progressive multifocal leucoencephalopathy (PML), an inflammatory disease of the central nervous system (CNS), which is caused by JC virus reactivation after therapeutically limiting leucocyte access to the CNS, for example during natalizumab therapy against multiple sclerosis (MS) [70]. In order to understand JC virus specific immune control better, HIS mice were

intraperitoneally injected with JC virus [71]. This resulted in persistent infection and the virus could be detected in blood and urine for more than three months. While infection with a PML derived virus was more aggressive and led to more frequently detected viral titers and elevated IgM responses, no PML or CNS infection could be observed. Therefore, it remains unclear if HIS mice can be used to recapitulate PML pathogenesis.

3.4. Orthomyxovirus infections (influenza)

Another virus with mixed human and mouse tropism after infection of HIS mice is the orthomyxovirus influenza A virus. This segmented RNA virus causes lung infections and pandemics, especially when its genome segments rearrange to new combinations that have not, or not for a long time, been seen by the human population. Also its tropism in human cells was not characterized in detail, infection with live attenuated influenza A virus resulted in influenza antigen presentation on both $CD1c^+$ and $CD141^+$ dendritic cell (DC) subsets [72]. However, only $CD1c^+$ DCs were able to prime influenza specific $CD8^+$ T cell responses that can efficiently home back to epithelial tissues, including the lung. Moreover, influenza A virus infection elicited innate immune recognition in HIS mice, particularly when the lung myeloid compartment was better reconstituted after transgenic introduction of human GM-CSF and IL-3 into HIS mice [73]. Therefore, lung epithelial cell infection by influenza A virus can interact with reconstituted human immune system components in HIS mice, even so it remains unclear how many and which human leucocytes are directly infected with the virus under these conditions.

4. Immune surveillance in mice with reconstituted human immune responses

Overall, HIS mice develop within the three months after human $CD34^+$ HPC injection that most investigators wait for reconstitution before experimentation, a fairly immature human immune system. Indeed the B cell compartment consists mainly of transitional and naïve B cells [74,75]. Furthermore, the NK cell compartment harbors CD56 negative cells and the frequency of killer

immunoglobulin like receptor (KIR) positive cells is only between 10 and 20% [27,76]. These characteristics are reminiscent of immune compartments in human cord blood, indicating that HIS mice have the immune systems of newborns. Accordingly, they are compromised in their ability to make humoral immune responses and primarily IgM antibodies against viruses can be found after infection. This was reported for huNSG, huBRG and BLT mice after HIV, HSV-2, adeno-, EBV, dengue and JC virus infection [16,40,43,62,66,77,78]. Consistent with these findings, the B cell areas of secondary lymphoid tissues of HIS mice do not show germinal center development [79], but can develop these structures after prolonged or repeated immunization [75,80]. In these structures, B cell differentiation beyond transitional and naïve B cells can be found and significant numbers of follicular helper T cells are present [75]. Also in line with a young immune system, NK cells efficiently restrict primary viral infections, like EBV, which are better handled in children than adults, while further differentiation by IL-15 injection compromises this innate immune control in HIS mice [27]. In general, cell mediated immune responses are much better developed in HIS mice than humoral immune responses. These can be initiated by DC populations, which are efficiently reconstituted in HIS mice and can be expanded by Flt3 ligand injection [79,81]. Both conventional and plasmacytoid human DCs respond to pathogen associated molecular patterns (PAMPs), which bind to toll-like receptors (TLRs), with the specificity that is expected from the more restricted and DC subset specific TLR expression in humans [79]. Human cytokines and chemokines that are produced by these DC populations as well as innate lymphocytes that rapidly respond to their activation can be measured one to three days after dengue virus infection, and 11 hours after TLR agonist administration [66,79]. IFN-α2, IL-10 and CXCL10 correlated with the detected dengue viral titers [66]. Interestingly, mouse and human cytokines could be independently stimulated by TLR ligands, depending on the species specific TLR distribution [79]. Apart from the DCs' ability to activate NK cell responses [76], they can also elicit $CD4^+$ T cell responses [79] and presumably are involved in $CD8^+$ T cell priming. Such $CD8^+$ T cell responses have been reported during

EBV, HIV, adeno-, influenza and dengue virus infection of HIS mice [26,40,62,63,66,72,78,82-84]. The presence of HLA class I transgenes or HLA expressing human thymic tissue seems to favor the development of $CD8^+$ T cell responses during viral infections, which recognize dominant T cell epitopes defined in human virus carriers [26,62,82-84]. Mutational escape from these suggest that they are protective in vivo during HIV infection [83]. More directly, $CD4^+$ and $CD8^+$ T cells have been depleted with specific antibodies prior to some viral infections of HIS mice. These depletion studies indicate that during EBV infection both $CD4^+$ and $CD8^+$ T cells seem to contribute to immune control [26,85]. Furthermore, $CD8^+$ T cells have been implicated in HIV and adenovirus specific immune control in HIS mice [40,86]. However, protective T cell specificities need to be defined in these human in vivo systems of viral infections in more detail in order to identify suitable antigens for vaccination. Overall, HIS mice seem to mount both innate and adaptive cellular immune responses to viral infections. While these are probably not able to control the respective pathogens as well as human individuals, they exert some degree of immune control and can therefore be interrogated for their protective functions in vivo. Moreover, additional genetic manipulations might be able to overcome some of the most significant short-comings of HIS mice, including secondary lymphoid tissue structure formation, cytokine and chemokine incompatibilities between mouse and man, and limited reconstitution of mucosal sites. Such improvements might then broaden the usefulness of HIS mice for vaccine development, allowing formulations to be tested also for the induction of humoral immune responses and protection at mucosal sites.

5. Conclusions and outlook

HIS mice constitute a valuable in vivo model for important human pathogens with a restricted tropism for the human hematopoietic lineage. They sustain infection with a number of these (HIV, EBV, HCMV, dengue and HTLV-1 for example). In addition, human cell-mediated immune responses can be studied in these models. However, many more hurdles have to be overcome

to develop HIS mice into robust in vivo model systems for human immune responses. The biggest is probably to obtain several genetically defined sources of human hematopoietic progenitor cells (HPCs) for their reconstitution. Induced pluripotent stem (iPS) cells bear great promise to become such sources [87], but efficient differentiation into HPCs for HIS reconstitution has not been achieved yet. The other short-comings, like deficient humoral immune responses and limited mucosal homing of reconstituted human immune system components are starting to be overcome [9,75], but require much more work to develop robust systems. Finally, more tools for human HPC manipulation need to be developed in order to manipulate and monitor human immune responses to viral infections more specifically in HIS mice. These include T cell receptor transgenes to allow the development of reporter T cell populations during viral infection, and recombinant viral vectors to knock-down, knock-out or overexpress certain genes in the developing hematopoietic lineage. Together with recombinant manipulation of the investigated human pathogens [27,28,88], the manipulation of human immune system components will generate potent preclinical models for relevant human diseases, which might yield results that can be more easily translated into the clinic.

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Figure legend

Figure 1: Human viral infections in mice with reconstituted human immune system components. NOD-*scid* $\gamma_c^{-/-}$ (NSG), NOD Rag1$^{-/-}$ $\gamma_c^{-/-}$ (NRG), BALB/c Rag2$^{-/-}$ $\gamma_c^{-/-}$ (BRG) or NOD-*scid* with a human fetal thymus and liver organoid (LT) mice are injected with human HPCs for human immune system component reconstitution (HIS mice). Usually this reconstitution is allowed to proceed for three months. After analyzing reconstitution efficacy in the peripheral blood, HIS mice are infected with human viruses by intravenous, intraperitoneal or subcutaneous injection. The DNA viruses HBV, HSV-2, HCMV, EBV, KSHV and adenovirus, as well as the RNA viruses HIV, HTLV-1, dengue virus, HCV, JC virus and influenza A virus have been used for HIS mouse infections. These infections usually do not continue for more than 3 months. After this time period viral titers, virus induced pathology and primarily cell-mediated virus-specific immune responses are characterized.

Table 1. Viral pathogens of HIS mice.

Virus	Pathology in HIS mice	Key reference[1]
Hepatitis B virus (**HBV**)	Liver disease	[41]
Herpes simplex virus 2 (**HSV-2**)	Inflammation at mucosal infection site	[16]
Human cytomegalovirus (**HCMV**)	GM-CSF mediated reactivation from myeloid cells	[17]
Epstein Barr virus (**EBV**)	Tumor formation, hemophagocytic lymphohistiocytosis, erosive arthritis	[89]
Kaposi Sarcoma associated herpesvirus (**KSHV**)	Persistent infection in B cells	[39]
Adenovirus	Liver disease	[40]
Human immunodeficiency virus (**HIV**)	$CD4^+$ T cell depletion	[43]
Human T cell leukemia virus 1 (**HTLV-1**)	Akute T cell leukemia (ATL) like T cell expansions	[59]
Dengue virus	Leucocyte infection	[66]
Hepatitis C virus (**HCV**)	Liver disease	[69]
John Cunningham (**JC**) virus	Persistent infection	[71]
Influenza A virus	Lung infection	[72]

[1] For clarity only one key reference has been listed

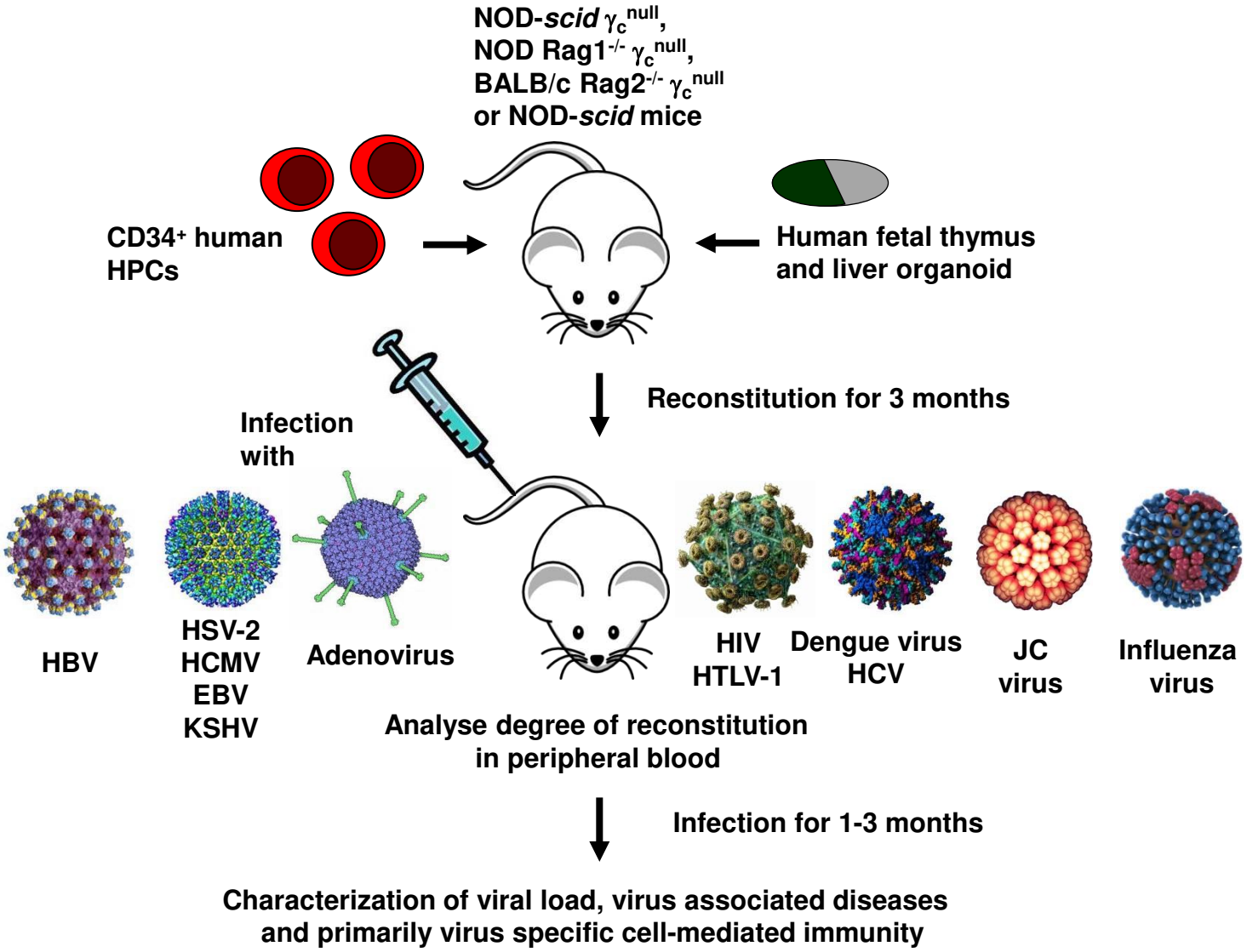
NOD-*scid* γ_c^{null},
NOD Rag1$^{-/-}$ γ_c^{null},
BALB/c Rag2$^{-/-}$ γ_c^{null}
or NOD-*scid* mice
CD34$^+$ human
HPCs
Human fetal thymus
and liver organoid
Reconstitution for 3 months
Infection
with
HBV
HSV-2
HCMV
EBV
KSHV
Adenovirus
HIV
HTLV-1
Dengue virus
HCV
JC
virus
Influenza
virus
Analyse degree of reconstitution
in peripheral blood
Infection for 1-3 months
Characterization of viral load, virus associated diseases
and primarily virus specific cell-mediated immunity

Figure 1