Modeling malaria parasites’ life cycle in humanized mice

Valérie SOULARD

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Faculté de Médecine Pierre et Marie Curie
Site Pitié-Salpêtrière
91 Bvd de l'Hôpital, 75013 PARIS
Still a major health problem

40% of the world’s population exposed to varying degrees of malaria risk in around 100 countries

Over 200 million people suffer from malaria annually, resulting in about 700,000 deaths, 90% in Africa of whom 85% are children under 5 years (World Malaria Report 2013)
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Malaria is caused by the protozoan parasite *Plasmodium*
5 species infect humans: *P. falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi*
but *P. falciparum* and *P. vivax* account for more than 95% of the total number of cases of malaria

*P. falciparum* the most deadliest

*P. vivax* (as *P. ovale*) less virulent but causes relapses because of persistent dormant hepatic forms called hypnozoites
Life cycle of *Plasmodium* spp

- **Schizont**
- **Sporozoite**
- **Hypnozoite**
- **Hypnozoite**

**HEPATIC STAGE**

- **Sporozoite**
- **Hypnozoite**
- **Schizont**
- **Merozoites**

Adapté de Mueller I., The Lancet Infectious Diseases, 2009
Life cycle of *Plasmodium* spp

HEPATIC STAGE

- Schizont
- Hypnozoite
- merozoites

BLOOD STAGE

- Gametocytes

Adapted from Mueller I., The Lancet Infectious Diseases, 2009
Malaria Control

- Insecticide-impregnated bed nets: resistance
- Drugs against liver/blood stages: resistance
- Vaccine: in development...

**HEPATIC STAGE**

- Schizont
- Hypnozoite
- Gametocytes

**BLOOD STAGE**

- Sporozoite
- Merozoites

BUT need reliable tools and models to perform drug screening, identify vaccine targets, etc...

*In vitro and in vivo* models

Adapted from Mueller I., The Lancet Infectious Diseases, 2009
**Tools and models to study human Malaria**

**Context:** the study of the *Plasmodium* species that infect humans is largely hampered by the narrow host-species restriction of these pathogens.
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**In vitro**
- **Hepatic stage:** *P. falciparum / P. vivax / P. cynomolgi* infection of human/simian primary hepatocytes

![Primary culture of hepatocytes](image)

*Dembélé, Gego et al., PlosOne 2011*

- **Blood stage:** Infection of human red blood cells (RBC), *P. falciparum*

**In vivo**
- Non-human primate models for full life cycle of *P. falciparum* (cost, expertise, feasibility)
- *P. falciparum* blood stage in RBC-humanized mice (*Moreno et al., Int J Parasitol. 2006*)
- Incomplete *in vivo* *P. falciparum hepatic stage* in liver-humanized mice (*Morosan et al., JID 2006*)
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Establish a **humanized mouse model**

for the *in vivo* study of human Malaria parasites life cycle
Liver-humanization: transplant human hepatocytes into immunodeficient mice that are depleted from endogenous murine hepatocytes

Liver humanization in the TK-NOG model

Immunodeficient to favorize xenotransplantation

Transgenic for HSV-Thymidine Kinase (TK) under Albumin promoter to allow depletion of endogenous hepatocytes and make room for transplanted ones

Hasegawa et al., BBRC 2011
**Liver humanization in the TK-NOG model**

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- inducible liver injury (tuning)
- hepatocyte transplantation in 8 wk-old adult mice
- no continuous chemical treatment applied to the mice
1. Complete life cycle of *P. falciparum* in vivo in humanized TK-NOG mice
Complete life cycle of *P. falciparum* in vivo – hepatic stage

**Human hepatocyte transplantation**

**Infection with *P. falciparum* sporozoites of mice ≥ 1.5 mg/ml hAlb (20%)**

**Sacrifice at different time points of hepatic development**

**Analysis of hepatic development by parasite immunostaining on liver frozen sections**
Complete life cycle of *P. falciparum* in vivo – hepatic stage

Human hepatocyte transplantation → Infection with *P. falciparum* sporozoites

Sacrifice at different time points of hepatic development

Analysis of hepatic development by parasite immunostaining on liver frozen sections

**HSP70**

**DAPI**

**MERGE**

day 5

![Day 5 Images](image1)

day 7

![Day 7 Images](image2)
Complete life cycle of *P. falciparum* in vivo – hepatic stage

- Sacrifice at different time points of hepatic development
- Analysis of hepatic development by parasite immunostaining on liver frozen sections

- 60 μm on day 6 in Humans (Shortt *et al.*, Trans R Soc Trop Med Hyg, 1951)
- 60 μm to 100 μm on day 6 in chimpanzee livers (Meis *et al.*, Exp Para, 1990)
- compared to *in vitro* hepatic schizonts: 40μm at day 6 (Mazier, Nature, 1985)
Complete life cycle of *P. falciparum* in vivo – hepatic stage

**Human hepatocyte transplantation**

**Infection with *P. falciparum* sporozoites**

Analysis of hepatic development by parasite immunostaining on liver frozen sections

**day 7-mature *P. falciparum* schizonts**

**EXP-1**

**DAPI**

**MERGE**

**MSP-1**

**DAPI**

**MERGE**

**AMA-1**

**DAPI**

**MERGE**
Complete life cycle of *P. falciparum* in vivo – hepatic stage

Latest steps of hepatic parasite development

Mazier D., Nature Reviews Drug Discovery 2009

The majority of *P. yoelii* merozoites exit the liver intact, adapt a relatively uniform size of 12–18 μm, and contain 100–200 merozoites.

Complete life cycle of *P. falciparum* in vivo – hepatic stage

Latest steps of hepatic parasite development

**Do we generate infectious hepatic merozoites *in vivo***?

- Majority of *P. yoelii* merozoites exit the liver intact, adapt a relatively uniform size of 12–18 μm, and contain 100–200 merozoites.

*Image credit: Mazier D., Nature Reviews Drug Discovery 2009*
Set up of hRBC engraftment in TK-NOG mice:  
- obtain high level of humanization in peripheral blood (where encounter btw hepatic merozoites and hRBC will occur)

Further Improvements of the *P. falciparum* Humanized Mouse Model

Ludovic Arnold™, Rajeev Kumar Tyagi™, Pedro Meija™, Claire Swetman, James Gleeson, Jean-Louis Pérignon, Pierre Drulhe*

![Graphs and images showing parasiteemia and HuRBC percentage over time.]

Treatment with chlodronate-liposomes to eliminate macrophages

- as much as possible, **avoid additional immunosuppressive treatment**

⇒ *Angulo-Barturen et al., PlosOne, 2008: daily i.p. injection of hRBC*
Set up of hRBC engraftment in TK-NOG mice:
- obtain high level of humanization in peripheral blood (where encounter btw hepatic merozoites and hRBC will occur)
- as much as possible, avoid additional immunosuppressive treatment

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Up to 90% of humanization in liver and RBC compartment can be achieved using this protocol in TK-NOG mice.
Complete life cycle of *P. falciparum in vivo* – Transition from hepatic stage to blood stage

- Human hepatocyte transplantation
- Human RBC engraftment in mice ≥ 5 mg/mL hAlb (60%)
- Infection with *P. falciparum* sporozoites
- Sampling of 200uL venous blood for *in vitro* culture and/or *in vivo* follow-up
- Analysis of GIEMSA stained blood smears

**in vitro**

- Number of parasites / 2uL
- Days of *in vitro* culture

**in vivo**

- Parasitemia, %
- Day post-infection *in vivo*

RBC humanization does not interfere with *P. falciparum* hepatic development. Transition from liver to blood stage was achieved *in vitro* and *in vivo* in all the mice tested.

**Hepatic merozoites generated *in vivo* are infectious**

Robustness of the model

Can gametocytes differentiate and mature *in vivo*?
Liver- and RBC-humanized TK-NOG mice support differenciation and maturation of *P. falciparum* gametocytes *in vivo*
Complete life cycle of *Plasmodium falciparum* in vivo in humanized TK-NOG mice

P. falciparum

Hepatic stage

Sporozoite

HSP70

MSP-1

AMA-1

Gametocytes

Blood stage
Complete life cycle of *Plasmodium falciparum* in vivo in humanized TK-NOG mice

- **In vivo validation of drug**
- **In vivo CD8 killing?**

- **In vivo genetic studies**
- **In vivo validation of *P. falciparum* attenuated live vaccines**

**P. falciparum**

- Transmission to mosquitoes

What are the phenotypes/genotypes expressed at the blood stage?

Antibiotic treatment

Emergence of blood parasitemia? *in vitro and in vivo* analysis
2.
Is the TK-NOG model suitable for the study of the hypnozoite?
in vivo hepatic stage of *Plasmodium* relapsing species

- *P. vivax*
- *P. ovale*
- *P. cynomolgi*
**in vivo hepatic stage of Plasmodium relapsing species**

- *P. vivax* / *P. ovale*
- *P. cynomolgi*

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* Dembélé, Gego et al., PlosOne 2011

- *in vitro* model for liver stage of *P. vivax/P. ovale* hypnozoite (long term cultures of human primary hepatocytes)
- No *in vitro* culture for asexual / sexual blood stage parasites
in vivo hepatic stage of Plasmodium relapsing species – P. ovale

Human hepatocyte transplantation → Infection with P. ovale sporozoites → Sacrifice at different time points to search for schizonts and hypnozoites

Analysis of hepatic development by parasite immunostaining on liver frozen sections
in vivo hepatic stage of *Plasmodium* relapsing species – *P. ovale*

Human hepatocyte transplantation

Infection with *P. ovale* sporozoites

Sacrifice at different time points to search for schizonts and hypnozoites

Analysis of hepatic development by parasite immunostaining on liver frozen sections

**P. ovale** hepatic development

5μm diameter round, uninucleate « hypnozoite-like » parasites
**in vivo hepatic stage of Plasmodium relapsing species – P. ovale**

Sacrifice at different time points to search for schizonts and hypnozoites

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**P. ovale hepatic development**

- **Day 8**
  - 5um diameter round, uninucleate « hypnozoite-like » parasites

- **Day 21**
  - « delayed-schizonts » attesting that some parasites persisted in the liver and resumed their hepatic development from day 13 of infection

**Confirms that small forms identified at day 8 are hypnozoites**
The TK-NOG mice also support the hepatic development of *Plasmodium* relapsing species

**P. ovale**
2 different clinical isolates

Hepatic stage

• *In vivo* validation of drug

Soulard et al., *Nat Com* 2015
P. ovale
2 different clinical isolates

Hepatic stage

• *In vivo* validation of drug

The TK-NOG mice also support the hepatic development of *Plasmodium* relapsing species

Soulard et al., Nat Com 2015
The TK-NOG mice also support the hepatic development of *Plasmodium* relapsing species.

**Human hepatocyte transplantation** → **Human retics engraftment in mice ≥ 5 mg/mL hAlb (60%)** → **Infection with *P. vivax* sporozoites** → **Blood sampling**

- **Goal:** obtain *P. vivax* blood stage development *in vivo*
CIMI, Equipe 12
Audrey LORTHIOIS
Henriette BOSSON-VANGA
Clémentine ROUCHER
Jean-François FRANETICH
Maurel TEFIT
Thierry HOUPERT
Mallaury BORDESSOULLES
Monique BAUZOU
Georges SNOUNOU
Alicia MORENO
Dominique MAZIER

CIMI, Equipe 11
Sylvie BRIQUET, Véronica RISCO
Olivier SILVIE

Centre d’expérimentation Fonctionnel
Serban MOROSAN, Christelle ENOND

Service de chirurgie Digestive, Hépato-Bilieu-
Pancréatique et Transplantation Hépatique
Laurent HANNOUN

Service d’Anatomo-Pathologie
Annette LESOT, Gilles LE NAOUR
Frédérique CAPRON

Plateforme d’Imagerie Cellulaire
Claire LOVO, Aurélien DAUPHIN

Plateforme de Cytométrie en Flux
Bénédicte HOAREAU, Catherine BLANC

University Medical Center, Nijmegen,
Netherlands
Robert SAUERWEIN, Geert-Jan VAN GEMERT

CNR Paludisme
Marc THELLIER, Nathalie CHARTREL,
Liliane CICERON

CIEA, Kawasaki, Japan
Hiroshi SUEMIZU
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