Immunity to liver-stage Plasmodium falciparum in peripheral and tissue-resident immune cells

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(Refer to Protocol Section 2)Primary Objectives• To establish an in vitro assay to study recognition and killing of P. falciparum-infected hepatocytes by:o CSP-specific cytolytic CD8+ T cellso hepatic and peripheral innate/innate-like…

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Protozoal infectious disorders
Study type	Observational invasive

Summary

ID

NL-OMON49766

Source
ToetsingOnline

Brief title
Liver-stage T cell and innate cell immunity (LYTIC)

Condition

- Protozoal infectious disorders

Synonym
malaria, Plasmodium falciparum

Research involving
Human

Sponsors and support

Primary sponsor: Radboud Universitair Medisch Centrum
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: innate cell, liver, malaria, T cell

Outcome measures

Primary outcome

(Refer to Protocol Section 8)

Study parameters/endpoints

Main study endpoints:

• Establishment of an in vitro assay to study recognition and killing of P.

falciparum-infected hepatocytes by:

o cytolytic CD8+ T cells

o hepatic and peripheral innate/innate-like lymphocytes

Secondary outcome

(Refer to Protocol Section 8)

Secondary study endpoints:

• Recognition (IFNγ and CD107a expression) and killing (lysis or apoptosis) of

P. falciparum-infected hepatocytes by cytolytic CD8+ T cell line

• Differences in recognition and killing of P. falciparum-infected hepatocytes

between liver-resident and peripheral blood lymphocytes

• Identity of the individual lymphocyte (sub-)populations which contribute to

recognition and killing of P. falciparum-infected hepatocytes

Study description

Background summary

(Refer to protocol Section 1)

Malaria caused by the parasite Plasmodium falciparum poses a huge burden to public health in endemic regions, particularly among children and pregnant women. The parasite life cycle begins with infectious mosquito bites, injecting sporozoite-stage parasites that migrate to the liver and develop within hepatocytes for ~6-7 days. This liver-stage is asymptomatic. Mounting sterile immunity to liver-stage malaria parasites results in abrogation of further pathology, and has therefore served as the basis for many vaccine candidates. However, conventional vaccine strategies to generate liver-stage immunity have not been highly successful to date and a better understanding of how immunity takes place in the liver will be required to improve the design of future vaccines.

We have recently developed an in vitro model of P. falciparum liver-stage parasite development using freshly isolated human hepatocytes obtained from liver sections of patients undergoing medically-indicated surgery. This model has been successfully used by us to study aspects of liver-stage biology. We have moreover been able to isolate and study the phenotype and function of liver-resident immune cells from these same liver sections.

In murine and human studies, cytolytic CD8+ T cells are important for sterile immunity. One of the natural targets of cytolytic immunity to the liver-stage is the circumsporozoite protein (CSP). We have developed an HLA-A2-restricted CSP-specific cytolytic T cell clonal line capable of lysing cells which have been artificially loaded with CSP, though this has not been demonstrated in P. falciparum-infected hepatocytes. Demonstrating functional activity of this T cell line against infected hepatocytes would permit investigation of a wide range of other questions highly relevant to malaria immunity. The contribution of other cells to liver antimalarial immunity is even less well understood. The liver microenvironment is enriched in innate immune cells, particularly natural killer (NK) and innate-like gd T cells. Both the cell types have been indicated in murine studies to have a role in antimalarial protection. It is unclear how these cells affect liver-stage immunity.

In this study we will use the in vitro fresh human hepatocyte model of P. falciparum liver-stage infection in combination with both the CSP-specific cytolytic T cell clone and donors* own lymphocytes from peripheral blood and liver to investigate whether human immune cells are able to mount functional responses to liver-resident P. falciparum parasites.

Study objective

(Refer to Protocol Section 2)

Primary Objectives
• To establish an in vitro assay to study recognition and killing of P. falciparum-infected hepatocytes by:
o CSP-specific cytolytic CD8+ T cells
o hepatic and peripheral innate/innate-like lymphocytes
Secondary Objectives:
• To assess recognition and killing of P. falciparum-infected hepatocytes by CSP-specific cytolytic CD8+ T cell line
• To assess the differences in recognition and killing of P. falciparum-infected hepatocytes between liver-resident and peripheral lymphocytes
• To identify the individual lymphocyte (sub-)populations which contribute to recognition and killing of P. falciparum-infected hepatocytes
Exploratory Objectives:
• To assess at which time point during intra-hepatocytic development (early, middle or late) P. falciparum-infected hepatocytes are most optimally recognised and killed
• To determine difference in recognition and killing of P. falciparum-infected hepatocytes between parasite strains
• To compare recognition and killing of P. falciparum-infected hepatocytes in different zonal hepatocyte types
• To characterise immunological pathways involved in recognition and killing of P. falciparum-infected hepatocytes by lymphocyte (sub-)populations

Study design

(Refer to Protocol Section 3)

This is a single-centre investigator-initiated exploratory study. Participants will be recruited building upon an existing collaboration with the Department of Surgery, through which we routinely receive anonymized liver tissue which would otherwise be considered medical waste, for in vitro P. falciparum culture from patients undergoing medically-indicated partial liver-resection for underlying disease. For the current study, upon initial scheduling for surgery, written informed consent will be obtained to draw 6mL of blood to determine if the participant has the HLA-A2 phenotype compatible with the cytolytic T cell line. Immediately prior to surgery, 24mL of blood will be drawn, where possible via an existing intravenous or arterial line for isolation of peripheral blood mononuclear cells (PBMCs). No study procedures will interfere with routine clinical care for the participants* underlying disease.
Hepatocyes will be isolated from part of the available liver tissue for in vitro P. falciparum culture and innate/innate-like lymphocytes will be isolated

from the remaining liver tissue and PBMCs. In participants who express HLA-A2, in vitro hepatocyte cultures will be used to assess recognition and killing of intra-hepatocytic parasites. In all subjects with sufficient material, we will assess recognition and killing by liver-resident and peripheral innate(-like) lymphocytes. Read-out will be by variety of immunological techniques including, immunofluorescence microscopy, flow cytometry, qPCR and ELISA/multiplex bead array.

Study burden and risks

(Refer to Protocol Section 11)

There is no direct benefit to study participants. Malaria poses a significant risk to global health and a vaccine is urgently needed to combat the burden of disease. Development of a vaccine against the liver stage would prevent malaria-related morbidity and mortality entirely. Unfortunately, very little is known about liver-stage immunity. An in vitro liver stage platform to investigate immunity to P. falciparum in the liver would advance the field significantly by enabling more in-depth studies of the correlates of protection and factors which can modify the host immune response.

In the proposed study, adult patients scheduled for medically-indicated partial liver resection for underlying disease will undergo one 6mL blood draw to determine HLA-A2 phenotype and another 24mL blood draw on the day of surgery, where possible through an existing intravenous or arterial line. The risks associated therewith are minimal. The liver tissue obtained for this study would otherwise be discarded as medical waste and thus represent no additional risk to participants.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Patients (M/F) over 18 years of age undergoing medically-indicated partial liver resection for underlying disease and who have provided written informed consent.

Exclusion criteria

Patients who have received immunosuppressive and/or cytostatic agents within the past 3 months, with the exception of topical or inhaled steroids.

Patients who are known to have infection with humanimmunodeficiency virus (HIV), hepatitis B virus (HBV) or hepatitis C virus (HCV), or other known clinically-relevant immunodeficient states.

Study design

Design

Study type: Observational invasive

Masking:	Open (masking not used)
Control:	Uncontrolled
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	13-12-2022
Enrollment:	45
Type:	Actual

Ethics review

Approved WMO Date:	18-05-2020
Application type:	First submission
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)
Approved WMO Date:	07-08-2024
Application type:	Amendment
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL72410.091.19