

2017-07-01

# Controlled human malaria infection leads to long-lasting changes in innate and innate-like lymphocyte populations

---

M. Mpina, N.J. Maurice, M. Yajima, C.K. Slichter, H.W. Miller, M. Dutta, M.J. McElrath, K.D. Stuart, S.C. De Rosa, J.P. McNevin, P.S. Linsley, S. Abdulla, M. Tanner, S.L. Hoffman, R. Gottardo, C.A. Daubenberger, M. Prlc. 2017. "Controlled Human Malaria Infection Leads to Long-Lasting Changes in Innate and Innate-like Lymphocyte Populations" *Journal of Immunology*, Volume 199, Issue 1, pp.107-118. <https://doi.org/10.4049/jimmunol.1601989>  
<https://hdl.handle.net/2144/48588>

*"Downloaded from OpenBU. Boston University's institutional repository."*



Published in final edited form as:

*J Immunol.* 2017 July 01; 199(1): 107–118. doi:10.4049/jimmunol.1601989.

## Controlled human malaria infection leads to long-lasting changes in innate and innate-like lymphocyte populations

Maximilian Mpina<sup>\*,†,‡‡</sup>, Nicholas J Maurice<sup>‡,‡‡</sup>, Masanao Yajima<sup>‡,††,‡‡</sup>, Chloe K Slichter<sup>‡,§,‡‡</sup>, Hannah W Miller<sup>‡,‡‡</sup>, Mukta Dutta<sup>‡</sup>, M. Juliana McElrath<sup>‡,§</sup>, Kenneth D Stuart<sup>¶</sup>, Stephen DeRosa<sup>‡</sup>, John P. McNevin<sup>‡</sup>, Peter S Linsley<sup>||</sup>, Salim Abdulla<sup>#</sup>, Marcel Tanner<sup>\*,†</sup>, Stephen L Hoffman<sup>\*\*</sup>, Raphael Gottardo<sup>‡</sup>, Claudia A. Daubenberger<sup>\*,†,§§</sup>, and Martin Prlic<sup>‡,§,§§</sup>

\*Swiss Tropical and Public Health Institute, Basel, Switzerland †University of Basel, Basel, Switzerland ‡Fred Hutchinson Cancer Research Center, Vaccine and Infectious Disease Division, Seattle, WA 98109, USA §Department of Global Health, University of Washington, Seattle, WA 98195, USA ¶Center for Infectious Disease Research, Seattle, WA, 98109, USA ||Benaroya Research Institute, Seattle, WA, USA #Ifakara Health Institute, Bagamoyo Research and Training Centre, Bagamoyo, Tanzania \*\*Sanaria Inc. Rockville, MD, USA ††Boston University, Department of Mathematics & Statistics, Boston, MA 02215, USA

### Abstract

Animal model studies highlight the role of innate-like lymphocyte populations in the early inflammatory response and subsequent parasite control following *Plasmodium* infection. IFN $\gamma$  production by these lymphocytes likely plays a key role in the early control of the parasite and disease severity. Analyzing human innate-like T cell and natural killer (NK) cell responses following infection with *Plasmodium* has been challenging since the early stages of infection are clinically silent. To overcome this limitation, we examined blood samples from a controlled human malaria infection (CHMI) study in a Tanzanian cohort, in which volunteers underwent CHMI with a low or high CHMI dose of *Plasmodium falciparum* sporozoites (PfSPZ challenge). The CHMI differentially affected NK, natural killer T (iNKT) and mucosal-associated invariant T (MAIT) cell populations in a dose-dependent manner resulting in an altered composition of this innate-like lymphocyte compartment. While these innate-like responses are typically thought of as short-lived, we found that changes persisted for months after the infection was cleared leading to significantly increased frequencies of MAIT cells 6 months post-infection. We used single-cell RNAseq and TCR  $\alpha\beta$  chain usage analysis to define potential mechanisms for this expansion. These single-cell data suggest that this increase was mediated by homeostatic expansion-like mechanisms. Together these data demonstrate that CHMI leads to previously unappreciated long-lasting alterations in the human innate-like lymphocyte compartment. We discuss the consequences of these changes for recurrent parasite infection and infection-associated pathologies

§§Corresponding authors: Claudia A. Daubenberger: Claudia.Daubenberger@unibas.ch, phone: 0041 612848217, Fax: 004161 2848111; Martin Prlic: mprlic@fhcrc.org, phone: 001 206 667 2216, Fax: 001 206 667 2209.

‡‡These authors contributed equally to this work

**Disclosure:** Sanaria Inc. manufactured the PfSPZ Challenge. Thus, all authors associated with Sanaria Inc. have potential conflicts of interest. There are no other conflicts of interest.

and highlight the importance of considering host immunity and infection history for vaccine design.

---

## Introduction

Infection with the apicomplexan parasite *Plasmodium falciparum* causes malaria and resulted in an estimated number of 214 million clinical cases (range: 149–303 million) and 438 000 deaths (range: 236 000–635 000) globally in 2015 (1). Children are particularly vulnerable until the age of 5 when they become more resistant to severe forms of malaria. *P. falciparum* sporozoites are transmitted to the human host during feeding of infected female *Anopheles* mosquitoes. These few sporozoites travel through blood vessels to the liver where they develop into the symptomatically silent pre-erythrocytic life cycle stage. Immunization with high numbers of attenuated *P. falciparum* sporozoites can induce protection against malaria infection (2-7). Importantly, resistance to disease is rather short-lived due to development of short-lived immunological memory (8). Over time, repeated exposure will eventually lead to decreased disease symptoms, however resistance against subsequent infections is limited. Pre-erythrocytic stage specific protective immunity requires a combination of liver-resident adaptive cellular immune mechanisms including cytotoxic CD8 T cells and CD4 T cells that secrete IFN $\gamma$  and provide help to sporozoite-neutralizing antibody producing B cells (8, 9), but other immune processes may be of importance as well. Innate-like lymphocyte responses precede adaptive immune responses and animal model data suggest that these early immune responses are critical for the outcome of subsequent adaptive immune responses (8).

Innate-like lymphocytes have been studied in a wide variety of infections due to their ability to rapidly secrete IFN $\gamma$  and TNF $\alpha$  and other effector molecules that control early inflammatory responses and pathogen load (10, 11). These innate and innate-like subsets include Natural Killer (NK) cells, Natural Killer T (iNKT) cells and Mucosal-Associated Invariant T (MAIT) cells. NK cells (CD3<sup>-</sup> CD56<sup>+</sup>) are abundant in human blood (~13% of lymphocytes) and liver (~31% of lymphocytes) (12). NK cell function is controlled by a balance of inhibitory (recognizing MHC class I) and activating signals such as stress-induced ligands from the ULBP family (13). MAIT cells (CD3<sup>+</sup> CD161<sup>hi</sup> V $\alpha$ 7.2<sup>+</sup>) make up 1-8% of T cells in blood and mucosal tissues and 20-45% of T cells in the liver (14, 15). The MAIT cell TCR consists of an invariant TCR $\alpha$  chain (V $\alpha$ 7.2) and a limited range of possible  $\beta$  chains (16, 17), but MAIT cells have recently been shown to have greater TCR heterogeneity than initially expected (18-20). The MAIT TCR recognizes antigen in the context of the major histocompatibility complex-related protein 1 (MR1), which includes bacterial metabolites of the riboflavin synthesis pathway (21). We demonstrated that inflammatory and TCR signals synergize to induce MAIT cell effector function (22), although inflammatory signals can be sufficient to activate MAIT cells (23, 24). iNKT cell (CD3<sup>+</sup> V $\alpha$ 24J $\alpha$ 18<sup>+</sup>) abundance is substantially lower in humans compared to the mouse model system. iNKT cells are typically ~0.02% of T cells in blood and ~0.5% of T cells in the liver (25). The iNKT cell TCR consists of an invariant TCR $\alpha$  chain (V $\alpha$ 24J $\alpha$ 18) and a limited range of possible  $\beta$  chains (typically V $\beta$ 11) and recognizes lipids presented by the MHC class I-like protein CD1d (26).

A shared feature of these cell populations is their ability to quickly respond to inflammatory signals such as IL-12, IL-15 and IL-18 (26, 27). A consequence of this inflammation-mediated activation is the acquisition of effector function including expression of IFN $\gamma$  and granzyme B (grzB) (23). IFN $\gamma$  is of particular interest as it seems critical in mediating immunity against the parasite (28-35). Importantly, while inflammation appears limited during the liver stage of malaria, the pro-inflammatory response is pronounced during the asexual blood stage with an increase in IL-12 and IL-18 serum concentrations (36-39). Thus, the initial inflammatory environment elicited by the infection may be sufficient to activate these cell populations leading to secretion of effector molecules such as IFN $\gamma$  and TNF $\alpha$ . Importantly, animal model data suggest that the early inflammatory environment and particularly IFN $\gamma$  seem to play an important role in the ensuing parasite control as well as disease progression, including development of cerebral malaria (10, 40, 41). It is currently unknown how accurately the mouse model mimics these innate-like lymphocyte responses in human infection since - to our knowledge - the function and fate of NK and innate-like T cells following *Plasmodium* infection of humans have not been elucidated in detail.

To address this question we specifically examined changes in the NK cell, NKT cell and MAIT cell populations using samples from a controlled human malaria infection (CHMI) study in a Tanzanian cohort of young adult males (age 20 - 35) exposed to aseptic *P. falciparum* sporozoites (PfSPZ challenge) (42). We found that CHMI significantly affected the NK and MAIT cell population frequencies and that changes in the MAIT cell population persisted at least 168 days post infection. To further interrogate these changes in MAIT cell frequency, we used a novel method to reconstruct TCR  $\alpha\beta$  chain usage from single-cell RNAseq data. Analysis of these data suggests that the MAIT cell population rebound after the blood stage parasitemia is driven by homeostatic expansion mechanisms. Together, these data demonstrate for the first time that CHMI induces long-lasting changes in the composition of the innate-like lymphocyte compartment with the most pronounced changes in the MAIT cell population. We discuss the implications of these data in the context of recurrent infection, pathology and vaccine development.

## Materials and Methods

### Ethics statement

Peripheral blood mononuclear cells (PBMC) analyzed here were collected during the CHMI trial conducted in Tanzania and described in Shekalaghe et al. (42). This CHMI was conducted as single center, randomized, double blind controlled trial enrolling volunteers following predefined inclusion and exclusion criteria. All volunteers gave written informed consent before screening and being enrolled in the study and the trial was performed in accordance with Good Clinical Practices, an Investigational New Drug (IND) application filed with the U.S. Food and Drug Administration (US FDA) (IND 14267), and an Investigational Medical Product Dossier (IMPD) filed with the Tanzanian Food and Drug Administration (TFDA). The protocol was approved by institutional review boards (IRBs) of the Ifakara Health Institute (IHI/IRB/No25) and the National Institute for Medical Research, Tanzania (NIMR/HQ/R.8a/Vol.IX/1217), the Ethikkommission beider Basel (EKBB), Basel,

Switzerland (EKBB 319/11). The protocol was also approved by TFDA (Ref. No. CE. 57/180/04A/50), and the trial was registered at ClinicalTrials.gov (NCT01540903).

### Samples analyzed

Cryopreserved samples including only the participant ID (but no personal or identifiable information) from this study were sent from the Ifakara Health Institute to the FHCRC for analysis with approval by the institutional IRB (#7349). In the  $2.5 \times 10^4$  PfSPZ group, one volunteer had to be removed from the analysis due to pre-mature malaria treatment and only volunteers who developed asexual blood stage parasites after administration of Sanaria PfSPZ Challenge were included in the analysis. Venous blood was drawn prior to the intradermal injection PfSPZ Challenge (day 0) and at subsequent time-points (days 9, 28, 56 and 168) (42). Volunteers were monitored for onset of the asexual blood stage parasitemia by examination of thick blood smears by microscopy and treated with a curative dose of Coartem® following national guidelines. A very limited number of samples were also available from blood drawn at the time of microscopy based asexual blood stage parasitemia diagnosis. Importantly, although these volunteers were not malaria-naïve, they had not had a documented malaria episode in the past 5 years. PBMC samples were stored in liquid nitrogen and then shipped to the Fred Hutchinson Cancer Research Center, Vaccine and Infectious Disease Division for analysis by flow cytometry, RNAseq or used for *ex vivo* stimulation assays. Lymphocyte counts of volunteers participating in this CHMI have been collected using the Sysmex XS-800i machine.

### Flow cytometry

For the phenotypic identification of lymphocyte populations, bulk PBMC or sorted MAIT cells were stained with Aqua Live/Dead Fixable Dead Cell Stain (Invitrogen). Our gating strategy and representative FACS plots are shown in Supplemental Figure 2 and our 4 different FACS analysis panels are listed as tables in Supplemental Figure 3.

### Cell sorting

PBMC were stained with the described sort panel and sorted on a FACSria (BD) into complete RP10. Populations sorted were CD8<sup>+</sup> MAIT (CD3<sup>+</sup>CD8<sup>+</sup>CD161<sup>hi</sup>Vα.7.2<sup>+</sup>) cells. All sorts were performed on the FACS Aria II.

### Single-cell RNAseq processing, sequencing, and alignment

After flow sorting, single cells were captured on the Fluidigm C1 Single-Cell Auto Prep System (C1), lysed on chip, and subjected to reverse transcription and cDNA amplification using the SMARTer Ultra Low Input RNA Kit for C1 System (Clontech, Mountain View, CA). Sequencing libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA) according to C1 protocols (Fluidigm). Barcoded libraries were pooled and quantified using a Qubit Fluorometer (Thermo Scientific Life Technologies, Grand Island, NY). Single-read sequencing of the pooled libraries was carried out either on a HiScanSQ or a HiSeq2500 sequencer (Illumina) with 100-base reads, using TruSeq v3 Cluster and SBS kits (Illumina) with a target depth of >2.5 M reads. Sequences were aligned to the UCSC Human Genome Assembly version 38, gene expression levels

quantified using RSEM and TPM values loaded into R for analyses. We used the MAST analysis platform(23) for subsequent single-cell analysis steps. Quality control parameters for the single-cell RNAseq data included expression of at least 500 genes, alignment rate bigger than 80%, library size > 10000 and exon rate bigger than 30% (all 4 criteria have to be met to for a single cell to be included). MAIT cells were analyzed directly after sorting and were not stimulated prior to single-cell RNAseq processing.

### MAIT cell Stimulation Assay

2,000-20,000 CD8<sup>+</sup> MAIT cells were left untreated or were treated with IL-12 (eBioscience), IL-15 (eBioscience), and IL-18 (MBL) at 100ng/mL or a combination of the cytokines and anti-CD3/CD28-coupled beads (Invitrogen). At 20h GolgiPlug (BD) was added at a 1:1000 final dilution. For intracellular staining, cells were fixed with Cytofix/Cytoperm (BD) and permeabilized with Perm/Wash (BD) per manufacturers instructions and stained for intracellular cytokines. Samples were run on an LSRII (BD) and analyzed using FlowJo (Tree Star).

### Statistical analyses

Statistical significance comparing control and treated subjects was done by using a Type-II ANOVA for a linear mixed effect model with patient level random intercepts with alpha level of 5%. R functions lmer and Anova in lme4 and car packages were used for the calculations. Comparison of time-points to each other was done using a paired t-test with false discovery rate (FDR) correction.

## Results

### Innate-like lymphocyte frequencies change in a PfSPZ infection dose-dependent manner

To study the innate-like lymphocyte response to PfSPZ infection, we analyzed samples from a trial conducted in African, malaria-pre-exposed volunteers with intradermal application of  $1 \times 10^4$  (10K) and  $2.5 \times 10^4$  (25K) purified, cryopreserved non-attenuated *P. falciparum* sporozoites (PfSPZ) to two groups of each 12 volunteers, respectively. 11 of 12 volunteers in the 10K PfSPZ group and 10 of 11 evaluable volunteers in the 25K PfSPZ group developed asexual blood stage parasitemia after intradermal injection of PfSPZ (42). Blood was drawn prior to the intradermal challenge (day 0) and at subsequent time-points (days 9, 28, 56 and 168) thereafter (42). Volunteers were monitored for onset of the blood parasitemia stage by blood smear and treated with Coartem® following a positive blood smear to clear the infection. A very limited number of samples were also available from blood drawn at the time point of blood parasitemia diagnosis. Importantly, although these volunteers were not malaria-naïve, they had not had a documented malaria episode in the past 5 years. Thus, the design of this study allowed us to ask how NK, iNKT and MAIT cell function and frequencies change early (day 9) following CHMI and longitudinally examine the duration of these changes in a cohort that is highly relevant for malaria vaccine efforts.

We initially asked if an infection with 10K or 25K PfSPZ affects the composition of the innate-like lymphocyte compartment in the venous blood by flow cytometry. We examined the frequency of MAIT cells (CD3<sup>+</sup> Vα.7.2<sup>+</sup> CD161<sup>hi</sup>), NK cells (CD3<sup>-</sup> CD56<sup>+</sup>), and iNKT

cells (CD3<sup>+</sup> Vα24Jα18<sup>+</sup>) on days 0 and 9 post-infection, and then after the Coartem® treatment on days 28, 56 and 168. Gating strategies used and representative flow cytometry plots are shown in Suppl. Fig. 2.

We observed a significant increase in the frequency of MAIT cells within the CD8<sup>+</sup> T cell population 168 days post-infection ( $p=0.0174$ ) in the 25K challenge dose group. Significant changes in frequency of MAIT cells within the CD8<sup>+</sup> T cell population following infection with the 10K challenge dose was not observed (Fig. 1A, left vs. right panel) indicating that MAIT cells respond in an infection dose-dependent manner. This was recapitulated when calculating the absolute number of CD8<sup>+</sup> MAIT cells in the blood (Suppl. Fig. 1). Importantly, the absolute number of CD3<sup>+</sup> T cells (cells per microliter blood) did not significantly change over time indicating that this population serves as a suitable reference point to display T cell subset frequencies (Suppl. Fig. 1). We next examined the frequencies of NK cells and iNKT cells (Fig. 1B, C). NK cell frequencies in the lymphocyte population were reduced significantly on day 28 ( $p=0.0005$ ) and 56 ( $p=0.0083$ ) post infection, particularly in the high challenge group (Fig. 1B). NK cell frequencies are shown as % of the CD3<sup>+</sup> lymphocyte population, but the same result is seen when analyzing NK cell numbers per microliter blood (Suppl. Fig. 1C). In contrast to NK and MAIT cells, iNKT cell frequencies did not change significantly following the infection regardless of the PfSPZ dose (Fig. 1C). Given the changes of the MAIT cell population following CHMI in the 25K challenge group, and considering their functional potential (IFN $\gamma$  production) and abundance in the liver (up to 40% of all T cells), we primarily focused on the more abundant CD8<sup>+</sup> MAIT cell subset for the rest of the study to determine the consequences of CHMI on the MAIT cell population.

### MAIT cell frequencies change following PfSPZ infection

We examined the frequency of MAIT cells (CD3<sup>+</sup> Vα7.2<sup>+</sup> CD161<sup>hi</sup>; CD8<sup>+</sup> and CD8<sup>-</sup> subsets) on days 0 and 9 post-infection, during the blood parasitemia stage and then after the Coartem® treatment on days 28, 56 and 168. To better dissect the changes in the MAIT cell population, we looked at the frequency of all (CD8<sup>+</sup> and CD8<sup>-</sup>) MAIT cells within the T cell (CD3<sup>+</sup>) compartment (Fig. 2A) and then subdivided the population further into the CD8<sup>+</sup> (Fig. 2B) and the CD8<sup>-</sup> (Fig. 2C) subset. We found that the frequency of MAIT cells within the CD3<sup>+</sup> population dropped significantly during the blood parasitemia stage ( $p=0.0302$ ) and then rebounded thereafter with MAIT cell frequencies on day 168 exceeding the initial day 0 baseline level ( $p=0.0072$ , Fig. 2A). This pattern was also observed when the MAIT population was further divided into the CD8<sup>-</sup> and CD8<sup>+</sup> MAIT cell subset (Fig. 2B-C). Importantly, availability of the blood-stage parasitemia samples was limited to this MAIT cell analysis experiment and thus these samples could not be included in any subsequent experiments.

### MAIT cell activation occurs in a limited manner during the course of the infection

Exposure to pro-inflammatory cytokines is sufficient to induce MAIT cell activation (23, 24). Release of the parasites from the liver into the bloodstream is associated with a pro-inflammatory immune response, which could activate MAIT cells. Thus, we first addressed whether there are signs of MAIT cell activation on day 9 post-infection. CD69 is an early

marker of T cell activation and although an increase in CD69 surface expression is often used as an indicator of TCR signaling, inflammatory signals are also sufficient to induce expression (43). We examined surface expression levels of CD69 and found no significant changes on day 9 or later (Fig. 3A). Similarly, the frequency of CD25<sup>+</sup> and CD40L<sup>+</sup> expressing MAIT cells did not increase significantly on days 9 and 28 following challenge with PfSPZ (Fig. 3B, C). CD25<sup>+</sup> MAIT cells were significantly decreased on day 56 ( $p=0.0011$ ) (Fig. 3B). Finally, NKG2D expression did not change significantly post-infection (Fig. 3D). Together, these flow cytometry data suggest that *ex vivo* MAIT cell activation at the interrogated time-points is minimal.

### MAIT cell effector function is limited on day 9 post-infection

To determine if there is early (day 9) acquisition of effector function we examined the *ex vivo* expression of TNF $\alpha$ , IL-17, IFN $\gamma$  and grzB by intracellular cytokine staining and flow cytometry (Fig. 4A-D). We found no evidence of significant *ex vivo* MAIT cell effector function on days 9, 28, 56 and 168 post-infection. We considered two possibilities for the lack of *ex vivo* effector function: it could be due to a lack of activating inflammatory signals or, alternatively, due to parasite-induced impairment of function. To distinguish between these two possibilities, we next tested how MAIT cells isolated before and after blood stage parasitemia respond to TCR and cytokine stimulation *ex vivo*.

### MAIT cells remain functional following infection with PfSPZ

We isolated MAIT cells from 5 donors by FACS and examined if the ability of MAIT cells to respond to *ex vivo* stimulation was changed on day 9 and 28 post-infection compared to day 0. We measured the ability of MAIT cells to express IFN $\gamma$ , grzB and IL-17A/F following stimulation with pro-inflammatory cytokines (IL-12/15/18) or a combination of T cell receptor signals delivered by anti-CD3/CD28 coated beads and IL-12/15/18. Regardless of the time-point post-infection, MAIT cells responded to both stimulation conditions and almost uniformly expressed grzB (Fig. 5A) and IFN $\gamma$  (Fig. 5B). A much smaller fraction expressed IL-17A or IL-17F at all 3 time-points (Fig. 5C, D). Together these data suggest that MAIT cells remain responsive to TCR and cytokine mediated stimulation on day 9 or 28 post-infection. To determine if we can find some indication of MAIT cell activation early after infection, we next examined MAIT cells from one donor by single-cell RNAseq.

### Single-cell RNAseq analysis of MAIT cells

We isolated MAIT cells by FACS from donor 20064 and used the C1 Fluidigm system to interrogate changes in gene expression signatures on a single-cell level comparing days 0, 9 and 28 after CHMI. Using an analysis method we previously developed (23), we found that some, but not all MAIT cells express either grzB or IFN $\gamma$  transcript on day 9 post infection, but not on days 0 and 28 (Fig. 6A) suggesting that some MAIT cells are indeed partially activated on day 9 post-infection.

To acquire a more global understanding of the MAIT cell changes we used the single-cell transcriptome data to compare the transcriptome of these single cells at days 0, 9 and day 28 post-infection. We found that the MAIT cell population displayed a distinct RNA expression profile on each day (Fig. 6B) suggesting that MAIT cells do respond to the PfSPZ challenge

by day 9 and do not return to baseline transcriptional status by day 28. To better understand the nature of these changes, we next reconstructed the TCR $\alpha$  and TCR $\beta$  sequences from the transcriptome and analyzed the CDR3 region to identify clonal diversity (Fig. 6C and Suppl. Fig. 4). We did not observe significant changes in the relative distribution of MAIT cell clones by day 28 suggesting that the expansion of MAIT cells is not dominated by a distinct TCR bearing clonal population. We found an increase in the co-expression of IL-7R $\alpha$  and the  $\gamma$  chain (needed for signal transduction and shared with other cytokine receptors) on days 9 and 28 (Fig. 6D). While IL-7 is typically associated with promoting homeostatic proliferation (44), recent studies suggest that IL-7 can induce MAIT cell cytotoxic function (45, 46). The increase of mRNA encoding for the  $\gamma$  chain on MAIT cells was confirmed on the protein level by flow cytometry staining (Fig. 6E). Given this potential change in responsiveness to cytokine signals, we next wanted to determine if the expanded (post day 28) MAIT cell population is distinct in terms of trafficking properties from the pre-infection MAIT cell population.

### Trafficking properties in MAIT cells

Finally, to determine if tissue trafficking properties of MAIT cells change post-infection, we examined chemokine receptor expression following CHMI by FACS. To specifically determine liver- and tissue-homing potential of MAIT cells during CHMI, we analyzed expression of CCR6, CXCR6, and CXCR3. Briefly, CCR6 is a key receptor to enable trafficking to inflamed tissues and CXCR6 plays an essential role for trafficking to the liver (47-49). The majority of MAIT cells expressed CCR6 and CXCR6 on their surface prior to CHMI. We found that expression of CCR6 and CXCR6 was overall fairly stable over time (Fig. 7A-B). Only a small fraction of MAIT cells expressed CXCR3, which has recently been shown to orchestrate migration of antigen-specific T cells (50). We observed an initial significant decrease (day 0 vs. 28,  $p=0.0078$ ) in the frequency of CXCR3<sup>+</sup> CD8<sup>+</sup> MAIT cells, followed by an increase over time nearing initial expression frequency (Fig. 7C).

### Discussion

Controlled human malaria infection (CHMI) is a powerful approach to study immune responses following defined exposure to *P. falciparum* in different human populations (51). We report here that a single intradermal infection with aseptic, purified, cryopreserved *P. falciparum* sporozoites (PfSPZ challenge) has profound, long-lasting impacts on the innate-like lymphocyte compartment in peripheral blood of malaria pre-exposed, adult Tanzanian volunteers. MAIT cells in malaria naïve volunteers undergoing similar CHMI approaches have to our knowledge not been studied (51). We observed a significant decrease in the frequency of blood circulating MAIT cells following intradermal infection with 25K PfSPZs during early blood stage parasitemia (day 11 – 18 post-infection). This was followed by a MAIT cell frequency rebound, which exceeded the initial baseline frequency and was sustained for months after the infection (Fig. 1 and Fig. 2; Suppl. Fig. 1). The long lasting (168 days) increase in MAIT cell frequency that we observed is remarkable, because an acute infection or vaccine application typically only temporarily changes the innate immune system before it returns back to baseline. In contrast, long-lasting changes are seen in the adaptive immune system due to antigen-specific adaptive memory immune responses. This

was elegantly demonstrated by Tsang and colleagues on a systems biology level in context of the influenza vaccine (52). Our data suggest that an acute infection with PfSPZ did not just result in a temporary immune perturbation, but instead had a long-lasting impact on MAIT cells. Importantly, these changes occurred in an infection dose-dependent manner in volunteers infected with 25K, but not 10K PfSPZ (Fig. 1). These two doses resulted in *P. falciparum* infections with different geometric mean asexual erythrocytic stage parasitemia pre-patent periods of 15.4 days (10K) and 13.5 days (25K) by microscopy ( $p=0.023$ ) and 12.2 days (10K) and 11.1 days (25K) by qPCR ( $p=0.076$ ) (42). This kinetic difference is presumably due to the release of higher numbers of merozoites from the liver in the 25K PfSPZ group resulting in a shorter pre-patent period in comparison to the 10K PfSPZ group. Malaria treatment decision was based on detection of asexual blood stage parasites in the blood by microscopy. Given the difference in detection sensitivity of qPCR and microscopy, the immune system had likely been exposed to asexual blood stage parasites for around 2.5 to 3 days in both groups before initiation of Coartem® treatment. Based on this similarity in the duration of the blood stage phase of infection in the two dosage groups, the infection dose-dependent differences we observed (Fig. 1) are likely due to an increased inflammatory response in the liver following infection with 25K PfSPZ as opposed to a prolonged infection at the blood stage.

Interestingly, the NK cell frequency decreased significantly early after a 25K PfSPZ infection similarly to MAIT cells (Fig. 1B), but then slowly (by day 168) returned to baseline levels. This decrease contrasts data from an animal model demonstrating that NK cells in the blood expand robustly and early after infection (40). Support for the notion that NK cells contribute to the anti-parasite immune response comes from a recent study that provided evidence that human NK cells bind to and eliminate infected red blood cells using cell contact-dependent mechanisms (53). Additional human studies will be required to determine which aspects of the mouse model system can be used to mimic human NK cell responses. Finally, iNKT cell frequencies were stable regardless of the challenge dose (Fig. 1C). We argue that this is unexpected given the many similarities of iNKT and MAIT cells in terms of pro-inflammatory cytokine responsiveness (26). Future studies will need to address why MAIT but not iNKT cells are affected by CHMI.

To examine if the MAIT cell function is altered in addition to frequency, we next examined MAIT cells for expression of markers that indicate recent activation (CD69, CD25) as well as *ex vivo* expression of cytokines (IL-17, TNF $\alpha$ , IFN $\gamma$ , grzB). MAIT cells did not show a strong increase in expression of biomarkers that indicate activation at early or later time-points (Fig. 3) or displayed *ex vivo* effector function on day 9 or later post infection (Fig. 4). Importantly, MAIT cells remained fully responsive to activating signals *ex vivo* (Fig. 5). These data demonstrate that MAIT cell function is not lost and argue against the existence of inhibitory signals that impair MAIT cell function following PfSPZ infection. This is in contrast to reports that suggest parasite specific adaptive immune responses are impaired by the presence of asexual blood stage parasites (11). Determining that MAIT cells remain functional is highly relevant, because mechanisms that activate MAIT cells in the liver could be of interest as a potential therapeutic strategy against the liver-stage disease. MAIT cells, due to their high abundance in the liver (up to 45% of T cells), may be involved in maintaining liver homeostasis during malaria parasite infections and might provide early

anti-parasite effector mediators (such as IFN $\gamma$  production) before the adaptive immune response is recruited and fully developed. The size of the MAIT cell population in the liver likely exceeds the size of the adaptive, antigen-specific T cell population that could be induced by vaccination. In this context it is also important to consider that MAIT cells have high expression levels of CCR6 and CXCR6 allowing MAIT cells to traffic to the liver. Importantly, we found evidence of partial activation by single-cell RNAseq (Fig. 6A) suggesting that there are some activating - but yet unknown - stimuli early after infection. It is noteworthy that despite minimal initial activation, the MAIT cell transcriptome did not return to baseline in the time frame tested as we still found transcriptional differences on day 28 post-infection (Fig. 6B). Future studies will need to examine the MAIT cell transcriptome at later time points after infection to determine how long this functional alteration lasts.

Next, we used these single-cell RNAseq MAIT cell transcriptome data to reconstruct TCR $\alpha$  and  $\beta$  chain usage in the MAIT cell population on days 0, 9 and 28 from the same donor. Although MAIT cells express an invariant  $\alpha$  chain, there are differences in the CDR3 junctions that allowed us to track distinct MAIT cell clones (Fig. 6C). Given that we observed an increase in MAIT cell frequency starting by day 28 in an infection-dose dependent manner, we asked how the TCR  $\alpha\beta$  distribution changed at these 3 time points. We wanted to distinguish if the increase on day 28 is due to a relocation of the same MAIT cell population back into circulation after its sharp decline on the day of parasitemia or indicative of replenishing of the MAIT cell population via homeostatic mechanisms or *de novo* generation from the thymus. Our single-cell RNAseq data did not reveal significant changes to the TCR repertoire between days 0, 9 and 28 indicative of an overall MAIT cell expansion rather than a clonotype driven response (Fig. 6C). Interestingly, a previous study reported a sustained expansion of  $\gamma\delta$  T cells following a primary CHMI in naive volunteers, but it is still unclear if this expansion is polyclonal or clonally driven (51, 54). Importantly, in context of the increased IL-7R $\alpha$  and  $c\gamma$  co-expression on day 28, we argue that the increase in the MAIT cell population is triggered by homeostatic expansion. Homeostatic expansion occurs in response to available growth factors and is typically driven by cytokines (such as IL-7 and IL-15), whose receptor utilizes the common  $\gamma$  chain (55, 56). Increased responsiveness to IL-7 through enhanced IL-7R $\alpha$  expression could be of specific interest as it also positively affects MAIT cell effector function (45, 46).

Given the temporary decrease of MAIT cell frequency in blood in the 25K PfSPZ group, we considered that MAIT cells could migrate to other tissues or, alternatively, undergo apoptosis. A decrease in blood MAIT cell frequencies following infection is not unusual and has been reported in several other instances including active tuberculosis (57) and HIV (58-60), although the fate of the MAIT cells in these situations has not been fully resolved. In an effort to determine whether migration may play a role in these MAIT cell changes, we examined the expression patterns of 3 highly relevant chemokine receptors for homing to the liver and other tissues: CCR6, CXCR6 and CXCR3 (Fig. 7). We did not find any significant differences in the expression pattern of CCR6 and CXCR6 with only a temporary decrease of CXCR3 expression early after infection. This shows that MAIT cells remain responsive to chemotactic signals, but does not provide insight into the relationship of blood and tissue MAIT cells. Ultimately it would be necessary to examine the human liver tissue to understand the dynamics of the MAIT cell response. MAIT cells are rare in mice, however a

recently developed mouse model with MAIT cell frequencies that approximate human frequencies opens new possibilities for studying MAIT cells in an animal model (61).

How biologically relevant are these changes in the MAIT cell and NK cell population? NK cells play an important and non-redundant role in host defense against viruses, particularly herpesviruses. Although the frequency of NK cells eventually normalized again, it raises the question if the PfSPZ-induced NK cell decrease may have a temporary general negative impact on host immunity given the critical role of NK cells for protection against herpesvirus infections (62). This is particularly interesting in context of Epstein-Barr virus (EBV) infections, since co-infection is a major risk factor for developing Burkitt's lymphoma and exposure to malaria can affect immune responses against EBV (63-65). Finally, MAIT cells have been suggested to play a role in antibacterial immunity through sensing of MR1-bound microbial products, but appear quite sensitive to inflammatory cues in the absence of antigen. Thus, MAIT cells may act as an amplifier of early pro-inflammatory signals and an increase in MAIT cells may lead to a more profound subsequent pro-inflammatory response. In context of malaria, these changes in the early immune response to the parasite may either be beneficial and contribute to clearance, but may also exacerbate pathologies that have been associated with excessive inflammatory responses. It is important to keep in mind that we studied the immune response following a defined single malaria infection event initiated by purified, cryopreserved sporozoites of a defined genotype, NF54 and treatment occurred upon detection of the blood stage of infection. Under natural conditions, humans are exposed to multiple infectious mosquito bites carrying a range of different parasite genotypes and treatment is typically not immediate. Thus, the well-defined CHMI infection approach allows us to study the immune response in the context of the liver- and early blood stage of infection, which will help interpret data from more complex natural infection studies.

Changes in the initial immune response affect the ensuing adaptive immune response, which is of relevance in the context of vaccine efficacy. Our data demonstrate changes in the NK and MAIT cell compartment months after CHMI. Additional studies are now required to address how these changes alter the outcome of repeated malaria infections (delivered by CHMI or natural infections) that could be beneficial or detrimental for malaria induced pathology, viral or bacterial co-infections and vaccine take and responsiveness in individuals with recent history of malaria exposure, particularly infants and children (66).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

We thank the James B. Pendleton Charitable Trust for their generous equipment donation. We thank Daryl Morris for a preliminary statistical analysis of the data and Gabriela Diaz for assistance in the sample preparation. We thank Vivian Gersuk, Kimberly O'Brien and Quynh-Anh Nguyen for excellent technical support for the single-cell RNAseq experiments. We thank all volunteers and staff of the Ifakara Health Institute, Bagamoyo Research and Training Centre, Sanaria Inc., and Swiss Tropical and Public Health Institute who contributed so much to the successful conduct of the CHMI.

**Funding:** Funding was provided by NIH grants DP2 DE023321 (to MP), 2T32AI007509-16 (to CKS), 1U19AI089986 (to KDS), U01 AI089859-05 (Pilot Grant to MP) and the Swiss Tropical and Public Health Institute (to CD). The development, manufacturing, and quality control release and stability studies of the Sanaria PfSPZ Challenge were supported in part by an NIAID Small Business Innovation Research grant, 5R44AI058375.

## References

1. Organization, W. H.. World malaria report 2015. Geneva, Switzerland: WHO Press; 2015.
2. Epstein JE, Paolino KM, Richie TL, Sedegah M, Singer A, Ruben AJ, Chakravarty S, Stafford A, Ruck RC, Eappen AG, Li T, Billingsley PF, Manoj A, Silva JC, Moser K, Nielsen R, Tosh D, Cicatelli S, Ganeshan H, Case J, Padilla D, Davidson S, Garver L, Saverino E, Murshedkar T, Gunasekera A, Twomey PS, Reyes S, Moon JE, James ER, Kc N, Li M, Abot E, Belmonte A, Hauns K, Belmonte M, Huang J, Vasquez C, Remich S, Carrington M, Abebe Y, Tillman A, Hickey B, Regules J, Villasante E, Sim BK, Hoffman SL. Protection against *Plasmodium falciparum* malaria by PfSPZ Vaccine. *JCI insight*. 2017; 2:e89154. [PubMed: 28097230]
3. Ishizuka AS, Lyke KE, DeZure A, Berry AA, Richie TL, Mendoza FH, Enama ME, Gordon IJ, Chang LJ, Sarwar UN, Zephir KL, Holman LA, James ER, Billingsley PF, Gunasekera A, Chakravarty S, Manoj A, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, K CN, Murshedkar T, DeCederfelt H, Plummer SH, Hendel CS, Novik L, Costner PJ, Saunders JG, Laurens MB, Plowe CV, Flynn B, Whalen WR, Todd JP, Noor J, Rao S, Sierra-Davidson K, Lynn GM, Epstein JE, Kemp MA, Fahle GA, Mikolajczak SA, Fishbaugher M, Sack BK, Kappe SH, Davidson SA, Garver LS, Bjorkstrom NK, Nason MC, Graham BS, Roederer M, Sim BK, Hoffman SL, Ledgerwood JE, Seder RA. Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. *Nature medicine*. 2016; 22:614–623.
4. Lyke KE, Ishizuka AS, Berry AA, Chakravarty S, DeZure A, Enama ME, James ER, Billingsley PF, Gunasekera A, Manoj A, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, Kc N, Murshedkar T, Mendoza FH, Gordon IJ, Zephir KL, Holman LA, Plummer SH, Hendel CS, Novik L, Costner PJ, Saunders JG, Berkowitz NM, Flynn BJ, Nason MC, Garver LS, Laurens MB, Plowe CV, Richie TL, Graham BS, Roederer M, Sim BK, Ledgerwood JE, Hoffman SL, Seder RA. Attenuated PfSPZ Vaccine induces strain-transcending T cells and durable protection against heterologous controlled human malaria infection. *Proceedings of the National Academy of Sciences of the United States of America*. 2017
5. Mordmuller B, Surat G, Lagler H, Chakravarty S, Ishizuka AS, Lalremruata A, Gmeiner M, Campo JJ, Esen M, Ruben AJ, Held J, Calle CL, Mengue JB, Gebru T, Ibanez J, Sulyok M, James ER, Billingsley PF, Natasha KC, Manoj A, Murshedkar T, Gunasekera A, Eappen AG, Li T, Stafford RE, Li M, Felgner PL, Seder RA, Richie TL, Sim BK, Hoffman SL, Kremsner PG. Sterile protection against human malaria by chemoattenuated PfSPZ vaccine. *Nature*. 2017; 542:445–449. [PubMed: 28199305]
6. Sissoko MS, Healy SA, Katile A, Omaswa F, Zaidi I, Gabriel EE, Kamate B, Samake Y, Guindo MA, Dolo A, Niangaly A, Niare K, Zeguime A, Sissoko K, Diallo H, Thera I, Ding K, Fay MP, O'Connell EM, Nutman TB, Wong-Madden S, Murshedkar T, Ruben AJ, Li M, Abebe Y, Manoj A, Gunasekera A, Chakravarty S, Sim BK, Billingsley PF, James ER, Walther M, Richie TL, Hoffman SL, Doumbo O, Duffy PE. Safety and efficacy of PfSPZ Vaccine against *Plasmodium falciparum* via direct venous inoculation in healthy malaria-exposed adults in Mali: a randomised, double-blind phase 1 trial. *The Lancet Infectious diseases*. 2017
7. Seder RA, Chang LJ, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, Holman LA, James ER, Billingsley PF, Gunasekera A, Richman A, Chakravarty S, Manoj A, Velmurugan S, Li M, Ruben AJ, Li T, Eappen AG, Stafford RE, Plummer SH, Hendel CS, Novik L, Costner PJ, Mendoza FH, Saunders JG, Nason MC, Richardson JH, Murphy J, Davidson SA, Richie TL, Sedegah M, Sutamihardja A, Fahle GA, Lyke KE, Laurens MB, Roederer M, Tewari K, Epstein JE, Sim BK, Ledgerwood JE, Graham BS, Hoffman SL. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. *Science*. 2013; 341:1359–1365. [PubMed: 23929949]
8. Crompton PD, Moebius J, Portugal S, Waisberg M, Hart G, Garver LS, Miller LH, Barillas-Mury C, Pierce SK. Malaria immunity in man and mosquito: insights into unsolved mysteries of a deadly infectious disease. *Annual review of immunology*. 2014; 32:157–187.

9. Doolan DL, Hoffman SL. The complexity of protective immunity against liver-stage malaria. *J Immunol.* 2000; 165:1453–1462. [PubMed: 10903750]
10. Hansen DS, D’Ombrain MC, Schofield L. The role of leukocytes bearing Natural Killer Complex receptors and Killer Immunoglobulin-like Receptors in the immunology of malaria. *Current opinion in immunology.* 2007; 19:416–423. [PubMed: 17702559]
11. Stevenson MM, Riley EM. Innate immunity to malaria. *Nature reviews Immunology.* 2004; 4:169–180.
12. Doherty DG, O’Farrelly C. Innate and adaptive lymphoid cells in the human liver. *Immunological reviews.* 2000; 174:5–20. [PubMed: 10807503]
13. Orr MT, Lanier LL. Natural killer cell education and tolerance. *Cell.* 2010; 142:847–856. [PubMed: 20850008]
14. Dusseaux M, Martin E, Serriari N, Peguillet I, Premel V, Louis D, Milder M, Le Bourhis L, Soudais C, Treiner E, Lantz O. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. *Blood.* 2011; 117:1250–1259. [PubMed: 21084709]
15. Jo J, Tan AT, Ussher JE, Sandalova E, Tang XZ, Tan-Garcia A, To N, Hong M, Chia A, Gill US, Kennedy PT, Tan KC, Lee KH, De Libero G, Gehring AJ, Willberg CB, Klenerman P, Bertoletti A. Toll-like receptor 8 agonist and bacteria trigger potent activation of innate immune cells in human liver. *PLoS pathogens.* 2014; 10:e1004210. [PubMed: 24967632]
16. Porcelli S, Yockey CE, Brenner MB, Balk SP. Analysis of T cell antigen receptor (TCR) expression by human peripheral blood CD4-8- alpha/beta T cells demonstrates preferential use of several V beta genes and an invariant TCR alpha chain. *The Journal of experimental medicine.* 1993; 178:1–16. [PubMed: 8391057]
17. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, Affaticati P, Gilfillan S, Lantz O. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. *Nature.* 2003; 422:164–169. [PubMed: 12634786]
18. Gold MC, McLaren JE, Reistetter JA, Smyk-Pearson S, Ladell K, Swarbrick GM, Yu YY, Hansen TH, Lund O, Nielsen M, Gerritsen B, Kesmir C, Miles JJ, Lewinsohn DA, Price DA, Lewinsohn DM. MR1-restricted MAIT cells display ligand discrimination and pathogen selectivity through distinct T cell receptor usage. *The Journal of experimental medicine.* 2014; 211:1601–1610. [PubMed: 25049333]
19. Eckle SB, Birkinshaw RW, Kostenko L, Corbett AJ, McWilliam HE, Reantragoon R, Chen Z, Gherardin NA, Beddoe T, Liu L, Patel O, Meehan B, Fairlie DP, Villadangos JA, Godfrey DI, Kjer-Nielsen L, McCluskey J, Rossjohn J. A molecular basis underpinning the T cell receptor heterogeneity of mucosal-associated invariant T cells. *The Journal of experimental medicine.* 2014; 211:1585–1600. [PubMed: 25049336]
20. Lepore M, Kalinichenko A, Colone A, Paleja B, Singhal A, Tschumi A, Lee B, Poidinger M, Zolezzi F, Quagliata L, Sander P, Newell E, Bertoletti A, Terracciano L, De Libero G, Mori L. Parallel T-cell cloning and deep sequencing of human MAIT cells reveal stable oligoclonal TCRbeta repertoire. *Nature communications.* 2014; 5:3866.
21. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, Bhati M, Chen Z, Kostenko L, Reantragoon R, Williamson NA, Purcell AW, Dudek NL, McConville MJ, O’Hair RA, Khairallah GN, Godfrey DI, Fairlie DP, Rossjohn J, McCluskey J. MR1 presents microbial vitamin B metabolites to MAIT cells. *Nature.* 2012; 491:717–723. [PubMed: 23051753]
22. Slichter CK, McDavid A, Miller HW, Finak G, Seymour BJ, McNevin JP, Diaz G, Czartoski JL, McElrath MJ, Gottardo R, Prlic M. Distinct activation thresholds of human conventional and innate-like memory T cells. *JCI insight.* 2016; 1
23. Finak G, McDavid A, Yajima M, Deng J, Gersuk V, Shalek AK, Slichter CK, Miller HW, McElrath MJ, Prlic M, Linsley PS, Gottardo R. MAST: a flexible statistical framework for assessing transcriptional changes and characterizing heterogeneity in single-cell RNA sequencing data. *Genome biology.* 2015; 16:278. [PubMed: 26653891]
24. Ussher JE, Bilton M, Attwod E, Shadwell J, Richardson R, de Lara C, Mettke E, Kurioka A, Hansen TH, Klenerman P, Willberg CB. CD161<sup>++</sup> CD8<sup>+</sup> T cells, including the MAIT cell subset, are specifically activated by IL-12+IL-18 in a TCR-independent manner. *European journal of immunology.* 2014; 44:195–203. [PubMed: 24019201]

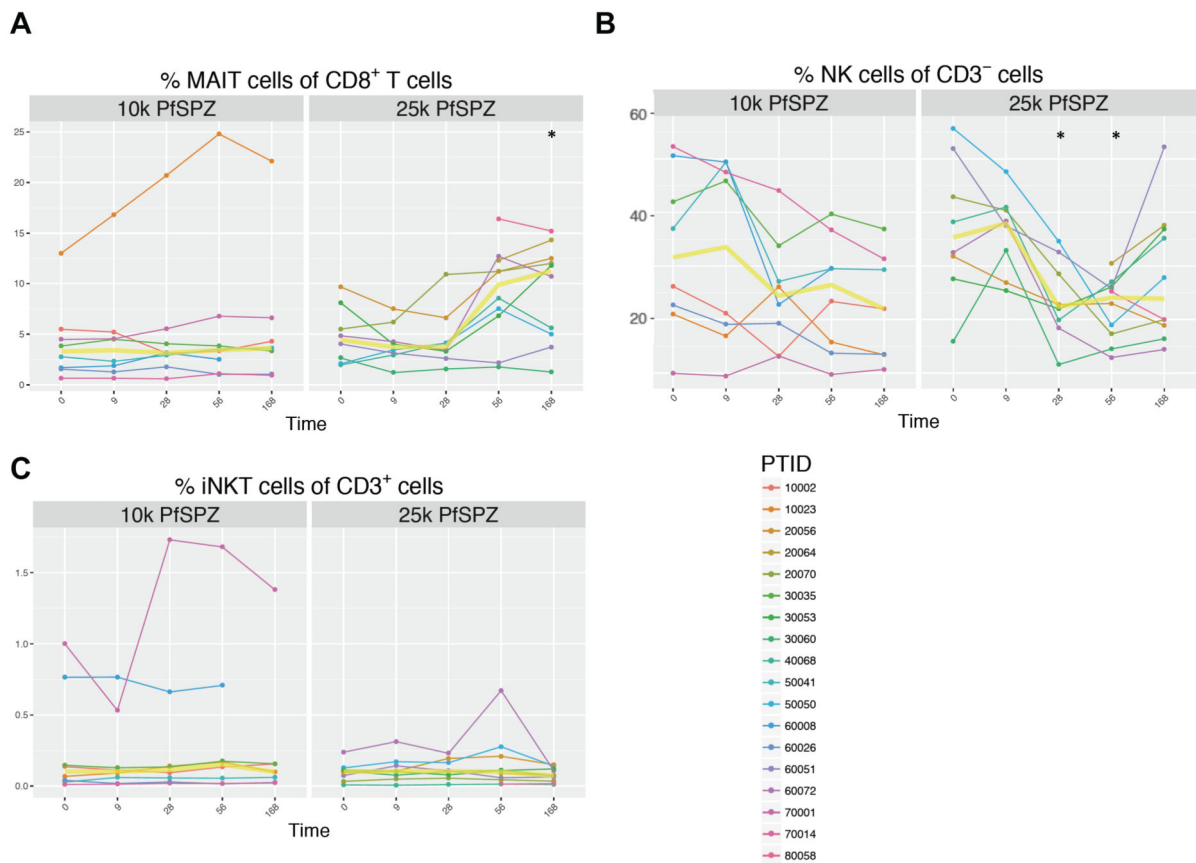
25. Kenna T, Golden-Mason L, Porcelli SA, Koezuka Y, Hegarty JE, O'Farrelly C, Doherty DG. NKT cells from normal and tumor-bearing human livers are phenotypically and functionally distinct from murine NKT cells. *J Immunol.* 2003; 171:1775–1779. [PubMed: 12902477]
26. Chandra S, Kronenberg M. Activation and Function of iNKT and MAIT Cells. *Advances in immunology.* 2015; 127:145–201. [PubMed: 26073984]
27. Prlic M, Hohl TM. iNKTs foil fungi. *Cell host & microbe.* 2011; 10:421–422. [PubMed: 22100157]
28. Schofield L, Villaquiran J, Ferreira A, Schellekens H, Nussenzweig R, Nussenzweig V. Gamma interferon, CD8+ T cells and antibodies required for immunity to malaria sporozoites. *Nature.* 1987; 330:664–666. [PubMed: 3120015]
29. McCall MB, Sauerwein RW. Interferon-gamma--central mediator of protective immune responses against the pre-erythrocytic and blood stage of malaria. *Journal of leukocyte biology.* 2010; 88:1131–1143. [PubMed: 20610802]
30. Pombo DJ, Lawrence G, Hirunpetcharat C, Rzepczyk C, Bryden M, Cloonan N, Anderson K, Mahakunkijcharoen Y, Martin LB, Wilson D, Elliott S, Eisen DP, Weinberg JB, Saul A, Good MF. Immunity to malaria after administration of ultra-low doses of red cells infected with *Plasmodium falciparum*. *Lancet.* 2002; 360:610–617. [PubMed: 12241933]
31. Roestenberg M, Teirlinck AC, McCall MB, Teelen K, Makamdop KN, Wiersma J, Arens T, Beckers P, van Gemert G, van de Vegte-Bolmer M, van der Ven AJ, Luty AJ, Hermsen CC, Sauerwein RW. Long-term protection against malaria after experimental sporozoite inoculation: an open-label follow-up study. *Lancet.* 2011; 377:1770–1776. [PubMed: 21514658]
32. Roestenberg M, McCall M, Hopman J, Wiersma J, Luty AJ, van Gemert GJ, van de Vegte-Bolmer M, van Schaijk B, Teelen K, Arens T, Spaarman L, de Mast Q, Roeffen W, Snounou G, Renia L, van der Ven A, Hermsen CC, Sauerwein R. Protection against a malaria challenge by sporozoite inoculation. *The New England journal of medicine.* 2009; 361:468–477. [PubMed: 19641203]
33. Doodoo D, Omer FM, Todd J, Akanmori BD, Koram KA, Riley EM. Absolute levels and ratios of proinflammatory and anti-inflammatory cytokine production in vitro predict clinical immunity to *Plasmodium falciparum* malaria. *The Journal of infectious diseases.* 2002; 185:971–979. [PubMed: 11920322]
34. D'Ombra MC, Robinson LJ, Stanicic DI, Taraika J, Bernard N, Michon P, Mueller I, Schofield L. Association of early interferon-gamma production with immunity to clinical malaria: a longitudinal study among Papua New Guinean children. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2008; 47:1380–1387. [PubMed: 18947328]
35. Luty AJ, Lell B, Schmidt-Ott R, Lehman LG, Luckner D, Greve B, Matousek P, Herbich K, Schmid D, Migot-Nabias F, Deloron P, Nussenzweig RS, Kremsner PG. Interferon-gamma responses are associated with resistance to reinfection with *Plasmodium falciparum* in young African children. *The Journal of infectious diseases.* 1999; 179:980–988. [PubMed: 10068595]
36. Malaguarnera L, Pignatelli S, Musumeci M, Simpoire J, Musumeci S. Plasma levels of interleukin-18 and interleukin-12 in *Plasmodium falciparum* malaria. *Parasite immunology.* 2002; 24:489–492. [PubMed: 12654091]
37. Day NP, Hien TT, Schollaardt T, Loc PP, Chuong LV, Chau TT, Mai NT, Phu NH, Sinh DX, White NJ, Ho M. The prognostic and pathophysiologic role of pro- and antiinflammatory cytokines in severe malaria. *The Journal of infectious diseases.* 1999; 180:1288–1297. [PubMed: 10479160]
38. Lyke KE, Burges R, Cissoko Y, Sangare L, Dao M, Diarra I, Kone A, Harley R, Plowe CV, Doumbo OK, Szein MB. Serum levels of the proinflammatory cytokines interleukin-1 beta (IL-1beta), IL-6, IL-8, IL-10, tumor necrosis factor alpha, and IL-12(p70) in Malian children with severe *Plasmodium falciparum* malaria and matched uncomplicated malaria or healthy controls. *Infection and immunity.* 2004; 72:5630–5637. [PubMed: 15385460]
39. Walther M, Woodruff J, Edele F, Jeffries D, Tongren JE, King E, Andrews L, Bejon P, Gilbert SC, De Souza JB, Sinden R, Hill AV, Riley EM. Innate immune responses to human malaria: heterogeneous cytokine responses to blood-stage *Plasmodium falciparum* correlate with parasitological and clinical outcomes. *J Immunol.* 2006; 177:5736–5745. [PubMed: 17015763]

40. Kim CC, Parikh S, Sun JC, Myrick A, Lanier LL, Rosenthal PJ, DeRisi JL. Experimental malaria infection triggers early expansion of natural killer cells. *Infection and immunity*. 2008; 76:5873–5882. [PubMed: 18824529]
41. Hansen DS, Bernard NJ, Nie CQ, Schofield L. NK cells stimulate recruitment of CXCR3+ T cells to the brain during Plasmodium berghei-mediated cerebral malaria. *J Immunol*. 2007; 178:5779–5788. [PubMed: 17442962]
42. Shekalaghe S, Rutaihua M, Billingsley PF, Chemba M, Daubenberger CA, James ER, Mpina M, Ali Juma O, Schindler T, Huber E, Gunasekera A, Manoj A, Simon B, Saverino E, Church LW, Hermesen CC, Sauerwein RW, Plowe C, Venkatesan M, Sasi P, Lweno O, Mutani P, Hamad A, Mohammed A, Urassa A, Mzee T, Padilla D, Ruben A, Sim BK, Tanner M, Abdulla S, Hoffman SL. Controlled human malaria infection of Tanzanians by intradermal injection of aseptic, purified, cryopreserved Plasmodium falciparum sporozoites. *The American journal of tropical medicine and hygiene*. 2014; 91:471–480. [PubMed: 25070995]
43. Jiang J, Lau LL, Shen H. Selective depletion of nonspecific T cells during the early stage of immune responses to infection. *J Immunol*. 2003; 171:4352–4358. [PubMed: 14530360]
44. Prlic M, Lefrancois L, Jameson SC. Multiple choices: regulation of memory CD8 T cell generation and homeostasis by interleukin (IL)-7 and IL-15. *The Journal of experimental medicine*. 2002; 195:F49–52. [PubMed: 12070294]
45. Leeansyah E, Svard J, Dias J, Buggert M, Nystrom J, Quigley MF, Moll M, Sonnerborg A, Nowak P, Sandberg JK. Arming of MAIT Cell Cytolytic Antimicrobial Activity Is Induced by IL-7 and Defective in HIV-1 Infection. *PLoS pathogens*. 2015; 11:e1005072. [PubMed: 26295709]
46. Tang XZ, Jo J, Tan AT, Sandalova E, Chia A, Tan KC, Lee KH, Gehring AJ, De Libero G, Bertoletti A. IL-7 licenses activation of human liver intrasinusoidal mucosal-associated invariant T cells. *J Immunol*. 2013; 190:3142–3152. [PubMed: 23447689]
47. Germanov E, Veinotte L, Cullen R, Chamberlain E, Butcher EC, Johnston B. Critical role for the chemokine receptor CXCR6 in homeostasis and activation of CD1d-restricted NKT cells. *J Immunol*. 2008; 181:81–91. [PubMed: 18566372]
48. Jeffery HC, van Wilgenburg B, Kurioka A, Parekh K, Stirling K, Roberts S, Dutton EE, Hunter S, Geh D, Braitch MK, Rajanayagam J, Iqbal T, Pinkney T, Brown R, Withers DR, Adams DH, Klenerman P, Oo YH. Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1. *Journal of hepatology*. 2016; 64:1118–1127. [PubMed: 26743076]
49. Yamazaki T, Yang XO, Chung Y, Fukunaga A, Nurieva R, Pappu B, Martin-Orozco N, Kang HS, Ma L, Panopoulos AD, Craig S, Watowich SS, Jetten AM, Tian Q, Dong C. CCR6 regulates the migration of inflammatory and regulatory T cells. *J Immunol*. 2008; 181:8391–8401. [PubMed: 19050256]
50. Hickman HD, Reynoso GV, Ngudankama BF, Cush SS, Gibbs J, Bennink JR, Yewdell JW. CXCR3 chemokine receptor enables local CD8(+) T cell migration for the destruction of virus-infected cells. *Immunity*. 2015; 42:524–537. [PubMed: 25769612]
51. Scholzen A, Sauerwein RW. Immune activation and induction of memory: lessons learned from controlled human malaria infection with Plasmodium falciparum. *Parasitology*. 2016; 143:224–235. [PubMed: 26864135]
52. Tsang JS, Schwartzberg PL, Kotliarov Y, Biancotto A, Xie Z, Germain RN, Wang E, Olnes MJ, Narayanan M, Golding H, Moir S, Dickler HB, Perl S, Cheung F. Global analyses of human immune variation reveal baseline predictors of postvaccination responses. *Cell*. 2014; 157:499–513. [PubMed: 24725414]
53. Chen Q, Amaladoss A, Ye W, Liu M, Dummler S, Kong F, Wong LH, Loo HL, Loh E, Tan SQ, Tan TC, Chang KT, Dao M, Suresh S, Preiser PR, Chen J. Human natural killer cells control Plasmodium falciparum infection by eliminating infected red blood cells. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111:1479–1484. [PubMed: 24474774]
54. Teirlinck AC, McCall MB, Roestenberg M, Scholzen A, Woestenenk R, de Mast Q, van der Ven AJ, Hermesen CC, Luty AJ, Sauerwein RW. Longevity and composition of cellular immune responses following experimental Plasmodium falciparum malaria infection in humans. *PLoS pathogens*. 2011; 7:e1002389. [PubMed: 22144890]

55. Jameson SC. Maintaining the norm: T-cell homeostasis. *Nature reviews Immunology*. 2002; 2:547–556.
56. Prlic M, Kamimura D, Bevan MJ. Rapid generation of a functional NK-cell compartment. *Blood*. 2007; 110:2024–2026. [PubMed: 17554057]
57. Gold MC, Napier RJ, Lewinsohn DM. MR1-restricted mucosal associated invariant T (MAIT) cells in the immune response to *Mycobacterium tuberculosis*. *Immunological reviews*. 2015; 264:154–166. [PubMed: 25703558]
58. Cosgrove C, Ussher JE, Rauch A, Gartner K, Kurioka A, Huhn MH, Adelman K, Kang YH, Fergusson JR, Simmonds P, Goulder P, Hansen TH, Fox J, Gunthard HF, Khanna N, Powrie F, Steel A, Gazzard B, Phillips RE, Frater J, Uhlig H, Klenerman P. Early and nonreversible decrease of CD161<sup>+</sup>/MAIT cells in HIV infection. *Blood*. 2013; 121:951–961. [PubMed: 23255555]
59. Wong EB, Akilimali NA, Govender P, Sullivan ZA, Cosgrove C, Pillay M, Lewinsohn DM, Bishai WR, Walker BD, Ndung'u T, Klenerman P, Kasprovicz VO. Low levels of peripheral CD161<sup>+</sup>+CD8<sup>+</sup> mucosal associated invariant T (MAIT) cells are found in HIV and HIV/TB co-infection. *PloS one*. 2013; 8:e83474. [PubMed: 24391773]
60. Fernandez CS, Amarasena T, Kelleher AD, Rossjohn J, McCluskey J, Godfrey DI, Kent SJ. MAIT cells are depleted early but retain functional cytokine expression in HIV infection. *Immunology and cell biology*. 2015; 93:177–188. [PubMed: 25348935]
61. Cui Y, Franciszkiewicz K, Mburu YK, Mondot S, Le Bourhis L, Premel V, Martin E, Kachaner A, Duban L, Ingersoll MA, Rabot S, Jaubert J, De Villartay JP, Soudais C, Lantz O. Mucosal-associated invariant T cell-rich congenic mouse strain allows functional evaluation. *The Journal of clinical investigation*. 2015; 125:4171–4185. [PubMed: 26524590]
62. Orange JS. Natural killer cell deficiency. *The Journal of allergy and clinical immunology*. 2013; 132:515–525. quiz 526. [PubMed: 23993353]
63. Matar CG, Jacobs NT, Speck SH, Lamb TJ, Moormann AM. Does EBV alter the pathogenesis of malaria? *Parasite immunology*. 2015; 37:433–445. [PubMed: 26121587]
64. Chattopadhyay PK, Chelimo K, Embury PB, Mulama DH, Sumba PO, Gostick E, Ladell K, Brodie TM, Vulule J, Roederer M, Moormann AM, Price DA. Holoendemic malaria exposure is associated with altered Epstein-Barr virus-specific CD8(+) T-cell differentiation. *Journal of virology*. 2013; 87:1779–1788. [PubMed: 23175378]
65. Moormann AM, Snider CJ, Chelimo K. The company malaria keeps: how co-infection with Epstein-Barr virus leads to endemic Burkitt lymphoma. *Current opinion in infectious diseases*. 2011; 24:435–441. [PubMed: 21885920]
66. Moormann AM. How might infant and paediatric immune responses influence malaria vaccine efficacy? *Parasite immunology*. 2009; 31:547–559. [PubMed: 19691558]

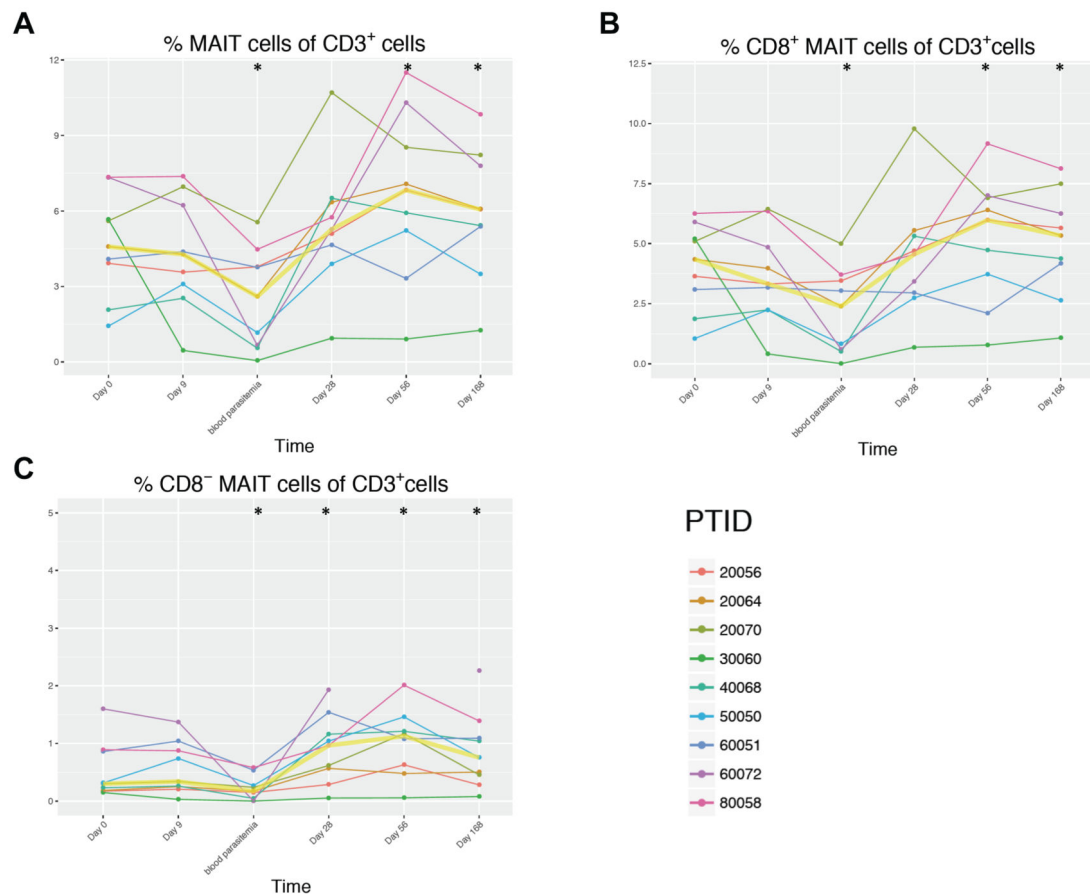
## Abbreviations

<b>CHMI</b>	controlled human malaria infection
<b>grzB</b>	granzyme B
<b>PfSPZ</b>	<i>Plasmodium falciparum</i> sporozoites



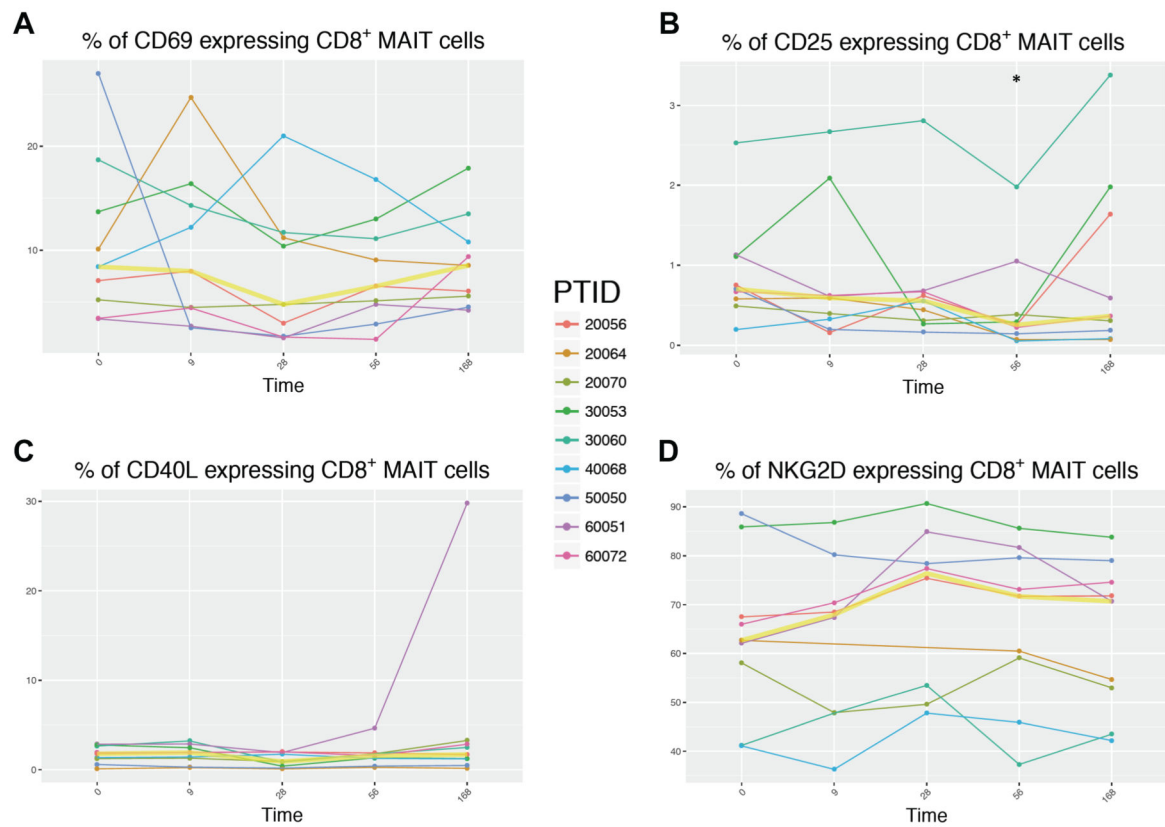
**Fig. 1. Changes in the innate-like T lymphocyte and NK cell compartment following intradermal injection of PfSPZ**

Changes in the (A) frequency of MAIT cells within the CD8<sup>+</sup> T cell population, the (B) NK cell population (shown as frequency in CD3<sup>-</sup> lymphocyte population) and (C) iNKT cell population are shown for the low dose challenge group (10k PfSPZ, left panel) and the high dose challenge group (25k PfSPZ, right panel). Significant changes at time points following PfSPZ injection (compared to day 0) are indicated with a \*. Statistical significance comparing control and treated subjects was done by using a Type-II ANOVA for a linear mixed effect model. Comparison of time-points to each other was done using a paired t-test with FDR. The yellow line in each graph represents the median. Volunteer IDs are listed with each volunteer depicted in a distinct color.



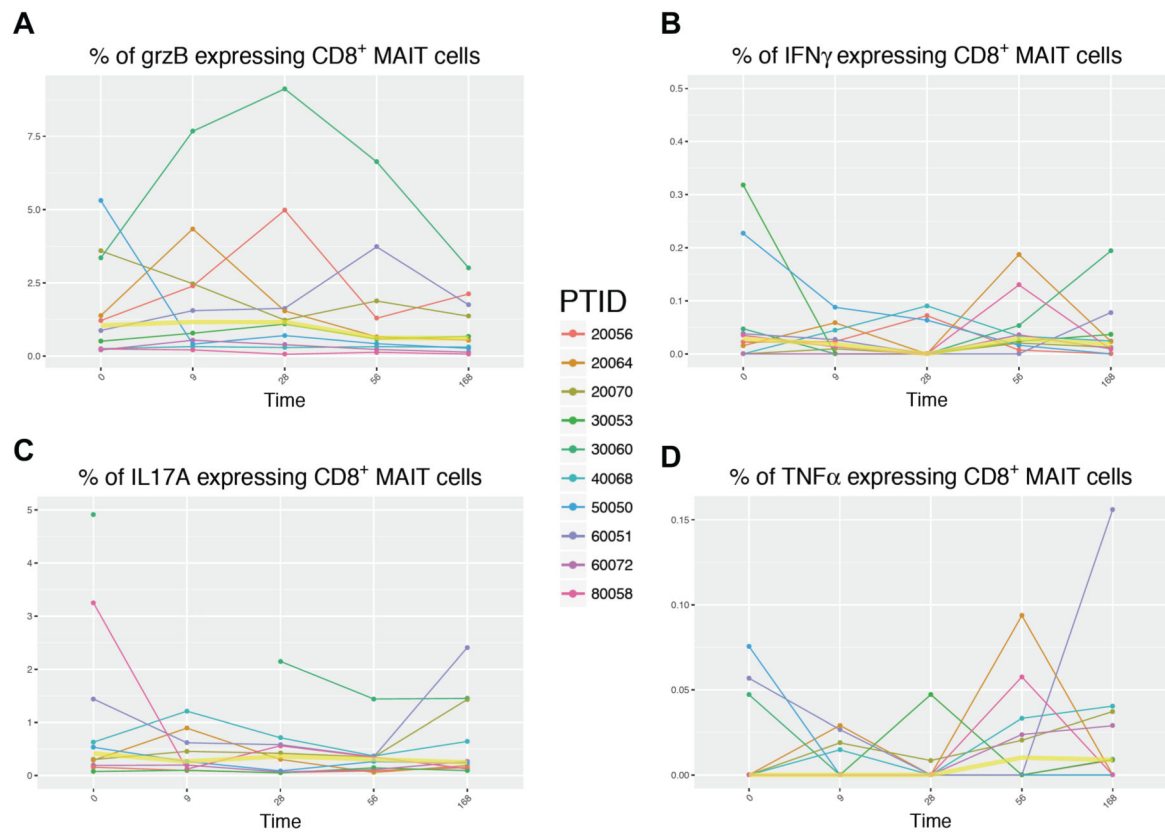
**Fig. 2. MAIT cell frequency is altered following intradermal injection with PfSPZ**

Shown are changes in the frequency of MAIT cells over time for each individual volunteer. Significant changes are indicated with a \* and p values were determined by t test. (A) The % of MAIT cells in the CD3<sup>+</sup> population is shown in the 25K PfSPZ group in the blood. Significant changes compared to the day 0 baseline were observed at the blood parasitemia stage (p=0.0302), on day 56 (p=0.0022) and on day 168 (p=0.0072). (B) The frequency of CD8<sup>+</sup> MAIT cells and (C) CD8<sup>-</sup> MAIT cells within the CD3<sup>+</sup> cell population is shown. Statistical significance comparing control and treated subjects was done by using a Type-II ANOVA for a linear mixed effect model. Comparison of time-points to each other was done using a paired t-test with FDR. Significant changes compared to the day 0 baseline were observed for CD8<sup>+</sup> MAIT cells at the blood parasitemia stage (p=0.0278) and on day 168 (p=0.0239). Significant changes were detected for CD8<sup>-</sup> MAIT cells on days 28 (p=0.0003), 56 (p=0.0008) and on day 168 (p=0.0002). The yellow line in each graph represents the median. Volunteer IDs are listed with each volunteer depicted in a distinct color.



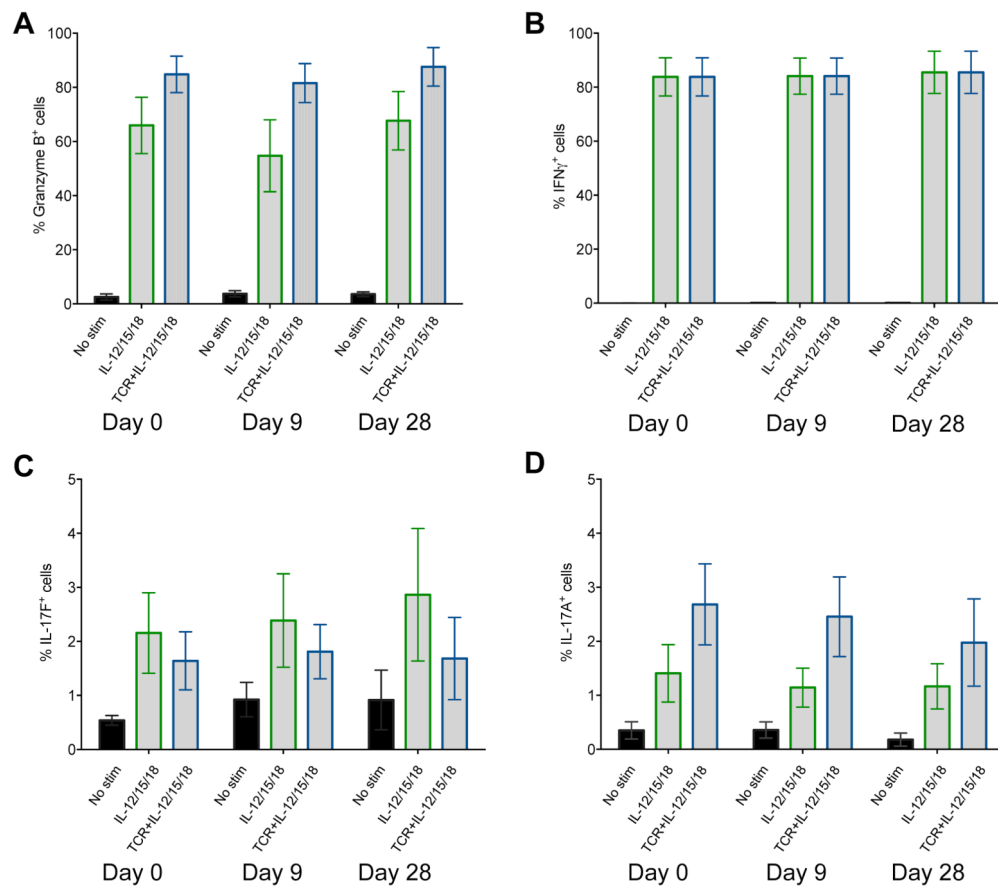
**Fig. 3. Limited MAIT cell activation early after infection**

Expression frequency of (A) CD69, (B) CD25, (C) CD40L and (D) NKG2D is shown for CD8<sup>+</sup> MAIT cells in the high dose (25K PfSPZ) group. Significant changes compared to day 0 are indicated with a \*. Statistical significance comparing control and treated subjects was done by using a Type-II ANOVA for a linear mixed effect model. Comparison of time-points to each other was done using a paired t-test with FDR. The yellow line in each graph represents the median. Volunteer IDs are listed with each volunteer depicted in a distinct color.



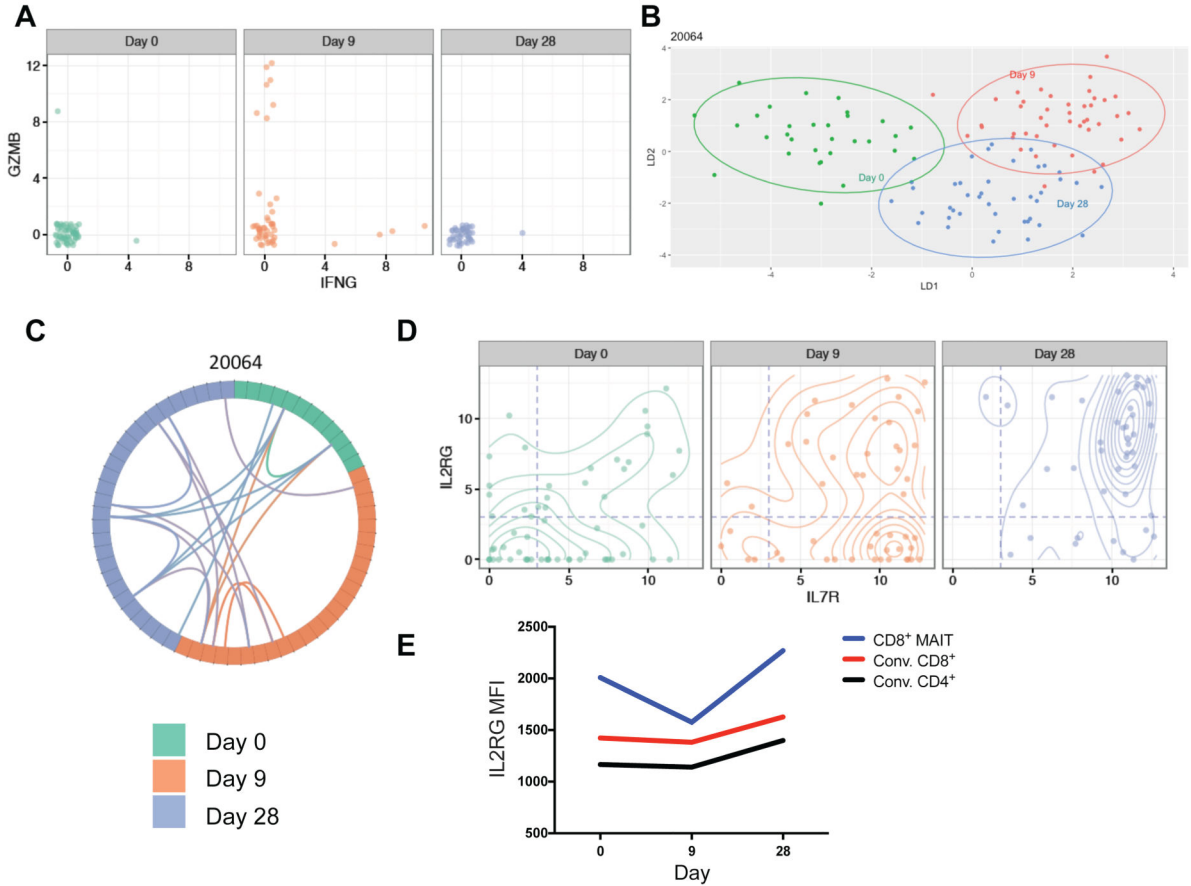
**Fig. 4. Minimal MAIT cell effector function early after infection**

Expression of (A) grzB, (B) IFN $\gamma$ , (C) IL-17A and (D) TNF $\alpha$  on both MAIT cell subsets was measured in the 25K PfSPZ inoculated cohort. The yellow line in each graph represents the median. Statistical significance comparing control and treated subjects was done by using a Type-II ANOVA for a linear mixed effect model. Comparison of time-points to each other was done using a paired t-test with FDR, but not significant changes were detected. Volunteer IDs are listed with each volunteer depicted in a distinct color.



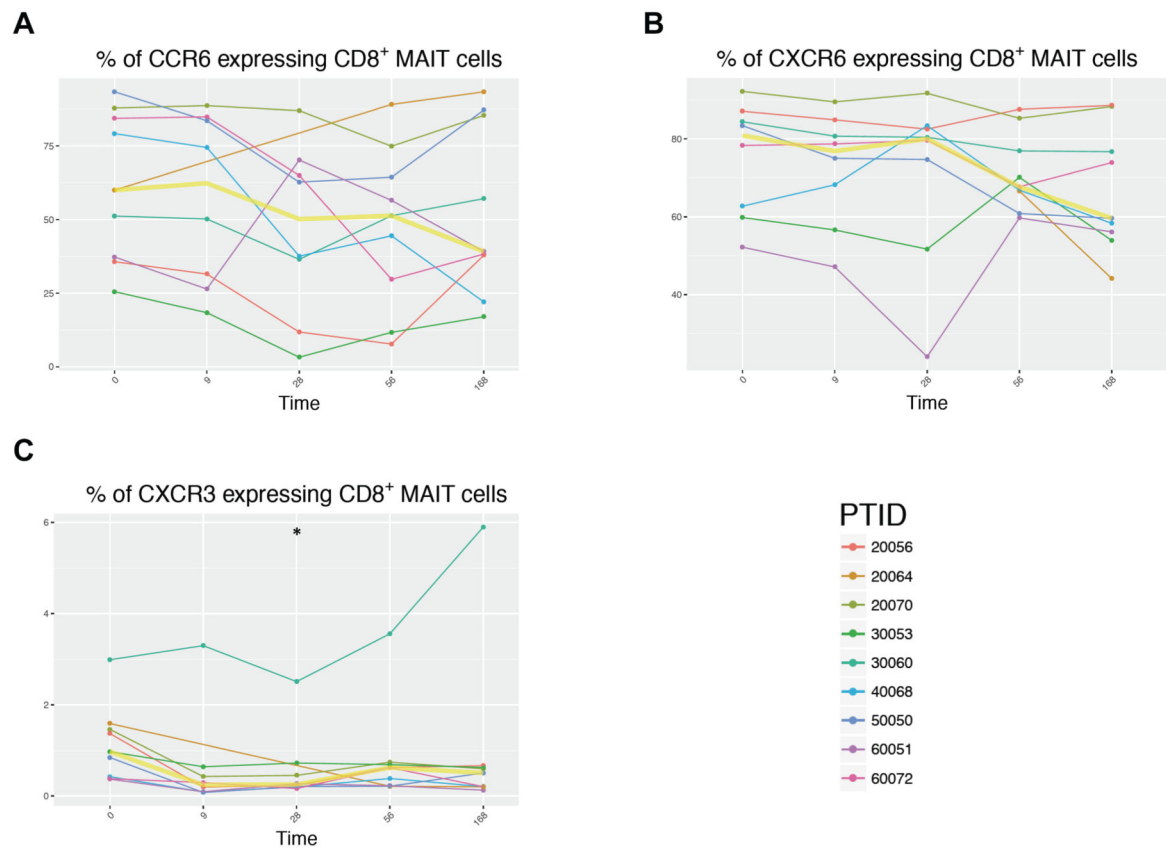
**Fig. 5. MAIT cells remain functional and responsive to stimulation ex vivo**

FACS-purified CD8<sup>+</sup>CD161<sup>hi</sup>V $\alpha$ 7.2<sup>+</sup> cells were stimulated with cytokines (IL-12/15/18), a combination of a TCR signal plus cytokines (TCR + IL-12/15/18) or left unstimulated for 24 hours (no stim). Expression of (A) grzB, (B) IFN $\gamma$ , (C) IL-17F and (D) IL-17A was measured in 5 randomly chosen donors on days 0, 9 and 28. MAIT cells responded to each stimulation condition, but we did not detect significant changes when comparing MAIT cell responses to cytokine IL-12/15/18 (green bars) or TCR+IL-12/15/18 (blue bars) stimulation between the different time-points (days 0, 9 and 28).



**Fig. 6. Single-cell RNAseq analysis of MAIT cells reveals partial activation and changes in the composition of the MAIT cell population**

CD8<sup>+</sup> MAIT cells were sorted by FACS for single-cell RNAseq expression analysis by the Fluidigm C1 system. Cells sorted from donor 20064 on day 0, 9 and 28 are depicted in green, orange and blue, respectively. (A) IFN $\gamma$  and grzB message are detected in some MAIT cells on day 9 post-infection but not on days 0 and 28. (B) Linear discriminant analysis of single MAIT cells sorted from days 0, 9 and 28 post sporozoite challenge. (C) Clonality in the MAIT cell population was examined by analyzing CDR3 usage from single-cells by reconstructing their TCR $\alpha$  and TCR $\beta$  chains. Segments in the circle represent individual cells yielding a TCR sequence. Arcs connect cells sharing TCR clonotypes (TRA and/or TRB V gene, J gene and CDR3 junction). (D) Changes in mRNA co-expression of IL7R $\alpha$  and  $\gamma$  chain (CD132) are shown in a contour plot. (E) The surface expression of CD132 measured by flow cytometry increases on day 28 validating the single-cell RNAseq data (donor 20064 used for both D and E).



**Fig. 7. Changes in chemokine receptor expression months after the infection**

The frequency of (A) CCR6, (B) CXCR6 and (C) CXCR3 expressing CD8<sup>+</sup> MAIT cells was determined by FACS in the 25K PfSPZ inoculated cohort. Statistical significance comparing control and treated subjects was done by using a Type-II ANOVA for a linear mixed effect model. Additional comparison of time-points to each other was done using a paired t-test with FDR. The yellow line in each graph represents the median.