Published in final edited form as:

Annu Rev Genet. 2015 ; 49: 21–45. doi:10.1146/annurev-genet-112414-054823.

Genetic Dissection of the Host Tropism of Human-Tropic Pathogens

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Abstract

Infectious diseases are the second leading cause of death worldwide. Although the host multitropism of some pathogens has rendered their manipulation possible in animal models, the human-restricted tropism of numerous viruses, bacteria, fungi, and parasites has seriously hampered our understanding of these pathogens. Hence, uncovering the genetic basis underlying the narrow tropism of such pathogens is critical for understanding their mechanisms of infection and pathogenesis. Moreover, such genetic dissection is essential for the generation of permissive animal models that can serve as critical tools for the development of therapeutics or vaccines against challenging human pathogens. In this review, we describe different experimental approaches utilized to uncover the genetic foundation regulating pathogen host tropism as well as their relevance for studying the tropism of several important human pathogens. Finally, we discuss the current and future uses of this knowledge for generating genetically modified animal models permissive for these pathogens.

Keywords

pathogen host tropism; virus; bacteria; genetic engineering; humanized mice; animal models

INTRODUCTION

A recent update on the global burden of disease estimated that approximately 16% of deaths in females and 17% in males can be attributed to infectious diseases, making this the second leading cause of death worldwide next to cardiovascular diseases (154). The pathogens responsible for these diseases are a diverse group of viruses, bacteria, and parasites, many of which broadly affect human populations. A staggering number of individuals—approximately 500 million—are chronically infected with hepatitis B (HBV) or C (HCV) viruses, leaving them with an increased risk for developing severe liver disease. Despite health education efforts, there are still 33 million individuals living with HIV, leading to 1.7

DISCLOSURE STATEMENT

Dr. Ploss is co-inventor on a patent covering the use of human occludin to generate animal models for HCV. All authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

million AIDS-related deaths every year. Of the approximately 400 million people who contract dengue virus (DENV) annually, almost 100 million present with clinical symptoms. Forty percent of the world's population is infected with human cytomegalovirus (HCMV), which can be lethal in newborns and immunocompromised individuals. Parasitic infections also significantly contribute to the global burden of disease. In 2013 alone, approximately 120 to 280 million people were diagnosed with malaria caused by *Plasmodium* parasites, resulting in more than half a million deaths. Additionally, bacterial pathogens continue to remain an important health issue worldwide. Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, killed 1.5 million of the 9 million individuals infected in 2013. *Vibrio cholerae*, the causative agent of cholera, is responsible for 100,000–120,000 deaths per year. Importantly, the continuing emergence of multidrug-resistant (MDR) bacteria can double a patient's death rate compared with patients infected by the same nonresistant bacteria.

Many of the pathogens responsible for these diseases and infections are highly restricted in their species tropism, infecting only humans and closely related nonhuman primates (NHPs) (Figure 1). This restricted tropism creates significant difficulties in understanding the biology of these pathogens in vivo and seriously hampers the development of effective therapeutics. To overcome such difficulties, the development of small animal models recapitulating the life cycle of these pathogens is critically needed. However, the generation of such in vivo platforms requires the identification of the molecular determinants governing the species-specific tropism of these human-tropic pathogens.

The host tropism of a pathogen can be dictated in a variety of ways. A given organism may not possess appropriate molecular factors or may have incompatible orthologs of factors needed for successful infection, such as receptors for a specific virus to enter into a host cell or a bacterium to properly adhere and invade. Additionally, the presence of host restriction factors can block steps of the pathogen's life cycle, such as its replication within host cells. Finally, the varying abilities of a pathogen to evade and/or disrupt the immune response of a given host can also contribute to species tropism.

Although understanding the limited host range of many human-tropic pathogens remains a daunting task, there are a growing number of techniques available for tackling this significant and highly relevant problem. In this review, we cover a variety of methods that have been implemented both in vitro and in vivo to tease apart the various molecular needs of human-tropic pathogens and the barriers that exist in nonpermissive species. Although it is impossible for the following discussion to be fully comprehensive, the aim is to focus on particularly telling examples, primarily in the study of viral and bacterial pathogens.

APPROACHES TO DISSECT THE LIMITED HOST RANGE OF PATHOGENS

Over the past decades, several approaches have been developed to improve our understanding of the host range of pathogens. Identifying the pathogen and host determinants that regulate the host tropism of pathogens has been of particular interest. Several experimental strategies have been implemented for this purpose, such as cell

engineering, generation of host-adapted pathogens through direct engineering or assisted evolution, and genome-wide genetic screens both in vitro and in vivo (Figure 2).

Uncovering Host Regulators of Pathogen Infection

From the use of interspecies heterokaryons to genome-wide loss-of-function genetic screens, an important number of experimental approaches have been employed to uncover major host regulators of pathogen infection. In this section, we present an overview of such methods with an emphasis on their use for genetically dissecting the host tropism of human-tropic pathogens.

Interspecies heterokaryons—The existence of species-specific factors and genetic determinants that promote or restrict pathogen infection is now a well-established fact. Interspecies heterokaryons (IHs), large cells generated through the experimental induction of fusion of cell types belonging to different species, have been intensively used for almost two decades to determine whether there are positive or negative regulators of a pathogen's life cycle within a given species (Figure 2). Briefly, an aspect of a pathogen's life cycle, such as replication, can be assessed in IHs formed between permissive and nonpermissive cells. A pathogen that can replicate in these IHs suggests that the nonpermissive cell line is lacking a specific factor or has an incompatible ortholog. In contrast, the absence of replication indicates that potential restricting factors are present within this cell line. The early observation that HIV-1 infection varied across animal species, even among closely related primate species, hinted that restriction factors present in some species might regulate HIV-1 host tropism (84). Among all the possible approaches, IHs were heavily utilized to strengthen the evidence for the existence of such factors in primates, ultimately enabling their identification. For example, the inability of HIV-1 to infect macaques is attributed in part to specific orthologs of proteins that target the HIV-1 capsid (22, 47, 48, 68, 101). However, the human orthologs but not the primate orthologs of other restriction factors can be specifically antagonized by the HIV-1 viral accessory proteins Vif (81, 131) and Vpu (147).

Similarly, HCV also has a narrow host tropism, restricted to humans and chimpanzees (82). Mouse cell lines are refractory to HCV infection due to restrictions at the level of viral entry and replication (76, 107, 143). In 2011, heterokaryons between human and mouse liver cell lines were generated to identify whether dominant negative restriction factors in mouse cells interfered with the HCV life cycle. However, the completion of the HCV life cycle within IHs demonstrated that the narrow tropism of HCV is more likely due to the absence of necessary, human-specific factors in mouse liver cells (38). Influenza viruses have also been examined for their host-restricted tropism. The genetic plasticity of influenza viruses is tightly linked to their ability to replicate within a broad panel of animal species (135). However, the determinants regulating the susceptibility of a given host to influenza viruses are not well understood. For example, the polymerase of avian influenza A virus has poor efficiency in human cells, thus restricting viral replication (86). Yet IHs of human and avian cells still support viral replication, ultimately demonstrating that humans lack a positive regulator of replication needed for effective viral polymerase activity (90).

cDNA complementation screens and comparative transcriptomics—Although the use of IHs is valuable for predicting the presence of positive or negative regulators of pathogen infection, additional approaches are needed to precisely identify the nature of these regulators (Figure 2). Complementary DNA (cDNA) library screens and comparative transcriptomics (through cDNA subtraction or gene arrays) between permissive and nonpermissive cells have been valuable tools to identify critical host-specific determinants of pathogenic infection. The first approach identified HIV-1 restriction factors involved in pre-integration stages of viral replication. The expression in HIV-1 permissive cells of a cDNA library derived from primary rhesus macaque lung fibroblasts identified TRIM5 α as one such factor (137). The macaque orthologs of TRIM5 α were shown to target the HIV-1 capsid and inhibit early steps of infection, thus highlighting this protein as a strong determinant of HIV-1 host tropism. Similarly, following identification of the HCV entry factors CD81 (103), scavenger receptor class B type I (SR-BI) (120), and claudin-1 (CLDN1) (36), murine cell lines expressing these molecules were nonetheless nonpermissive for HCV entry, highlighting the need for an additional entry factor(s). A cDNA screen using a library derived from a permissive human hepatoma cell line (Huh7.5) was applied to a mouse cell line overexpressing the human orthologs of CD81, SR-BI, and CLDN1. This screen identified occludin (OCLN) as an additional necessary factor, and its expression, along with that of CD81, SR-BI, and CLDN1, in mouse and nonpermissive human cell lines enabled HCV entry (107). More importantly, this screen demonstrated that HCV entry requires the human orthologs of both CD81 and OCLN, although SR-BI and CLDN1 could be of human or mouse origin. These findings, in combination with the identification of other human factors necessary for HCV replication and viral particle production (4, 61), permitted the development of engineered murine cell lines that allowed completion of the entire HCV life cycle in vitro (37, 148).

Comparative transcriptomics have identified key regulators of HIV-1 host tropism by uncovering restriction factors during the post-integration stages of infection. A cDNA subtraction screen, using cell lines susceptible and nonsusceptible to infection with HIV-1 lacking the accessory protein Vif, identified APOBEC3G as a restriction factor counteracted by Vif in the primary cell target of HIV-1, the human CD4⁺ T cell (126). Importantly, HIV-1 Vif is unable to counteract primate APOBEC3G orthologs (83). The restriction factor CD317 (also known as tetherin), which inhibits HIV-1 particle release, was also identified by comparative transcriptomics using a microarray approach (95). In cells treated with interferon- α (IFN- α), the absence of the HIV-1 protein Vpu induced an accumulation of HIV-1 particles at the cell surface (40, 64, 93, 94). Microarray analyses of cell lines treated or untreated with IFN- α revealed that Vpu counteracts the inhibitory action of human tetherin. However, primate tetherin is unaffected by Vpu. Indeed, the simian counterpart of HIV-1, simian immunodeficiency virus (SIV), uses a different accessory protein, Nef, to counteract primate tetherin orthologs (84, 163).

Whole-genome genetic screens—Many of the discoveries made via cDNA complementation assays and comparative transcriptomics were guided by earlier experimental observations and hypotheses. However, even in the absence of preliminary experimental evidence, determinants of host tropism can still be uncovered via other routes.

For this purpose, whole-genome genetic loss-of-function screens are of particular interest (Figure 2). Although small interfering RNA (siRNA) and small hairpin RNA (shRNA) screens have proven useful for identifying host factors important for pathogen propagation, the varying knock-down efficiencies and off-target effects of these techniques pose challenges. More recently, haploid screens or small noncoding RNA-based screens have found traction and address some of these shortcomings.

Haploid screens: The diploid genomes of mammals are an important limitation in large-scale genetic screens that aim to robustly disrupt gene expression and identify genes involved in key biological processes. Haploid screens have recently emerged as an innovative strategy to overcome this limitation. Using a human chronic myeloid leukemia cell line harboring a haploid karyotype except for chromosome 8, a large-scale genetic screen was established to identify host genes involved in susceptibility to pathogens. To disrupt gene expression, a retrovirus-mediated gene inactivation strategy was employed whereby tags are inserted into genes, thus permitting the identification of depleted genes. Importantly, this screen identified host factors important for influenza infection as well as those involved in the cytotoxic effects of bacterial toxins (13).

Haploid screens have also contributed to the understanding of host factors regulating viral entry. In a genome-wide haploid genetic screen, the cholesterol transporter Niemann-Pick C1 (NPC1) was identified as a critical host factor for Ebola virus (EBOV) entry into host cells (15). Another hemorrhagic fever virus, Lassa virus, infects its target cells by binding to glycosylated α -dystroglycan (α -DG). A recent haploid screen identified several host factors involved in the glycosylation of α -DG (58). Interestingly, despite virus engagement with α -DG, avian cells remain resistant to Lassa virus infection while human and rodent cells are permissive to virus propagation. A second haploid screen found that the sialyltransferase ST3GAL4 was critical for the interaction between Lassa surface glycoproteins and the host's lysosome-resident protein LAMP1 (57) during viral entry. This interaction depended on a single amino acid residue present in rodent LAMP1 but absent in the avian ortholog, thus possibly explaining the resistance of avian cells to infection.

Despite their utility, it is worth noting that the genetic instability of haploid cells may hamper the identification of important host factors. Haploid cell screens have long been limited by the small number of available cell types suitable for studying infection of particular human-tropic pathogens. However, the number of experimental systems and the resolution of haploid screening strategies have recently been improved (14). Additionally, the recent generation of haploid embryonic stem (ES) cells from mice (71) and macaques (157) could permit additional genetic screens for deciphering the determinants of mammalian susceptibility to pathogens, although this remains to be shown. If these ES cells cannot be utilized as is, they would need to be differentiated into a mature tissue type, even though many of these types do not continuously proliferate and thus are not amenable for high-throughput screens.

RNA-based screens: Targeting the host transcriptome represents a valuable strategy for uncovering host factors regulating the life cycle of challenging pathogens. In particular, studies using siRNA or shRNA libraries have identified regulators of viral infection, such as

HIV-1 (9, 67, 159, 166). Three studies using siRNAs in HIV-1-permissive cell lines unveiled a number of host dependencies critical for particular steps of the HIV-1 life cycle, such as viral entry and transcription (9, 67, 166). Although a comparative analysis demonstrated a lack of overlap between the hits of these three screens, their respective findings were consistent in terms of gene ontology (12). siRNA-based screens have also been applied to the search for factors regulating members of the family *Flaviviridae*, such as West Nile Virus (WNV), DENV, and HCV. Although several siRNA screens have focused on particular host targets involved in the HCV life cycle (5, 111, 118, 139), two major, whole-genome siRNA screens to date have been performed for uncovering HCV host dependencies (74, 140). One study focused on host factors regulating viral RNA replication through the use of cell lines expressing a selectable viral RNA—a so-called HCV replicon. Thereby, the authors were able to identify 96 factors regulating HCV replication (140). By contrast, a second study employed an infectious HCV clone able to replicate in cell culture to perform a whole-genome siRNA screen, thus allowing the identification of a number of important host factors regulating different stages of the HCV life cycle (74). The impact of these factors on defined steps of the viral life cycle was recently interrogated and challenged in vitro (75). Regarding the other members of the *Flaviviridae* family, similar siRNA screens also unveiled important host regulators of WNV and DENV infection. These factors were involved in endocytosis and intracellular trafficking as well as in antiviral responses in mammalian cells (69) and *Drosophila* (123, 158). Recently, an original in vivo siRNA screen approach delivered an siRNA library into mice using a replication-competent Sindbis virus, an RNA virus from the family *Togaviridae*. As a result of natural selection, viral variants carrying siRNAs advantageous for viral replication survived, thus uncovering direct effectors or negative host regulators of the antiviral response (146). In the future, genome-scale CRISPR-Cas9 knockout screens (124) (see Engineering Genomes In Vivo), which have a unique robustness compared to small RNA-based screens, may be employed to uncover new host regulators of pathogen infection.

Identifying and Challenging Pathogen Determinants Regulating Host Tropism

Although host species harbor an important set of factors that positively or negatively regulate pathogen tropism, pathogens also carry genetic determinants that strongly regulate their tropism. Various experimental approaches have been employed over the past decades to identify pathogen regulators of host tropism. Here, we describe some of the methods that identified important pathogen-mediated mechanisms regulating host tropism.

Pathogen adaptation—The adaptation of pathogens in vitro or in vivo to new host species remains a relevant strategy for identifying pathogen determinants that regulate host tropism (Figure 2). In 2010, an HCV molecular clone was serially passaged in a human cell line with limited expression of endogenous CD81 but ectopic expression of mouse CD81. Three mutations were identified in HCV envelope glycoproteins that allowed HCV to overcome its inability to use mouse CD81 (7). Another study, in which serum from HCV-infected patients was passaged in chimpanzees, selected for viral variants with low replication rates but high virus production and an increased fitness for viral persistence (117).

Rhesus macaques are resistant to HIV-1 infection due to the restriction factor, rhTRIM5 α , which targets the HIV-1 capsid protein following virus entry. Although HIV-1 can counteract the human version of TRIM5 α , the selection of HIV-1 capsid mutations through assisted evolution permitted rhTRIM5 α restriction to be overcome and subsequently allowed HIV-1 replication in primary rhesus macaque lymphocytes (133). Similar in vivo passaging of HIV-1 in pig-tailed macaques has resulted in another modified virus, which can antagonize macaque restriction factors, induce CD4⁺ T-cell depletion and cause AIDS-like disease (49). Recent gain-of-function experiments have also demonstrated, through serial passages in ferrets, the emergence and selection of avian H5N1 influenza A variants that can be spread between mammals via airborne transmission (52, 55). Several other studies have focused on the adaptation of avian influenza A viruses to replicate in human cells, with mutations identified in subunits of the polymerase complex, in nucleoproteins, and in the novel polymerase cofactor nuclear export protein (86). Using mosquitoes injected with serum from patients infected with Chikungunya virus (CHIKV), adaptive mutations within the CHIKV E1 envelope glycoprotein emerged in vivo. These mutations increased the fitness of CHIKV for replication in mammals and an additional mosquito species (85). Although EBOV is not lethal in experimentally infected immunocompetent mice (8), an EBOV Zaire strain serially passaged in these mice did prove lethal following intraperitoneal inoculation (10).

DENV infection can induce a broad range of clinical manifestations in humans from mild dengue fever to dengue hemorrhagic fever and dengue shock syndrome, with the last two sometimes being fatal. The development of suitable animal models has been hindered because DENV infection of wild-type mice results in a low level of replication and little viremia (162). Although it has not yet been possible to engineer immunocompetent mice susceptible to infection with DENV, mouse-adapted strains of DENV have been generated to robustly infect mice deficient for both IFN $\alpha\beta$ receptor and IFN γ receptor (AG129) (109). Nonadapted DENV serially passaged between mosquitoes and mice induced early death and increased vascular permeability in AG129 mice, although these clinical manifestations were not observed when AG129 mice were infected with the original DENV strain (128). The authors of this study reported that mutations within the E envelope glycoproteins likely mediate this phenotype. In addition, a critical amino acid mutation within NS4B of a mouse nonadapted DENV strain has also been reported as an important determinant of severe, human-like DENV pathogenesis in AG129 mice (42, 122).

Although AG129 mice are commonly used as an in vivo model for DENV infection, the restricted genetic diversity of the viral isolates capable of replicating in vivo and the highly immunocompromised status of the mice limit their utility. Recent work suggests that DENV infection of mice is antagonized by type I IFN signaling in specific myeloid cell populations because mice harboring a targeted disruption of the IFN $\alpha\beta$ receptor exclusively in dendritic cells and macrophages are susceptible to mouse-adapted DENV (167). To facilitate infection with optimally a broader range of mouse nonadapted viral strains, additional refinements of the model are needed.

Despite the feasibility of the pathogen adaptation approach, it should be noted that adaptive mutations that broaden a pathogen's host range may also alter its biological properties (e.g.,

altered host factor usage), potentially hampering the utility of species-adapted pathogens when aiming to conduct a relevant characterization of host-pathogen interactions in vivo.

Genetic engineering of pathogens—Genetic engineering is a convenient strategy for challenging host barriers to pathogenic infection, developing new infection systems, and creating more amenable, cost-effective animal models (Figure 2). As discussed above, HIV-1 replication in macaques is restricted by the inability of HIV to counteract the macaque orthologs of several restriction factors, such as APOBEC3G (83) and TRIM5α (137). Although SIV generally does not induce an AIDS-like disease in most primate species, the macaque-adapted SIV (SIVmac) strain can cause such disease in macaques and baboons. Thus, chimeric SIVmac-based viruses encoding single or several HIV-1 proteins (termed SHIV) have been of great interest in the past two decades for studying HIV-1-like pathogenesis and immune responses in vivo (35, 46, 54, 113). Among the SHIV models generated, SIVmac strains carrying HIV-1 envelope glycoproteins have emerged as one of the most powerful models for host antiviral responses. Recently, an in vivo screen using a mixture of unadapted SHIVs harboring HIV-1 envelope glycoproteins from patients identified variants able to cause AIDS-like disease in rhesus macaques (28). As SHIVs are essentially composed of the SIVmac genome, a virus encoding the HIV-1 genome and the SIVmac Vif proteins has also been generated (called simian tropic-human immunodeficiency virus or stHIV). Expressing SIVmac Vif can counteract the restriction factor APOBEC3G in pig-tailed macaques, with persistent infection detected for several months post-inoculation (50). However, no long-term CD4 depletion or AIDS-like disease could be observed, highlighting the need to counteract other restricting factors. Beyond HIV-1, a single amino acid mutation in the EBOV envelope protein that modulates EBOV host range tropism has been characterized. Interestingly, the mutation abrogates binding to human but not to murine NPC1 (39).

THE IMPACT OF INNATE IMMUNITY ON HOST TROPISM

As discussed above, the host tropism of a given pathogenic agent can be determined by a wide variety of factors, including the immune responses induced by infection. Thus, understanding the human immune response to pathogenic agents appears necessary for fully comprehending host tropism, for identifying points for clinical intervention, and for creating more relevant in vivo models. However, such an understanding remains highly challenging as the magnitude and kinetics of a host's innate immune response against a given pathogen are usually complex and highly specific to the host-pathogen combination.

Numerous viruses are equipped with strategies to specifically evade a host's innate immune response, thus contributing to host tropism. In some cases, researchers have altered the native tropism of a virus by manipulating its interactions with a host's immune response. This is exemplified by work on a nonhuman pathogen, the Poxvirus myxoma virus (MYXV). MYXV naturally infects rabbits, but wild-type mice are resistant to infection due to a strong innate immune response driven by type I IFN. However, MYXV is able to infect STAT1-deficient mice, as this disrupts type I IFN signaling (151). More generally, disruption of IFN signaling within mice (such as in type I IFN receptor-knockout mice) has been shown to render these animals susceptible to infection by a variety of different viruses at far lower

median lethal doses. These viruses include vesicular stomatitis virus (VSV) as well as the vaccinia, Semliki Forest, and lymphocytic choriomeningitis viruses (92).

Having in vivo models is especially relevant for human-tropic pathogens, many of which only infect humans and, in some cases, a limited number of NHP species. The hepatotropic virus HCV is a prominent example, with chimpanzees and humans being the only readily susceptible hosts. As discussed above, the HCV life cycle can be completed in mice that express the viral entry factors human OCLN and CD81 and also have blunted innate immune responses (31).

In establishing infection, the HCV-encoded protease NS3-4A cleaves the host cell's mitochondrial antiviral signaling (MAVS) protein (88) and Toll-interleukin 1 receptor-domain-containing adaptor-inducing IFN β (TRIF) (73). MAVS and TRIF are adaptor proteins of retinoic acid-inducible gene I (RIG-I) and Toll-like receptor 3 (TLR3), respectively. NS3-4A-mediated cleavage of TRIF and MAVS thus effectively interrupts downstream signaling in the RIG-I and TLR3 pathways, which are highly important in triggering the host's innate immune response. Differences in the NS3-4A cleavage site of MAVS that prevent the protein's cleavage in different NHPs may explain, at least in part, the inability of these species to be readily infected with HCV (102).

MAVS and its ability to propagate an immune response are also antagonized by PB2 of the influenza A polymerase complex, and sequence differences of even one residue in PB2 can influence the tropism of human and avian influenza viruses (41, 56, 134), as alluded to above. Finally, DENV is naturally unable to replicate in mice. However, disruption of IFN signaling can permit replication (2, 127). The viral nonstructural protein NS5 binds human STAT2, resulting in its degradation and thus permitting the virus to subvert IFN $\alpha\beta$ signaling (91). However, DENV NS5 cannot bind murine STAT2, allowing the host immune response to inhibit viral replication.

Other host factors have demonstrated evolution over time in response to viral antagonism. Although a given mutation may have been selected in response to an ancient virus, some of these adaptations can contribute to the species tropism of modern-day viruses. For example, when viral double-stranded RNA is detected by protein kinase R (PKR) in mammalian cells, PKR phosphorylates the eukaryotic translation initiation factor eIF2 α , shutting down protein synthesis. Vaccinia virus encodes an eIF2 α mimic, K3L, but the differential evolution of PKR, most likely in response to ancient viruses, has resulted in the varying resistance or susceptibility of primate species to K3L mimicry (34). Similarly, HIV infects humans but not all NHPs. As discussed in the section Approaches to Dissect the Limited Host Range of Pathogens, this tropism can be partially attributed to species-specific differences between restriction factor orthologs such as TRIM5 α , which has likely evolved in response to ancient retroviruses. The resultant orthologs of this protein in Old World monkeys and owl monkeys successfully target the HIV-1 capsid, but those in other New World monkeys and humans cannot (137).

Evasion of host immune responses also occurs following bacterial infections. The bacterium responsible for gonorrhea (which occurs only in humans), *Neisseria gonorrhoeae*, utilizes its

outer membrane porin molecules to bind host C4b-binding protein (C4BP), an inhibitor of the complement system. The complement system is a part of the immune system that facilitates the clearance of pathogens by complementing the action of antibodies. Although C4BP serves the host by preventing the destructive consequences of complement dysregulation, the porin molecules expressed by *N. gonorrhoeae* specifically bind human and chimpanzee C4BP. The bacterium is effectively eliminated in the serum of rodents and rabbits presumably because its porin molecule is unable to bind C4BP in these species (96). Similarly, the choline-binding protein A (CbpA, also called PspC) of *Streptococcus pneumoniae* specifically binds human C4BP (1) in addition to other human factors such as polymeric immunoglobulin receptor (pIgR) (164), factor H (FH) (25), complement C3 protein (132), and secretory IgA (SIgA) (45), contributing to the immune evasion of this pathogen in humans. For *Streptococcus pyogenes*, the interaction of its surface-exposed M protein with human CD46 contributes to the bacterium's host specificity, allowing it to bind human keratinocytes and subsequently invade epithelial cells (24, 115). Some human pathogens, such as *S. pneumoniae*, *Haemophilus influenzae*, *N. gonorrhoeae*, and *Neisseria meningitidis*, more actively disrupt the host immune response by cleaving a major mucosal antibody, human IgA1 (62, 105).

GENETIC HUMANIZATION AS A WAY TO OVERCOME SPECIES BARRIERS

Engineering Genomes In Vivo

To test whether expression of human-specific candidate genes and/or disruption of putatively interfering pathways can render previously nonpermissive species susceptible to human-specific pathogens, numerous genetic manipulations are often necessary (Figure 2). Methods suitable for engineering multicellular organisms with great precision have been refined over the past three decades. Several classical techniques, such as knockout, knockin, and transgenic organisms, have been used to investigate the specific roles of particular genes in disease. Knockouts can be achieved by deleting the first or several coding exons of a gene via homologous recombination, resulting in either a truncated protein or no protein at all. However, some genes are essential in an organism's development, viability, or fertility. As a result, conditional knockouts can be made by recombinase technology using the enzymes Cre, Dre, or Flp, knocking out the gene in a specific tissue and at a specific time. This strategy allows lethality to be circumvented and permits the study of a null phenotype. Conversely, genes can also be knocked in by integration of an exogenous gene at a specific, noncritical site via homologous recombination. This method ensures that any observed phenotype can be attributed with high confidence to the exogenous gene being expressed. Finally, transgenic animals expressing a foreign gene or genes can be created by (a) transfecting ES cells with the desired gene and then implanting these embryos into female mice or by (b) injecting the DNA of interest into the pronucleus of a fertilized embryo (21, 63).

All the above classical techniques are time-consuming and labor-intensive because of the extremely low frequency of recombination (ranging from 1 in 1×10^6 to 1 in 1×10^7) in higher-order eukaryotes. A major breakthrough came with the discovery that the efficiency of gene targeting through homologous recombination could be enhanced by at least two to

three orders of magnitude following the introduction of double-strand breaks (DSBs) in DNA by using rare-cutting endonucleases (116). Several genome-editing tools have since been developed that facilitate increases in DNA DSBs, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR-Cas9). All these techniques can be used to disrupt genes, leading to knockouts, insertions, corrections, and even chromosomal rearrangements. Although each of the above genome engineering tools facilitates DSBs, each does so by a distinct mechanism (see sidebars, ZFNs, TALENS, and the CRISPR-Cas9 System and Current Limitations of ZFNs, TALENS, and the CRISPR-Cas9 System).

ZFNs, TALENs, and the CRISPR-Cas9 system have been used to create gene knockouts and knockins in several mammalian organisms, including mice, rats, pigs, and monkeys. These techniques have especially facilitated more tractable genome engineering in the last three species, which had not been or were only minimally amenable to genetic manipulation. The success rate for gene knockout or knockin varies widely from as little as 10% up to 90% depending on the system and organism used. The CRISPR-Cas9 system has been used to generate mice with five disrupted genes in one step (152). By knocking out multiple genes in a one-step process, there is now the possibility of studying the interaction of multiple genes simultaneously. Using TALENs, the *Scn8a* gene was targeted for mutation to create a mouse model for early-onset epilepsy (60). CRISPR-Cas9 lentiviral knockout libraries for mice have been created along with a Cas9- and Cre-controllable Cas9 transgenic mouse, which will facilitate both knockouts and conditional knockout screens in mice (104, 119). These libraries contain both coding and noncoding regions, allowing the study of genes and their regulatory regions. In pigs, both ZFNs and TALENs have been used to create biallelic mutations in GGTA1 and the low-density lipoprotein receptor, respectively (16, 51). TALENs have also been used to knockout the rat immunoglobulin M locus (141). Recently, two genes, *Ppar-γ* and recombination activating gene 1 (*Rag1*), were ablated in cynomolgus monkeys in one step by injecting specific gRNAs and Cas9 mRNA into one-cell-stage embryos (97). This technology provides the tantalizing possibility of creating knockouts in NHPs, facilitating in-depth studies of gene functions in organisms closely related to humans. Additionally, a gene correction has been made using ZFNs by targeting an X-linked severe combined immunodeficiency mutation in the interleukin 2 receptor common γ chain gene in human cells (144).

Current and Future Potential Achievements Using Genetically Humanized Mice

Over the past 25 years, several achievements have been made in generating genetically engineered mouse models. Expression of specific human factors can increase the susceptibility of these mice to human-tropic viruses and bacteria. These models have led to major advances in our understanding of pathogenesis in vivo in addition to paving the way for new vaccines and treatment strategies (Figure 2). Here, we review some of the work on viral and bacterial pathogens that has benefited from the development of genetically humanized mice and suggest future directions for the studies of additional challenging pathogens.

Applications for studying viral pathogens—One of the first viral pathogens to benefit from the generation of humanized transgenic mice was the poliovirus. In 1990 and 1991, the human poliovirus receptor (PVR) was broadly expressed in vivo in mice, permitting viral replication of multiple poliovirus serotypes within the central nervous system (66, 114). The development of a paralytic poliomyelitis with clinical symptoms similar to those of humans within these mice confirmed the importance of PVR as a host determinant for poliovirus pathogenesis.

Additionally, this work highlighted transgenic mice as a unique way to study poliovirus neurovirulence and immunogenicity for the design of novel vaccine strategies. Indeed, in 2003, a collaborative study approved the use of this mouse model as an alternative to monkeys for testing the neurovirulence of oral poliovirus vaccines (OPVs) (33). However, these mice were only susceptible to infection following intraspinal, and not oral, inoculation. In 2007, PVR-transgenic mice deficient in the IFN α β receptor were generated. These mice were susceptible to poliovirus infection following oral inoculation, demonstrating the role of the innate immune response in regulating poliovirus pathogenesis in the alimentary tract. However, the efficiency of immune responses observed in this model following OPV inoculation was not as high as in the original PVR-transgenic mice (98).

A transgenic mouse model has also been developed for studying the measles virus (MV). Between 1996 and 1998, several groups developed human-transgenic mice expressing the human MV receptor CD46 (27, 53, 65, 106, 108). Ubiquitous expression of this receptor in mice rendered some tissues, such as the lungs and kidneys, permissive in vivo to MV and also activated B and T cells (53). Yet other cell types, such as macrophages, remained nonpermissive to MV infection, indicating the presence of murine-specific factors capable of restricting viral infection. Transgenic expression of human CD46 in the central nervous system of mice made them susceptible to MV infection and neurological disease (125). However, none of these studies were able to recapitulate human clinical features and disease in these mice, which remained incompletely permissive to MV infection. In 1999, human CD46 derived from a yeast artificial chromosome library was expressed in mice. Importantly, the location and level of human CD46 expression in these mice were the closest to those found in humans that had ever been reported. MV was able to replicate in both the central nervous system and in the immune system and could also spread between neurons, causing death in these animals 20 days postinfection (100).

As discussed above, HCV displays a very narrow host tropism, restricting its study in vivo. Different approaches have been taken to generate more tractable small animal models (149). The strategies can be broadly divided into viral and host adaptation approaches. One host adaptation strategy to render nonpermissive species susceptible to HCV in relevant tissues is the xenotransplantation of human liver cells into suitable xenorecipients such as mice. A second host adaptation strategy relies on the development of genetically engineered mouse models expressing human factors, permitting HCV replication within a partial or fully functional immune system context. The finding that viral entry, via the human host factors CD81 and OCLN (107), regulates HCV host tropism but viral assembly or replication (76) does not paved the way for generating genetically humanized mouse models permissive to HCV infection. Indeed, in 2011, adenovirus-mediated expression of the HCV entry factors

CD81 and OCLN in hepatocytes of immunocompetent Rosa26-Fluc mice allowed HCV uptake into murine hepatocytes in vivo (32). However, in this model, viral replication was not detectable and de novo viral particle production was not observed, likely because of murine innate and adaptive immune pressures (32). Two years later, the transgenic expression of the four canonical HCV entry factors (CD81, SR-BI, CLDN1, and OCLN) within Rosa26-Fluc mice harboring targeted disruptions in genes involved in antiviral innate defenses, such as the IFN α β receptor or STAT1, finally allowed low-level replication and de novo infectious particle production in these mice (31). A B- and T-cell response could also be observed. However, the magnitude and quality of this response was likely impaired by the murine STAT1 deficiency. Since then, a similar model based on transgenic expression of CD81 and OCLN in fully immunocompetent ICR (imprinting control region) mice has been reported. Sustained viremia was observed in these mice as well as hepatopathologic manifestations of infection (18), highlighting the potential impact of the murine genetic background on pathogen permissiveness. However, viral loads in serum were slightly lower than in humanized Rosa26-Fluc mice, and no anti-B-cell and anti-T-cell responses were reported.

By causing severe pneumonia in humans, the recently identified Middle East respiratory syndrome coronavirus (MERS-CoV) has a high mortality rate that can reach up to 40% (3). As mice and other small laboratory animals are not susceptible to MERS-CoV infection, the identification of dipeptidyl peptidase 4 as a functional entry receptor (110) for MERS-CoV in 2013 opened the way for the development of small human-transgenic animal models. In 2014, the adenoviral expression of the human dipeptidyl peptidase 4 in mice rendered these mice susceptible to infection (165). Specific CD8 T-cell responses were induced in these mice, and anti-MERS-CoV antibodies were able to protect these mice from a subsequent challenge. More importantly, this study demonstrated that adenovirus delivery of human host factors represents a relevant approach to rapidly generate suitable platforms for the evaluation of antiviral therapies and vaccines against emerging, life-threatening pathogens.

Applications for studying bacterial pathogens—Humanized mouse models have also been utilized for the in vivo study of human-tropic bacterial pathogens such as *Listeria monocytogenes*, *S. pyogenes*, and pathogenic *Neisseria*. *L. monocytogenes* is a Gram-positive, foodborne pathogen that causes listeriosis when the bacteria cross the intestinal barrier, enter the blood stream, and spread to other organs. Host selectivity is mediated by the bacterial proteins internalin A and B (InlA and InlB), which interact with human, but not murine, E-cadherin. Orally infected transgenic mice expressing either human E-cadherin or humanized murine E-cadherin develop listeriosis, showing similar pathologies as in humans and a high rate of mortality (29, 70).

The transgenic mouse model expressing CD46 has been important in the study of both *S. pyogenes* and pathogenic *Neisseria* (*N. gonorrhoeae* and *N. meningitidis*). CD46 interacts with the M protein of *S. pyogenes*, a major virulence factor important in bacterial infection (99). CD46 transgenic mice are more susceptible to *S. pyogenes* than are nontransgenic mice, and the transgenic animals develop high levels of bacteremia that often prove lethal (77, 87). Infection of CD46 transgenic mice with pilated *N. meningitidis*, either intraperitoneally or intranasally, leads to increased bacteremia in the blood as well as in the

cerebrospinal fluids compared with infection of nontransgenic mice (59). In addition, mortality was observed in CD46 transgenic mice, whereas all nontransgenic control mice survived. Finally, mice expressing human plasminogen experience increased pathogenesis when infected with *S. pyogenes* (138). Streptokinase is thought to have plasminogen-activating properties that facilitate the accelerated clearance of host fibrin, allowing *S. pyogenes* to penetrate and disseminate into host tissues more efficiently in transgenic mice than in nontransgenic mice. Not surprisingly, these transgenic mice have increased levels of bacteremia in a wide array of tissues.

Collectively, the continued development of genetically humanized mice will facilitate the in-depth study of human-tropic pathogens and their host interactions. A better understanding of the mechanisms of pathogenesis will provide a relevant platform for screening more efficacious therapeutic strategies.

Potential future directions—The limited host range of many other human-tropic pathogens makes them poorly understood in terms of pathogenic mechanisms. The genetic methods described above lend themselves to the systematic identification of positive and negative regulators governing a given pathogen's host range. In addition, for a number of parasitic, bacterial, and viral pathogens, essential human-specific host factors have already been identified, providing the blueprint for constructing genetically humanized mouse models.

EBOV and Marburg virus are both members of the family *Filoviridae*. EBOV has a very high mortality rate of 25–90%. However, no direct-acting treatments or vaccines are yet available. The narrow host range of EBOV, restricted to humans and NHPs, limits our understanding of EBOV pathogenesis. As described above, lethal EBOV infection of mice requires the use of a mouse-adapted EBOV strain (8), suggesting that primate-specific determinants regulate EBOV infection in vivo. The overexpression of NPC1, a recently identified receptor for filovirus entry (15), can cause originally nonpermissive cells to become susceptible to EBOV infection, indicating that NPC1 is a bona fide entry factor essential for EBOV uptake (89). Thus, the development of an animal model with inheritable susceptibility to nonadapted strains, such as a human NPC1 transgenic mouse, could offer an opportunity to better decipher the EBOV host determinants of infection and pathogenesis.

HBV is a small DNA virus with ~360 million chronic carriers worldwide. HBV has a very narrow organismal and cellular host range, robustly infecting only human and chimpanzee hepatocytes. Currently, human liver chimeric mice, i.e., xenorecipients harboring human hepatocytes, are the only models capable of recapitulating the entire HBV life cycle. However, the technical aspects of creating such mice are tremendous; thus, immunocompetent mice with inheritable susceptibility to HBV would be a valuable tool. Recently, human sodium taurocholate cotransporting polypeptide (hNTCP) was identified as a receptor for HBV entry (156). This finding has raised the possibility of developing a mouse model with inheritable susceptibility to HBV. The preS1 peptide, which is essential for HBV entry, can bind to hepatocytes of mice, rats, and dogs (121). These animals are not naturally susceptible to HBV infection, indicating a possible block following virus attachment in these species. Such restrictions might occur during HBV replication, as

covalently closed circular DNA (cccDNA)—the template for viral transcription important in establishing viral persistence—is not observed in transgenic mice expressing a 1.3× HBV genome (43). One previous study suggests that in the absence of hepatocyte nuclear factor 1α (HNF1α), cccDNA may form at low levels in HBV transgenic mice (112); this peculiar observation requires independent validation. Thus, the identification of additional human coreceptors and/or dominant negative murine factors that restrict HBV uptake is likely necessary in order to create a mouse model with inheritable susceptibility.

S. pneumoniae is an obligate human pathogen that causes several types of infections. As discussed in the section The Impact of Innate Immunity on Host Tropism, the *S. pneumoniae* protein CbpA is important in infection and plays a role in determining host specificity. Currently, there is no mouse model that is readily susceptible to *S. pneumoniae* infection. The knowledge that CbpA interacts only with human pIgR, secretory component, SigA, and FH (78–80, 164) provides a tantalizing possibility for developing humanized transgenic mice susceptible to *S. pneumoniae* infection. The development of such models would provide insight into the mechanism of *S. pneumoniae* infection and host response.

Plasmodium falciparum is an apoplexian protozoan parasite largely responsible for causing malaria in humans. Surrogate models for malaria exist, taking advantage of plasmodial species that readily infect rodents. However, rodent and human parasite strains substantially differ genetically, as more than 700 *P. falciparum* genes do not have orthologs in *Plasmodium chaubaudi*, *Plasmodium yoellii*, and *Plasmodium berghei* (17). These differences limit the predictive value of preclinical drug efficacy assessments in commonly used rodent models. *P. falciparum* has a complex life cycle, with obligate stages in mosquitoes and primates, including humans and great apes, but no other species. Transmitted through the bite of an infected mosquito, the parasite first establishes infections in humans in the liver, where the parasite matures and transitions to the blood stages before being taken up during the blood meal of a mosquito. Host factors governing tissue and host tropism of *P. falciparum* remain incompletely defined. Of the factors that seem to be important for completion of the parasite's life cycle, CD81 (130) and basigin (BSG) (150) appear to affect—at least in part—species-specific uptake into hepatocytes and erythrocytes, respectively. It remains to be tested whether expression of human CD81 and BSG in mice promotes parasite invasion. Given the complexity of the parasite's life cycle, additional, yet-to-be-defined factors are likely needed to overcome species barriers.

CONCLUDING REMARKS AND FUTURE CHALLENGES

In this review, we have discussed how genetically engineered mice can substantially aid the study of human-tropic pathogens. Tractable small animal models can also represent promising platforms for preclinical testing of novel therapeutic strategies. Indeed, several reports have already paved the way toward using such models for evaluating prophylactic or therapeutic strategies. For example, adenosine analogs have been shown to inhibit DENV infection within AG129 mice (161). Moreover, adenovirus-associated virus delivery of anti-HCV neutralizing antibodies in mice expressing the four HCV human entry factors (32) prevents HCV entry into mouse liver (26). However, some caveats must be considered in creating novel models and refining existing models.

Mice and humans diverged approximately 90 million years ago. Although physiologic and metabolic processes among mammalian species harbor an important degree of conservation, it is worth noting that the relatively strong evolutionary divergence between mice and humans represents a limitation for recapitulating human diseases in mouse models.

Indeed, the Mouse ENCODE Consortium recently aimed to shed light on mouse transcriptional and cellular regulatory mechanisms (160). The consortium was able to confirm the existence of a strong conservation of biological functions, gene regulatory networks, and chromatin states between mice and humans. However, it was also demonstrated that these two species exhibit important differences, such as (a) differential gene expressions despite relative conservation of gene sequences, (b) an important divergence of the *cis*-regulatory landscape (only 20% of mouse transcription factor occupancy sites have human orthologs), and (c) the existence of species-specific regulatory sequences enriched with repetitive DNA element sequences (20, 136, 160). Cumulatively, these observations highlight the important evolutionary divergence in terms of differential gene expression and transcriptional regulation between mice and humans.

In the future, an improved understanding of human-tropic pathogens may rely on the development of new human transgenic animal models based on animal species displaying less evolutionary divergence than mice. Indeed, proof-of-concepts of genetic engineering in several species have already been made. For instance, several rat models have been engineered for studying human diseases such as Alzheimer's (30), and pigs have been able to express ectopic human proteins for preventing bacterial infections (23). The recent application of a CRISPR-Cas9 RNA-mediated gene-targeting strategy in macaques, allowing the development of genetically modified cynomolgus monkeys, symbolizes one of these achievements (97).

Alternatively, humanization through tissue transplantation provides unprecedented opportunities to study human infectious diseases (72). Improved protocols and xenorecipient strains now allow the robust engraftment of human hepatocytes and components of a human immune system (HIS), following injection of human hepatocytes and hematopoietic stem cells, respectively. The resultant human liver chimeric mice are excellent models for studying human hepatotropic pathogens, such as hepatitis viruses or *P. falciparum* in vivo. HIS mice have a long-standing history, primarily as challenge models for human lymphotropic pathogens (39). To improve further utility of humanized xenotransplantation models and to allow (co)engraftment of other tissues, engraftment procedures and recipients need to be further refined. Importantly, it was recently demonstrated that human hepatocytes and hematopoietic stem cells can be coengrafted in a single murine recipient (6, 44, 153, 155), facilitating the analysis of pathogen-specific immune responses and immunopathogenesis (6, 153). However, our current ability to generate relevant mouse models harboring a HIS is still limited. Indeed, the absence or low reconstitution of human NK (natural killer) cells and myeloid cells, or an absence of human HLA (human leukocyte antigen) restriction, likely hampers human-specific immune responses within these mouse models. Thus, a considerable challenge relies now on the improvement of the HIS reconstitution and functionality within these mice (11, 129). In the future, the generation of HIS mice harboring deletions restricting murine immune cell development, allowing

expression of human-specific HLA molecules, and constitutive (or transient) expression of human-specific cytokines (19), could pave the way toward more suitable models for the study of immune responses against human-tropic pathogens as well as for the development of antibody-mediated vaccines as recently shown for HCV (26). Alternatively, although not yet demonstrated, such mice represent promising platforms for assessing the induction of protective immunity by a broad range of vaccine candidates against challenging pathogens, from soluble proteins harboring strong antigenic properties to live-attenuated pathogens.

In the next decades, the development of genetically humanized animal models, combined with the use of powerful genetic approaches for altering genomes, may shed light on molecular mechanisms governing the pathogenicity of human-tropic pathogens, potentially uncovering novel therapeutic and vaccine strategies for tackling challenging infectious diseases.

Acknowledgments

The authors thank all members of the Ploss lab for critical discussion of the manuscript. Work in the laboratory is in part supported by grants from the National Institutes of Health (2 R01 AI079031-05A1, 1 R01 AI107301-01, and 1 R56 AI106005-01), an American Cancer Society Research Scholar grant (RSG-15-048-01-MPC), and the Grand Challenge Program of Princeton University. J.M.G. and B.Y.W. are supported by cofunding from NIAID on iNRSA 5T32GM007388. M.v.S. is a recipient of a fellowship from the German Research Foundation (Deutsche Forschungsgemeinschaft). We apologize to all colleagues whose work could not be cited owing to space constraints.

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ZFNs, TALENs, AND THE CRISPR-CAS9 SYSTEM

Comprising both a DNA binding domain and an attached FokI nuclease domain (FND), ZFNs recognize 3-bp DNA sequences, and 3–6 ZFNs can generate a ZFN subunit that recognizes a 9–18-bp DNA sequence. Upon binding to the target site, the FNDs dimerize, resulting in a complex that generates a DSB. TALENs are composed of a TALE DNA binding domain and an FND. The TALE domain is made up of tandem arrays of amino acid repeats that specifically recognize a single DNA base pair. The nucleotide specificity of the TALE domain repeats is determined by two amino acids, named the repeat variable di-residues (RVDs). By combining these RVDs, almost any DNA sequence can be targeted. Upon binding DNA, the FND can create a DSB to facilitate gene knockout or knockin. The CRISPR-Cas9 complex consists of a guide RNA (gRNA) whose sequence is complementary to the genetic locus being targeted. The gRNA facilitates the CRISPR-Cas9 complex binding to the target gene, resulting in the Cas9 nuclease protein forming a DSB. Cas9 performs its nuclease activity as a monomer, as only one Cas9-gRNA complex is needed for DSB creation, whereas TALENs and ZFNs require two constructs to exert nuclease activity (63).

CURRENT LIMITATIONS OF ZFNs, TALENs, AND THE CRISPR-CAS9 SYSTEM

Although ZFNs, TALENs, and the CRISPR-Cas9 system all facilitate DSBs, each technology has its limitations. Some downsides of ZFNs include (a) no open-source database of ZFNs that cover all possible DNA triplet sites, making it difficult to design ZFNs for targeting any desired sequence; (b) several off-target effects that can lead to cytotoxicity; and (c) gene target sites must be high in GC content (145). In comparison, TALENs can be used to target any gene with high specificity and have a low rate of off-target effects. However, target sequences must start with a thymine and end with an adenine. CRISPR-Cas9 genome-editing technology has been progressing rapidly owing to the simple generation of gRNAs that can target any desired sequence, but a protospacer adjacent motif (PAM) site with a sequence of NGG or NAG must follow the target site. However, for all these techniques, it is important to corroborate that no off-target effects have occurred. With these limitations in mind, it is critical to choose the technique that best fits the particular application and organism being studied. The pros and cons of each of these genome engineering techniques are further reviewed in Reference 63.

SUMMARY POINTS

1. The host tropism of a pathogen is frequently regulated by complex interplays between host and viral determinants, which are under constant coevolutionary pressure.
2. Host tropism determinants can be uncovered through a variety of genetic approaches.
3. The understanding of these determinants can allow the generation of genetically humanized, cost-effective animal models for studying human pathogens.
4. Proof-of-concept has already been established for several important human pathogens, including *N. meningitidis*, poliovirus, and HCV.
5. In the future, the development of new technologies in genome engineering, combined with a better understanding of the host tropism of human pathogens, may allow the generation of novel robust animal models for their study.
6. Such animal models could underpin the development of new treatments and vaccines against the most challenging human pathogens.

FUTURE ISSUES

1. The generation of novel genetically humanized mouse models suitable for the study of challenging human pathogens such as EBOV, HBV, or DENV relies on the identification of host and viral factors regulating host tropism.
2. The relevant study of host-pathogen interactions in mice requires the generation of model organisms harboring fully functional human immune systems.
3. A major challenge lies in creating genetically engineered, HIS-engrafted mouse models with enhanced immune cell reconstitution immune cell reconstitution and impaired murine immune responses.
4. The genetic divergence between mice and humans underlies the need to generate new transgenic animal models based on other mammalian species that are evolutionarily closer to humans.

Figure 1. Diversity of pathogen host tropism. Viruses, bacteria, and parasites vary in their respective host ranges, with some only able to infect humans (*red*) while others can infect a wide range of vertebrates and invertebrates (*purple*). Each color, from red to purple, represents a particular level of host range diversity. Text included in colored rectangles indicates the animal groups included in each host range level. Only a few examples of pathogens are indicated for each level. The level of human-restricted pathogens (*red*) is enlarged as a red circle. Abbreviations: CCHFV, Crimean-Congo hemorrhagic fever virus; CMV, cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV-1, human immunodeficiency virus 1; HHVs, human herpes viruses; HEV, hepatitis E virus; TBEV, tick-borne encephalitis virus; VEEV, Venezuelan equine encephalitis virus.

Figure 2. Identification and applications of regulators of human-tropic pathogens. A graphic summary of the different methods used to discover host (*orange*) and viral (*blue*) determinants that regulate pathogen host tropism is shown. In some cases, the search for host factors can be guided by preliminary experiments, such as those using interspecies heterokaryons. In the absence of initial guiding experiments, whole-genome screening methods can be applied. The identification of pathogen factors can be determined via the screening of pathogen determinants (e.g., viral proteins, amino acids) by genome engineering (pathogen engineering), or pathogens can be serially passaged in a nonpermissive host to detect mutations that increase pathogen fitness (pathogen adaptation). The uncovering of host factors may also allow the generation of genetically modified pathogens able to overcome potential host restriction factors (*dotted line*). Collectively, the identification of host factors regulating the tropism of human pathogens, combined with the development of several genetic tools, can allow the generation of genetically humanized mice (or other suitable animal models) permissive for human-tropic pathogens. Such animal models represent a promising platform for the study of human-tropic pathogens in vivo, as well as for the development of vaccines or therapeutic strategies against challenging pathogens. Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus.