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Development of humanized mouse models to study human malaria parasite infection

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Abstract

Malaria is a disease caused by infection with *Plasmodium* parasites that are transmitted by mosquito bite. Five different species of *Plasmodium* infect humans with severe disease, but human malaria is primarily caused by *Plasmodium falciparum*. The burden of malaria on the developing world is enormous, and a fully protective vaccine is still elusive. One of the biggest challenges in the quest for the development of new antimalarial drugs and vaccines is the lack of accessible animal models to study *P. falciparum* infection because the parasite is restricted to the great apes and human hosts. Here, we review the current state of research in this field and provide an outlook of the development of humanized small animal models to study *P. falciparum* infection that will accelerate fundamental research into human parasite biology and could accelerate drug and vaccine design in the future.

Keywords

animal model; blood stage; humanized mice; infectious disease; liver stage; malaria; *Plasmodium falciparum*; *Plasmodium vivax*

The challenge

Malaria is a major global health problem. The disease disproportionately affects resource-poor, developing regions of the world [1]. Young children and pregnant woman in sub-Saharan Africa are most at risk from death caused by a malaria infection. *Plasmodium falciparum*, one of the five *Plasmodium* species that infect humans, is the most lethal parasite and contributes to the majority of deaths from the disease [1]. A combination of interventions including insecticide spraying and insecticide-treated bed nets for mosquito control, along with curative and preventative drug treatments, are currently the main methods of malaria control [2]. A fully protective malaria vaccine, after decades of research, is not yet available.

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P. falciparum is a pathogen that is difficult to study in the laboratory setting owing to its complicated life cycle (Box 1) and tropism for human tissue. The advances of *in vitro* culture, especially for the blood stages of the *P. falciparum* parasite – both asexual and sexual (the male and female gametocyte) – has allowed for unprecedented progress in our understanding of basic parasite biology and the development of antimalarial drugs. Significantly less is known about the *P. falciparum* liver stages. This is due not only to the fact that *in vitro* propagation of liver stages has proven difficult, but also that production of the hepatocyte-infectious form (the sporozoite) requires labor- and resource-intensive processes involving anopheline mosquito rearing and infection with gametocytes for sporozoite production.

Box 1

The *Plasmodium falciparum* life cycle

The *Plasmodium falciparum* life cycle in the human host starts with sporozoite inoculation into the skin by parasite-infected mosquitoes. After inoculation, sporozoites quickly reach the liver where they must infect hepatocytes in order to continue their life cycle. During the process of hepatocyte infection, the sporozoite surrounds itself with a membrane, the parasitophorous vacuole membrane, which is derived from the hepatocyte plasma membrane but greatly modified with parasite proteins. The parasitophorous vacuole membrane protects the parasite from its host cell and permits the parasite's unencumbered development. The magnitude of liver-stage growth and replication is striking; one parasite produces 20,000–40,000 red blood cell-infectious exoerythrocytic merozoites in just 7 days [2]. The growth is so massive that the liver stage causes a large expansion of its host hepatocyte to many times its original volume; often, the volume of the infected cell can increase 15-times, yet it appears that liver stages remain mostly undetected by the host immune system. This may be accounted for by the liver-stage parasite's ability to subvert host responses, thus hiding from the immune system or the immunosuppressive environment of the liver. Once released from the hepatocyte, the exoerythrocytic merozoites infect red blood cells, leading to cyclic asexual replication and causing all the pathologies associated with malaria.

Studies exploring fundamental host–parasite interactions on the cellular level, as well as a better understanding of the pathophysiological effects of infection, will bring us closer to developing successful antimalarial interventions. Significant knowledge of blood-stage biology in addition to the biology of the pre-erythrocytic stages (sporozoite and liver stage) has been generated by studying rodent malaria models (*Plasmodium berghei*, *Plasmodium yoelii* and *Plasmodium chabaudi*). A robust laboratory malaria model allows a more detailed analysis of the parasite but also its parasitic relationship with the host. Yet, not all aspects of *P. falciparum* biology can be modeled using rodent malaria. The parasites have diverged over hundreds of millions of years and *P. falciparum* exhibits a diversity of unique traits. It harbors many unique genes that lack orthologous genes in the rodent *Plasmodium* parasites, such as families of variant antigens. Aspects of the parasite's pre-erythrocytic biology are also unique, for example LSA-1, which has no ortholog in rodent malaria species [3], is a potential vaccine candidate [4]. Another complication of using rodent malaria models is the divergence of function between rodent and human malaria gene orthologs. For example, members of the ETRAMP family of parasitophorous vacuole membrane proteins, expressed in both rodent malaria parasites, as well as *P. falciparum*, are good examples where the orthologous genes have different times of expression and possibly different functions, as may be concluded from unsuccessful complementation studies [5-7]. This highlights the importance of studying the human parasites directly.

Facing the challenge of parasite drug resistance in malaria-endemic regions of the world, the lack of small-animal models for *P. falciparum* is a roadblock that might impede development of next-generation drugs. *In vitro* studies are very important for the discovery of new antimalarials, but the effect of the intervention must be tested not only on the parasite, but also evaluated for physiological consequences on the host. In addition, to move candidate interventions into the clinic efficiently, novel strategies are needed for rapid and cost-effective screening, selection and prioritization of the most promising candidates. The use of nonhuman primates allows more relevant preclinical studies to be performed, but their use is hampered by ethical and cost considerations. These limitations and the lack of suitable small-animal models create a challenging barrier to conducting essential preclinical investigations. In this review, we summarize the current state and challenges in the development of humanized mouse models to study *P. falciparum* infection.

Early small-animal models for *P. falciparum* blood-stage infection

The concept of developing a small-animal model for the blood stages of *P. falciparum* followed the discovery of mice with spontaneous immune deficiencies, which allow for engraftment with human red blood cells (hRBCs) – the obligate host cell for the asexual blood stages. The severe combined immunodeficiency (SCID) mouse harboring a point mutation in the *Prkdc* kinase (*Prkdc*^{SCID}), which interferes with the development of functional B and T lymphocytes, accepts xenogenic grafts of hRBCs and was used to establish *P. falciparum* blood-stage infection for up to 2 weeks [8]. Nevertheless, the initial studies were plagued with problems including difficulties in obtaining high levels of hRBC chimerism, as well as the rapid clearance of parasite-infected hRBCs by the mouse. Some of these problems have been analyzed and are mainly due to the substantial innate responses still present in these immunodeficient animals [9]. Macrophages and, in part, NK cells are responsible for the clearance of the parasite-infected and uninfected hRBCs [9]. Initially, mouse strains with more extensive immunodeficiencies were used to circumvent the problem. Mice bearing Beige, Nude and XID mutations (BNX or NIH III) [10], which are characterized by their lack of functional T cells, NK cells and T-cell-independent B lymphocytes, as well as nonobese diabetic (NOD)/SCID mice [11], which have additional immunity-related depletions due to the NOD background, together with pharmacological agents, splenectomy or radiation to suppress the remaining innate functions, have been used with limited success as functional models for *P. falciparum* blood-stage infection.

Recent models of improved *P. falciparum* blood-stage infection

Angulo-Barturen *et al.* were able to achieve a reproducible *P. falciparum* blood-stage infection in an immunosuppressed mouse model engrafted with hRBCs without any additional immunosuppressive interventions [12]. Two major differences in this improved mouse model could account for the success: first, the selection of the mouse background and, second, the *in vivo* selection of a *P. falciparum* strain that was adapted to replicate within the mouse. In the study, the NOD/SCID/ β_2 microglobulin (β_2m)^{-/-} deficient mouse was used [13]. In addition to the above described deficiencies of the NOD/SCID mouse strain, homozygosity for the β_2m^{null} allele results in the absence of MHC class I expression, loss of CD8⁺ T cells and NK cell activity, accumulation of iron in the liver and rapid clearance of human IgG1 [14]. It was previously shown that the NOD/SCID/ β_2m ^{-/-} background allows for an increased engraftment of human xenotransplants [14]. This mouse model was able to maintain high levels of hRBC chimerism with daily injections of hRBCs. Successful generation of *P. falciparum* blood-stage infection (~1% parasitemia) was achieved utilizing the 3D7 strain of *P. falciparum* that was adapted to continuous *in vivo* growth in the chimeric mice [12]. Mice were intravenously injected with parasite-infected hRBCs, which reduced to below detectable parasitemia, but then parasites emerged in the

NOD/SCID/ $\beta_2m^{-/-}$ mice that established a productive infection 2–3 weeks later. Such adapted parasites were further transmitted and expanded *in vivo* and reproducible exponential growth of the isolate was observed in all mice tested without the initial lag of detectable parasitemia. Interestingly, the selected variant of the *P. falciparum* 3D7 parasite strain could also grow in other murine strains engrafted with hRBCs, including the SCID and NOD/SCID models previously described. It remains unclear which adaptations in the humanized mouse-passaged parasite are responsible for stable growth. Injection of human serum and hypoxanthine showed further augmentation of parasite growth and selection of the *P. falciparum* strain and/or the engraftment of the hRBC in the NOD/SCID/ $\beta_2m^{-/-}$ mouse, furthering the success of the model. This significant improvement of *P. falciparum* blood-stage replication allowed for the experimental testing of antimalarials *in vivo* in a dose–response assay.

In a follow-up study, the authors successfully expanded the use of their mouse-adapted *P. falciparum* strains into a further mouse model of malaria – the nonmyelodepleted NOD/SCID/IL-2 receptor γ chain (IL2R γ)^{null} mouse [15]. Gene deletion of the IL2R γ , the common sub-unit of the IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 receptors, which are involved in the production of functional NK and T cells, results in mice lacking these cells [16]. This mouse strain supports a hRBC-infectious parasite burden up to tenfold higher than the engrafted NOD/SCID/ $\beta_2m^{-/-}$ mouse, and unlike the NOD/SCID/ $\beta_2m^{-/-}$ mouse, the NOD/SCID/IL2R γ ^{null} mouse does not retain residual NK cell function and is not prone to thymic lymphomas, which can dramatically diminish life span. This mouse model of blood-stage *P. falciparum* infection was shown to be responsive to the therapeutic effects of antimalarials such as chloroquine, artesunate and pyrimethamine.

Preadaptation of the parasite strain may be viewed as a significant limitation of the two studies described above, as various existing strains of *P. falciparum* parasites cannot be used unless they too are adapted to survive in the mouse. Furthermore, the adapted strains must have unique properties not observed in less adapted strains to be able to withstand the selective pressure of *in vivo* growth in the humanized mouse. The adapted *P. falciparum* 3D7 strain could also be of limited use due to the fact that it does not actively produce gametocytes and thus it is solely restricted to the study of asexual stages of the parasite.

Recently, Arnold *et al.* described the most comprehensive *P. falciparum* humanized mouse model to date, where various malaria parasite strains were used without prior parasite adaptation with reproducible results [17]. By using the NOD/SCID/IL2R γ ^{null} mouse strain also utilized by Jimenez-Diaz and colleagues in the above studies [15], the authors were able to infect the chimerized mice with various *P. falciparum* parasite strains and establish long-lasting para-sitemia with relatively high levels of hRBCs infected with the parasite. To establish this model of *P. falciparum* blood-stage replication, the immunodeficiency of the mice had to be augmented by the injection of liposomal-clodronate formulations to deplete murine phagocytic cells. This produced a mouse model of malaria in which 100% of infected mice showed development and expansion of the *P. falciparum*-infected hRBC population [17]. Furthermore, the model showed a high degree of synchronization of the parasite population and partial sequestration of infected hRBCs in the vasculature, a characteristic phenomenon observed in humans that is associated with severe disease. The long-lived and stable growth of the parasites allowed for at least partial development of the sexual stages of *P. falciparum* because mature gametocytes were observed. Nevertheless, gametocyte development in the humanized mouse model may still be challenging because the high turnover rate of infected hRBCs is not conducive to allow the long (2-week) maturation time of gametocytes.

Human liver chimeric mice for the study of *P. falciparum* liver-stage infection

The difficulty of working with *P. falciparum* liver stages is partly due to their highly restricted host-cell tropism. The parasite's complete development is absolutely restricted to human hepatocytes. Near-to-complete development of liver-stage parasites had previously been observed only in primary human hepatocytes [18]. More recently, however, the use of a hepatocytic cell line, HC-04, was reported, and allows the development of *P. falciparum* liver stages [19]. Unfortunately, it is difficult to infect this cell line with *P. falciparum* and does not efficiently support complete liver-stage development. A quest to develop a small-animal model, which can efficiently support liver-stage development *in vivo*, was initiated with the transplantation of human hepatocytes into immunosuppressed SCID mice [20]. Even though the model was plagued by low reproducibility, it provided a proof-of-concept that infecting human hepatocytes with *P. falciparum* in a surrogate host was possible. In order to promote hepatocyte expansion and to give the human cells a competitive growth advantage over the endogenous murine hepatocytes, genetic approaches were employed to inflict liver injury. Transgenic expression of uPA under the control of the liver-specific mouse albumin promoter was shown to be hepatotoxic [21,22]. When crossed to immunodeficient SCID or SCID/Beige backgrounds, homozygous Alb-uPA transgenic mice allow engraftment with human hepatocytes [23,24]. It was demonstrated that human liver-chimeric mice could be infected with *P. falciparum* sporozoites and support complete development of liver-stage parasites [25,26]. With regard to the SCID/Alb-uPA mouse, an immunomodulation protocol involving NK and macrophage depletion has been reported to improve both the level of human chimerism and *P. falciparum* liver-stage development [25], but even without this modulation, the SCID/Alb-uPA mouse can reproducibly allow for *P. falciparum* liver-stage development. It has also been shown by Sacci *et al.* that the liver-stage *P. falciparum* parasites can fully develop and exoerythrocytic merozoites released from infected hepatocytes can infect hRBCs if the infected hepatocytes are overlaid with hRBCs *ex vivo* [26].

The SCID/Alb-uPA human hepatocyte mouse was subsequently used to test the degree of attenuation of *P. falciparum* gene knockouts [27]. More recently, the SCID/Alb-uPA mouse was successfully used to investigate the development of *P. falciparum* parasites lacking LSA-1 [28]. The study, however, uncovered a shortcoming of the model: the model cannot be used to study the transition of the parasite from the liver stages to the blood stages within the mouse. Therefore, in cases where research into the late liver-stage function of an expressed gene requires investigating the ability of the liver-stage parasite to transition to the blood stage, the humanized mouse model requires further development to support this transition.

Although the human liver chimeric SCID/Alb-uPA mouse was successfully used to study *P. falciparum* liver-stage development, the model does present serious challenges. The uPA transgene expression is not only hepatotoxic but also causes hypocoagulopathy that results in a high neonatal mortality due to fatal hemorrhaging even before the animals can be transplanted. The severe liver injury of homozygous SCID/Alb-uPA pups requires engraftment surgery shortly after their birth, which, in combination with bleeding, further complicates the surgical procedures. Furthermore, the selective pressure needed to achieve and maintain a high level of human chimerism can only be achieved in homozygous SCID/Alb-uPA immunodeficient recipients, which suffer from infertility. Although the hypofertility phenotype can be overcome by reconstituting breeders with mouse hepatocytes [29], cumulatively, the afore-mentioned drawbacks, as well as the need for high-quality adult human hepatocytes for transplantation, translate into the generation of only modest

numbers of mice, which are extremely costly and a time-consuming endeavor. This limits the number of mice that can be used in experiments and precludes extensive studies [26]. Also, a more detailed investigation of the transition of liver stages into blood stages is limited by the fragility of the model and the rapid elimination of hRBCs from the circulation of SCID mice [30] if these human liver-chimeric mice were cotransplanted with human RBCs.

A new model to study liver-stage infection: the FAH^{-/-}Rag2^{-/-}IL2R^γnull mouse

To overcome some of the shortcomings of the SCID/Alb-uPA liver injury model, two groups have developed an alternative mouse model for human hepatocyte engraftment, in which liver injury is caused by genetic ablation of FAH [31,32]. FAH catalyzes the conversion of fumarylacetoacetate into fumarate and acetoacetate in the tyrosine catabolic pathway, and the targeted disruption of the *FAH* gene results in neonatal lethality due to acute liver failure [33]. However, FAH deficiency can be pharmacologically rescued by providing 2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) to the animals [34], which in turn also provides a means to conveniently induce liver injury when needed. FAH^{-/-} mice were crossed to a highly immunocompromised background, on which development of functional B, T and NK cells is blocked by disruptions of Rag2 and the IL2R^γ gene. FAH^{-/-}Rag2^{-/-}IL2R^γnull (FRG) mice can be efficiently engrafted with human adult hepatocytes, resulting in repopulation of the murine parenchyma with human cells in excess of 90% [35]. When compared with the SCID/Alb-uPA mouse model, the FRG mouse offers several advantages (Table 1). The FRG mice breed efficiently and are free of liver damage whilst drugged with NTBC and have long lifespans. Repopulation with human hepatocytes does not need to be carried out right after weaning and human hepatocytes from a donor FRG mouse can easily be transplanted into many recipient mice, allowing for a rapid expansion of human hepatocytes in repopulated mice [31]. This enables the creation of large numbers of animals that carry hepatocytes of the same human genetic background, which offers advantages for experimentation.

We have infected human hepatocyte-repopulated FRG mice with *P. falciparum* and observed complete liver-stage development [Vaughan AM *et al.*, Unpublished Data]. Mice were injected intravenously with *P. falciparum* sporozoites and subsequently sacrificed at various time points after injection and their livers analyzed by immunofluorescence assay for the presence of liver stages and by reverse transcription PCR for the presence of parasite transcripts. This demonstrated complete maturation of the liver stages 7 days after sporozoite inoculation. Additionally, primary human hepatocytes isolated from repopulated FRG mice were susceptible to sporozoite invasion and were able to support liver-stage growth. Thus, the FRG mouse paves the way for detailed analysis of *P. falciparum* liver stages. However, the 129/C57BL/6 background of the FRG mice currently precludes coengraftment with hRBCs, which are highly vulnerable to destruction by the remnants of the murine immune system, and therefore this model needs further modification to establish the complete parasite life cycle.

Future perspective

The use of humanized mouse models supporting parts of the *P. falciparum* life cycle is a great advancement allowing researchers to investigate blood-stage and liver-stage development *in vivo*, albeit separately at this point. This dramatically improves our ability to study host-parasite interactions and to test, preclinically, future anti-malarial interventions in a more physiological setting. The recent improvements of the *P. falciparum* blood-stage-permissive humanized mouse model, which can sustain growth of multiple parasite strains and the use of a similar mouse strain with human hepatocyte engraftment that supports *P.*

falciparum liver-stage development, brings us closer to the development of a humanized mouse model which will allow us to study the liver-stage to blood-stage transition and subsequent blood-stage replication in the same animal. This, however, has not yet been attempted and will require further modifications of the recipient strains and humanization protocols. Although SCID, SCID/Beige, Rag2^{-/-} and ILR γ ^{null} mutations severely impair development of primarily B, T and NK cells, phagocytes and the complement system remain largely unaffected. While the genetic backgrounds of both SCID/Alb-uPA and FRG liver injury models support the engraftment of human hepatocytes in the tolerogenic environment of the liver, they are not suitable for engraftment with hematopoietically derived cells. In this regard, it has been demonstrated that, in particular, the incompatibility of mouse SIRP α expressed on endogenous macrophages with human CD47 contributes largely to rapid graft destruction [36]. In turn, a natural polymorphism in the mouse SIRP α locus on the mouse NOD background [36], transgenic expression of human SIRP α in mice [37] or mouse CD47 on donor cells [38] drastically improve hematopoietic chimerism in humanized mice. However, even in highly immunocompromised xenorecipients with limited antixenogenic phagocytic activity, such as the NOD/SCID/ILR γ ^{null} mouse, rapid clearance of hRBCs occurs, mandating daily injections with hRBCs. Pharmacological ablation of phagocytic cells significantly improves human erythrocyte survivals suggesting that other non-SIRP α -CD47-mediated signals contribute to graft destruction [39]. This improved humanization regimen will render the chimeric animals susceptible to nonmouse-adapted strains of *P. falciparum* that support asexual blood stages of the parasite but will prove challenging for sexual stages, which need a much longer time to develop.

Repeated injections of hRBCs into suitable mouse recipients have yielded a robust platform to study *P. falciparum* blood stages. However, this regimen is laborious and logistically cumbersome and a humanized mouse model supporting *de novo* erythropoiesis would be desirable. Several immunodeficient mouse strains, such as NOD/SCID/IL2R γ ^{null}, NOD/Rag1^{-/-}/IL2R γ ^{null} and BALB/c/Rag2^{-/-}/IL2R γ ^{null} support development of many human hematopoietic lineages following transplantation of CD34⁺ hematopoietic stem cells (HSCs) (reviewed in [39]). While B and T cells, some NK cells and several dendritic cell subsets repopulate such humanized mice, differentiation towards the human myeloerythromegakaryocyte lineage is impaired [40-43]. It had been hypothesized that the limited biological cross-reactivity between critical mouse and human cytokines hinders differentiation of certain lineages (reviewed in [29]). In fact, expression of human IL-15 boosts NK cell development [44], IL-3 and GM-CSF improve macrophage engraftment [43], thrombopoietin improves human HSC maintenance [45] and, importantly, IL-3 and erythropoietin promote hRBC development [46]. Human *de novo* erythropoiesis can be further improved by ablating phagocytic cells using clodronate liposomes in HSC-injected mice in combination with expression of human IL-3 and EPO, which can result in an increase of up to 20% of circulating hRBCs in the periphery of mice [47]. Little is known about the hemoglobin (Hb) composition in human erythrocytes of humanized mice. Hb is consumed by *P. falciparum* during the intraerythrocytic stages, serving as an essential nutrient source for the parasite metabolism. A number of *P. falciparum*-encoded proteases, including plasmepsins, falcipains and falcilysin, as well as dipeptidyl peptidase-1 homologs, have been implicated in the breakdown of Hb (reviewed in [48]). While *P. falciparum* grows efficiently in RBCs containing adult Hb ($\alpha 2\beta 2$), growth of *P. falciparum* is retarded in cells containing fetal Hb ($\alpha 2\gamma 2$). It is conceivable that human RBCs derived from HSCs, which are commonly isolated from human umbilical cord blood or fetal livers, primarily retain fetal Hb. Thus, other HSC sources would have to be used and/or HSCs genetically engineered to efficiently switch to adult Hb. Consequently, additional modifications of xenorecipients and donor cells are needed to attain a hRBC chimerism sufficient to support continuous *P. falciparum* infection without further injections of hRBCs.

In conclusion, we have here reviewed the current status of mouse models that are being used to study both the blood-stage and liver stage of the human malaria parasite *P. falciparum*. These models have come a long way but further work needs to be carried out to markedly improve and ultimately combine them. Rather than inject mice with hRBCs, mice that efficiently support *de novo* erythropoiesis are required. This will be of great importance especially if studies of *Plasmodium vivax*, the second leading malaria-causing parasite, are to be undertaken in mouse models. *P. vivax* only invades reticulocytes and not mature erythrocytes. In addition, it forms dormant liver stages called hypnozoites, which can reactivate and lead to blood-stage infection months after the primary infection has been cleared. The dual humanization of the erythrocytic and hepatic compartments is likely to support the entire mammalian life cycle stages of human malaria parasites. This would enable studies of transmission from mosquito to mammal and back, but also the transition from liver to blood stages. A humanized mouse model for human malaria will be a valuable platform to assess the safety of live-attenuated vaccine strains, to test preclinically the efficacy of therapeutics blocking different parts of the parasite life cycle and may also facilitate genetic crosses to map drug resistance loci. Studies in the humanized mouse model will also be greatly enhanced with the use of transgenic parasites, such as green fluorescent protein- or luciferase-expressing parasites. These applications showcase the utility of a humanized animal harboring the specific tissue compartments required to render the animal susceptible to *P. falciparum* infection. However, in order to eventually study immune responses and to effectively prioritize vaccine candidates against *P. falciparum* infection in mice, it remains imperative to further improve human immune functionality in humanized mice models, which remains mediocre in current systems (reviewed in [39]). Human HSC-transplanted mice can be engrafted with components of a human immune system, but the overall cellularity is usually low, T cells are only inefficiently activated and recruited to inflamed tissues following infection, and while some low levels of IgM can be detected, class switching to IgG and affinity maturation usually does not occur. Expression of human cytokines to promote lineage differentiation, human MHCs to improve antigen recognition, chemokines and integrins to direct lymphocyte migration, cotransplantation of human thymic tissue to foster T-cell selection and modulations to improve the architecture of secondary lymphoid organs to improve immune cell priming, among other approaches, are actively being pursued to improve human immunity *in vivo*. While each modification of the humanization procedure may only result in incremental improvements, a combination of these approaches will possibly lead to more human-like immune responses.

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Executive summary

Malaria

- Malaria is a parasitic disease caused by infection with *Plasmodium* parasite species.
- Morbidity and mortality is caused by *Plasmodium falciparum* but also *Plasmodium vivax*.
- *P. falciparum* is difficult to study *in vivo* owing to its human tissue tropism.
- Rapid development of drug resistance to existing antimalarial drugs and the lack of a fully protective vaccine prompts the development of surrogate models allowing researchers to test new antimalarial interventions.

Animal models for *P. falciparum* blood-stage infection

- The severely immunodeficient mouse strain NOD/SCID/IL2R γ^{null} supports xenogenic grafts and can be injected with human red blood cells.
- *P. falciparum* strains have been developed that are adapted to infect and replicate within human red blood cells in murine NOD/SCID/IL2R γ^{null} recipients.
- Further pharmacological immunosuppression of the NOD/SCID/IL2R γ^{null} mouse allows for the propagation of many *P. falciparum* strains without prior adaptation and results in a long-lasting infection.

Future challenges

- Future challenges include:
 - The elimination of residual immune activity against transplanted tissue and cells infected with the human malaria parasite to allow for easier use of the model;
 - The development and optimization of a dually reconstituted humanized mouse model that supports propagation of both liver stages and blood stages of *P. falciparum* and allows liver-stage to blood-stage transition;
 - Further development of chimeric mice models that support *de novo* human erythropoiesis efficiently.

Table 1

Comparison between the Alb-uPA severe combined immunodeficiency and the FAH^{-/-}Rag2^{-/-}IL2R γ ^{null} mouse models.

		Alb-uPA SCID (Beige)	FAH ^{-/-} Rag2 ^{-/-} IL2R γ ^{null}
Liver injury	Mutation	uPA overexpression	FAH deficiency
	Occurrence	At birth	At birth, inducible upon NTBC withdrawal
Transplantation age		Within 3 weeks post-birth	Any (adult)
Human chimerism		Up to 100%	Up to 90%
Throughput		Low	Medium-high
Additional challenges		Hypofertility of homozygous transgenic mice, coagulopathy	Long-term NTBC withdrawal results in liver cancer, impaired kidney function
<i>Plasmodium falciparum</i>	Sporozoite infection	+	+
	Liver-stage development:		
	Early	+	+
	Mid	+	+
	Late (merozoite release)	+	+
Erythrocyte coengraftment possible		No	No

NTBC: 2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione; SCID: Severe combined immunodeficiency.