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► **To cite this version:**

Olivier Silvie, Rogerio Amino, Julius Clemence Hafalla. Tissue-specific cellular immune responses to malaria pre-erythrocytic stages. *Current Opinion in Microbiology*, 2017, 40, pp.160-167. 10.1016/j.mib.2017.12.001 . hal-01700959

HAL Id: hal-01700959

<https://hal.sorbonne-universite.fr/hal-01700959>

Submitted on 5 Feb 2018

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1 **Tissue-specific cellular immune responses to malaria pre-erythrocytic stages**

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13

14 **Highlights**

- 15 • Protective immunity against malaria can be achieved through immunisation with live
16 attenuated *Plasmodium* sporozoites and targets the parasite pre-erythrocytic stages.
- 17 • Naturally exposed individuals remain at risk of malaria despite multiple sporozoite
18 infections, and this could be explained by different mechanisms.
- 19 • Protective immunity relies primarily on effector CD8⁺ T cells targeting the parasite in
20 the liver.
- 21 • The generation of liver-resident parasite-specific memory CD8⁺ T cells is emerging as
22 a key determinant of protective immunity.

23

24

25

26 **Abstract**

27 Complete and long-lasting protective immunity against malaria can be achieved through
28 vaccination with invasive live attenuated *Plasmodium* sporozoites, the motile stage inoculated
29 in the host skin during a mosquito bite. Protective immunity relies primarily on effector CD8⁺
30 T cells targeting the parasite in the liver. Understanding the tissue-specific features of the
31 immune response is emerging as a vital requirement for understanding protective immunity.
32 The small parasite inoculum, the scarcity of infected cells and the tolerogenic properties of
33 the liver represent hurdles for the establishment of protective immunity in endemic areas. In
34 this review, we discuss recent advances on liver-specific features of immunity including
35 innate recognition of malaria pre-erythrocytic stages, CD8⁺ T cell interactions with infected
36 hepatocytes, antigen presentation for effective CD8⁺ T cell responses and generation of liver-
37 resident memory CD8⁺ T cells. A better understanding of the factors involved in the induction
38 and maintenance of effector CD8⁺ T cell immunity against malaria pre-erythrocytic stages is
39 crucial for the development of an effective vaccine targeting the initial phase of malaria
40 infection.

41

42

43 **Introduction**

44 Malaria, caused by *Plasmodium* parasites, is one of the leading causes of mortality and
45 morbidity in resource poor areas worldwide. Notwithstanding global control and elimination
46 efforts, >400,000 people still die annually due to malaria
47 (<http://www.who.int/malaria/publications/world-malaria-report-2016/report/en>). A highly
48 efficacious malaria vaccine remains elusive. *Plasmodium* sporozoites are injected in the host
49 skin by a female infected *Anopheles* mosquito. These sporozoites travel to the liver, invade
50 hepatocytes and develop into exo-erythrocytic forms (EEF), which generate thousands of
51 blood stage parasites. Targeting the malaria pre-erythrocytic stage is an ideal and attractive
52 strategy for malaria vaccination. Inhibiting liver infection and development of malaria
53 parasites can prevent both the disease-causing blood stages and the transmissible sexual
54 stages.

55 Humans, rhesus monkeys and mice exposed to multiple doses of γ -radiation-attenuated
56 sporozoites (RAS), the gold standard vaccine for malaria, can be fully protected against
57 normal sporozoite challenge (reviewed in [1]). Alternative attenuation strategies, such as
58 genetically attenuated parasites (GAP) or chemoprophylaxis with sporozoite infection (CPS),
59 also induce sterile protection (reviewed in [2]). Whilst the use of attenuated parasites is a
60 feasible approach for vaccination, they demand production of large quantities of infected
61 mosquitoes that is not easily scalable to mass vaccination in poor settings. But, if we can
62 discover the important features of a protective immune response, we can replicate these
63 phenotypes by sub-unit vaccination. RTS,S/AS01, the most advanced malaria sub-unit
64 vaccine candidate to date, is based on the circumsporozoite protein (CSP), the surface coat
65 antigen of sporozoites. Yet, despite being designed to elicit different arms of the immune
66 response, RTS,S/AS01 only provides partial protection in malaria-naïve and –experienced
67 individuals [3•].

68 In rodent models and rhesus monkeys, protection conferred by RAS vaccination is
69 largely dependent on effector CD8⁺ T cells (reviewed in [4]). Depletion of CD8⁺ T cells prior
70 to challenge of immunised mice and rhesus monkeys consistently abrogated protection [5,6].
71 *P. falciparum* (*Pf*) RAS vaccination of humans induces high numbers of sporozoite-specific
72 CD8⁺ T cells producing IFN- γ [7]. Understanding the key features of host-parasite
73 interactions and the induction of innate and adaptive immune responses, particularly parasite-
74 specific CD8⁺ T cells, is crucial for informing the development of an effective next generation
75 malaria vaccine.

76

77 **Immunisation with attenuated parasites versus natural infections: numbers matter**

78 Despite repeated infections, individuals in endemic areas do not develop sterilising
79 protection and those surviving episodes of childhood malaria remain vulnerable to
80 intermittent infections [8]. Several possibilities, including the small number of parasites
81 naturally transmitted by mosquitoes or the down-regulation of immunity by malaria blood
82 infection, can explain the reasons behind the contrasting outcomes with those experimentally
83 vaccinated with attenuated sporozoites (**Figure 1**).

84 In mice, only ~20-50 *P. yoelii* (*Py*) or *P. berghei* (*Pb*) sporozoites are inoculated in the
85 host skin during an infective bite and only a small fraction invades and develops inside
86 hepatocytes (reviewed in [9]). CD8⁺ T cell responses to CSP and sporozoites following *Py*
87 and *Pf* RAS immunisation, respectively, are dependent on antigen dose so low inoculum
88 equates to poor CD8⁺ T cell responses [7,10]. In the *Py* model, CD8⁺ T cell responses are not
89 readily increased by repeated immunisation [11,12]. To achieve sterile protection in humans,
90 more than 1,000 *Pf* infective bites (*Pf* RAS) are required [13]; this amount corresponds to
91 almost ten years of exposure to *Pf* in a high malaria transmission area [14] but administered in
92 a much shorter period. Sterile protection can also be achieved by the intravenous inoculation

93 of ~700,000 *Pf* RAS [7]. To protect humans under CPS, fewer *Pf* infective bites (~40) or
94 cryopreserved sporozoites (~150,000) are needed [15,16•]. For CPS, the host is exposed to
95 both pre-erythrocytic and blood stage (transient parasitemia) antigens, and sterile protection is
96 observed only against a *Pf* sporozoite challenge [17]. Comparable to findings in humans, CPS
97 induces efficient protection against *Pb* sporozoite infection, but not against blood stage
98 challenge. This sterilising protection is abolished after depletion of CD8⁺ T cells and is not
99 affected by the lack of mature B cells [18,19]. In contrast, CPS vaccination not only induces
100 high levels of antigen-experienced CD8⁺ T cells but also targets blood stages of *Py* and *P.*
101 *chabaudi* (*Pc*) [18,20–22]. In common, the two species used in these studies cause an acute
102 parasitemia that can be naturally controlled by non-vaccinated hosts, indicating a lower
103 stringency for the immune-control of the blood stage infection in comparison to the *Pb* and *Pf*
104 lethal strains. Late arresting *Py* GAPs have been shown to provide superior protective
105 immunity, suggesting a role of mid/late EEFs antigens in protection, and similar to RAS and
106 CPS, sterile protection is dependent on the immunising dose of attenuated sporozoites [23].
107 These data suggest that exposure to a broad antigenic repertoire, including antigens shared
108 between EEFs and blood stages, improves protection against the pre-erythrocytic stages.
109 Additionally, the absence or the rapid clearance of infected RBCs by RAS/GAP or CPS
110 vaccination, respectively, might impede the deleterious effect of blood infection on antigen
111 presentation [24], the numbers and functionality of CD8⁺ T cells [25] or the expansion of
112 regulatory T cells during a prolonged blood-infection [26•]. Overall, the delivery of high
113 doses of sporozoites seems to be a key requirement for the sterile protection elicited by
114 immunization using live attenuated sporozoites. This high antigenic load is likely associated
115 with overcoming the humoral and cellular effector thresholds necessary to sterilize the
116 sporozoite infection [27,28].

117

118 **Early responses to sporozoites and liver stages: innate immunity and hepatic responses**

119 Information on how sporozoites interact with the innate immune system remains
120 limited. *Pb* sporozoites induce the biphasic recruitment of CD11b⁺ Ly6C^{int} Ly6G^{hi}
121 polymorphonuclear neutrophils, CD11b⁺ Ly6C⁻ Ly6G⁻ resident myeloid cells and CD11b⁺
122 Ly6C^{hi} Ly6G⁻ inflammatory monocytes in the skin inoculation site and the proximal draining
123 lymph node (DLN), evoking a Th1 cytokine profile [29]. Migrating *Pb* sporozoites induce a
124 signalling cascade *in vitro* in primary murine hepatocytes with MyD88-mediated NF-KB
125 activation [30]. Although EEF development is clinically silent, accumulating evidence
126 suggest that, as parasites replicate in the liver, functional innate immune responses are
127 triggered that are dependent on both type I and II IFNs [31•,32•]. The type I IFN signaling
128 pathway is activated in the livers of mice intravenously infected with either *Pb* or *Py*, a
129 process that involves the cytosolic receptor melanoma differentiation-associated protein 5
130 (Mda5), suggesting sensing of parasite RNA, and requires the mitochondrial antiviral
131 signalling protein (Mavs) and the transcription factors interferon-regulatory factors-3 (Irf3)
132 and Irf7. Type I IFNs bind to Ifnar on hepatocytes and leukocytes, resulting in the subsequent
133 recruitment of leukocytes to the liver at the end of the hepatic infection. Indeed, following the
134 inoculation of *Py* GAP, type I IFN signalling is essential for the recruitment or expansion of
135 CD49b⁺CD3⁺ Natural Killer T (NKT) cells, one day after the peak of hepatic parasite release
136 in the blood circulation. These NKT cells reduce liver infection during a subsequent and
137 intertwined secondary *Py* sporozoite infection, presumably via the production of IFN- γ [32•].
138 However, type I IFN signalling does not impact the EEF growth after a primary *Pb* or *Py*
139 sporozoite infection [31•,32•]. Furthermore, earlier reports using *Pb* and *Py* suggest that NKT
140 cells have no role in protection against malaria pre-erythrocytic stages [33,34]. Notably, a
141 type I IFN response of much lower magnitude was observed when lower doses of parasites
142 were transmitted through mosquito bites [31•]. Hence, the significance of this response and its

143 relevance in humans still remains uncertain, as well as its impact on the acquisition of
144 immunity by vaccination using live attenuated sporozoites. During EEF development, the
145 parasite exploits diverse cellular pathways and several host and immune factors are
146 modulated, including Bcl-2, p53, IL-6, heme oxygenase and the autophagy machinery
147 (reviewed in [35,36] and [37]). The relationships amongst the modulation of host cell factors,
148 the innate immune system and the development of protective CD8⁺ T cells against pre-
149 erythrocytic stages remain to be established.

150

151 **Antigen capture and presentation leading to CD8⁺ T cell priming**

152 How parasite antigens are processed and presented for primary activation of antigen-specific
153 CD8⁺ T cells is not well understood, but likely depends on the nature and spatio-temporal
154 exposure of parasite-derived antigens. Sporozoites migrate through various cell types during
155 their journey from the skin to the liver. During cell traversal, sporozoites shed antigens in the
156 host cell cytosol, which can be processed and directly presented [38] or captured for cross-
157 presentation by dendritic cells (DCs). After dermal inoculation, a fraction of *Pb* sporozoites
158 actively migrate to the DLN [39], and can prime protective *Py*CSP-specific CD8⁺ T cells [40].
159 Lymph-node resident CD8 α ⁺ DCs capture antigens from migratory malaria sporozoites and
160 induce *Pb*CSP-specific CD8⁺ T cell responses [41]. Intravenous inoculation of live attenuated
161 sporozoites is a more efficient vaccination approach as compared to intradermal inoculation,
162 in both human and rodents models [7,42]. Various factors likely concur to protective
163 immunity induced by immunization with live attenuated sporozoites administered
164 intravenously (**Figure 2**). A recent study revealed that the lower protective efficacy of *Py*
165 GAP administered via the intradermal route is not linked to low hepatic parasite numbers, but
166 correlates with a shift towards regulatory immune responses [43•]. In particular, more
167 interleukin-10-producing B and T cells but fewer hepatic memory CD8⁺ T cells and CD8 α ⁺

168 DCs were found in the liver and skin DLNs after intradermal injection, as compared to
169 intravenous inoculation. Intravenous injection of *Pb* RAS leads to a CD8 α ⁺ DC-dependent
170 splenic priming of CD8⁺ T cells specific for an antigen expressed in pre-erythrocytic and
171 blood stages [44]. CD8 α ⁺ DCs accumulate in the liver after *Pb* RAS immunisation [45,46],
172 however a role for hepatic DCs in both priming of CD8⁺ T cells and protection remains poorly
173 characterised. In the liver, sporozoites traverse Kupffer cells (KC) and liver sinusoidal
174 endothelial cells (LSEC) prior to infecting hepatocytes [47]. In other systems, both KC and
175 LSEC can function as APCs and could present parasite antigens to CD8⁺ T cells, resulting in
176 either tolerance or enhanced immune responses in inflammatory conditions [48,49].

177 How antigens expressed exclusively during EEF development are presented to the
178 immune system is unclear. After invasion of hepatocytes, *Plasmodium* parasites replicate
179 within the PV membrane (PVM), which constitutes a barrier preventing access of antigens to
180 the host cell cytosol and the MHC class I presentation pathway. RAS and GAP invade
181 hepatocytes where they undergo arrested development into EEFs. Attenuation leads to
182 parasite death and possible breakdown of the PVM, which could also enhance antigen
183 presentation and priming of protective CD8⁺ T cells. Although antigen presentation by
184 hepatocytes tends to have a tolerising effect (reviewed in [50]), hepatocytes were shown to
185 prime CD8⁺ T cells specific for CSP [51]. Furthermore, a recent study revealed that
186 presentation of antigens expressed in hepatocytes leads to differentiation of systemically
187 primed CD8⁺ T cells into liver-resident memory cells that are critical for protection [52••].

188 During the effector phase of an efficient immune response, protective CD8⁺ T cells
189 recognize parasite-derived peptides displayed with MHC class I molecules on the surface of
190 infected hepatocytes, leading to parasite elimination [40,53,54]. In *Py*-infected hepatocytes,
191 antigen processing and presentation follows an endosomal-independent, TAP-dependent
192 pathway [53,55] requiring an intracellular source of parasite antigens. From thousands of

193 proteins expressed by sporozoites and liver-stages, so far, only a few antigens, conserved
194 among plasmodial species, are known to elicit a CD8⁺ T cell-dependent protection against a
195 sporozoite infection. Among them are the two most abundant surface proteins of sporozoites,
196 CSP [56] and the thrombospondin-related anonymous protein (TRAP/SSP2) [57]; a protein
197 involved in the wounding and traversal of host cells by ookinetes and sporozoites (CeITOS)
198 [58]; an asparagine-rich protein that regulates the initial development of liver stages (SLARP/
199 SAP1) and a putative serine hydroxymethyltransferase (SHMT) [59]. *Pf* liver-stage antigen 1,
200 liver-stage associated protein 2 and UIS3 antigens also elicit CD8⁺ T cell-dependent
201 protection in a challenge model where heterologous *Pb* sporozoites over-express these
202 antigens via the strong *uis4* promoter [60,61]. The expression of the OVA MHC-I epitope
203 fused to the HSP70 or the green fluorescent protein in the parasite cytosol via the constitutive
204 *hsp70* promoter can also lead to the elimination of infected hepatocytes by OVA-specific
205 CD8⁺ T cells [53], showing that cytosolic, membrane, secreted and PVM antigens can be
206 potentially presented on the surface of infected hepatocytes.

207

208 **Effector functions of protective CD8⁺ T cells**

209 Different effector molecules can be utilised by both effector and memory CD8⁺ T cells
210 to protect against infections, including IFN- γ , tumour necrosis factor (TNF), perforin,
211 granzyme, FasL and TNF-related apoptosis-inducing ligand (TRAIL). The mechanisms by
212 which CD8⁺ T cells inhibit the development of pre-erythrocytic stages remain poorly
213 understood. Studies using antigenically distinct *Pb* strains showed that bystander killing of
214 parasites does not occur during the CD8⁺ T cell response to malaria parasites [62], indicating
215 that elimination of infected parasites is likely mediated by direct recognition of infected
216 hepatocytes by antigen-specific CD8⁺ T cells. Systemic depletion of IFN- γ which is produced
217 not only by CD8⁺ T cells but also by CD4⁺ T, NK T and NK cells, consistently abolishes

218 sterile protection in rodent models immunised with *Pb* or *Py* RAS [5]; IFN- γ activates the L-
219 arginine-dependent inducible nitric oxide synthase (iNOS) pathway, which leads to the
220 production of nitric oxide (NO) that is toxic to the developing EEFs (reviewed in [1]). Lytic
221 factors appear to be dispensable in the effector function of CD8⁺ T cells against pre-
222 erythrocytic stages. Mice deficient for perforin, granzyme B or FasL and immunised with
223 either *Pb* or *Py* RAS are completely protected against sporozoite challenge [5,63]. However,
224 the roles for other immune mechanisms were not properly studied in these gene-deficient
225 animals. Several experiments have also been performed using peptide-stimulated activated
226 CD8⁺ T cells or vaccine-induced CD8⁺ T cells. Activated CD8⁺ T cells specific for a
227 cytoplasmic antigen in EEFs and generated by peptide-stimulation were shown to eliminate
228 developing parasites in the liver in the absence of IFN- γ [53]. Effector *Py*CSP-specific CD8⁺
229 T cells that are deficient of perforin, granzyme B or FasL, and generated following
230 vaccination with recombinant vaccinia virus, were capable of targeting the developing EEFs
231 [64]. Finally, an immunisation strategy involving priming with DCs and boosting with
232 recombinant *Listeria monocytogenes* to generate memory *Pb*CSP- or *Py*CSP-specific memory
233 cells showed the importance of both IFN- γ and TNF in protection against *Pb* and *Py*, whilst
234 perforin was only involved in protection against *Py*, providing evidence of species-specific
235 effector mechanisms for parasite killing [65].

236 Live cell imaging was utilised to dissect the fine mechanisms of CD8⁺ T cell
237 recognition of infected hepatocytes. Polyclonal CD8⁺ T cells from mice immunised with *Py*
238 GAP were shown to establish immunological synapses *in vitro* and utilise perforin to induce
239 massive apoptosis of infected hepatocytes, with no detectable production of IFN- γ and TNF
240 [66]. Intravital imaging revealed that *Py*CSP-specific effector CD8⁺ T cells (generated by
241 peptide-stimulation or a recombinant viral vaccination), as well as non-specific CD8⁺ T cells,
242 form clusters around infected hepatocytes, a process requiring G protein-coupled receptors

243 [53,67]. Targeting by *Py*CSP-specific effector CD8⁺ T cells showed heterogeneity in the death
244 phenotypes of the parasite, implying that multiple and redundant mechanisms are involved
245 [67]. Taken together, these findings uphold the view that elimination of infected hepatocytes
246 occurs in a multifaceted process.

247

248 **The role of liver-resident memory CD8⁺ T cells in protective immunity**

249 To induce sterilising immunity against EEFs, CD8⁺ resident or recruited to the liver
250 must locate and eliminate all parasites to prevent progression to the blood stage infection [62],
251 in a limited amount of time (2 days in mouse, 7-10 days in humans). CD8⁺ T cells must find
252 rare events: estimated at 1 out of 10⁹ hepatocytes in humans and 1 out of 10⁶ hepatocytes in
253 mice [4]. Consequently, extremely high numbers of circulating vaccine-induced effector
254 CD8⁺ T cells are required to scan, locate and kill infected hepatocytes in the short amount of
255 time the parasites are in the liver [28,68].

256 Accumulating evidence indicates a vital role for liver-resident CD8⁺ T cells in
257 protective immunity to pre-erythrocytic stages. Long-term protection after immunisation with
258 *Pb* RAS and CPS correlates with sustained IFN- γ responses of hepatic CD8⁺ memory T cells
259 [69]. Recent studies in non-human primates have also correlated liver CD8⁺ T cell numbers
260 with protective efficacy after intravenous RAS vaccination [70•]. Memory *Py*CSP-specific
261 CD8⁺ T cells express high levels of CXCR6 [71]. Poor CXCR6 expression in these cells
262 results in a reduction of both liver-associated memory and protective immunity [72],
263 suggesting a role of resident CD8⁺ T cells in protection. Intravital imaging documented the
264 presence of motile CD8⁺ T cells within the liver sinusoids of *Py* RAS vaccinated mice,
265 suggesting that memory T cells survey for liver infection by patrolling the sinusoids [73].
266 More recently, a study based on transgenic CD8⁺ T cells specific for a *Pb* antigen expressed
267 in pre-erythrocytic and blood stages, identified a population of memory CD8⁺ T cells in the

268 liver that express a distinct phenotype (CD69⁺ KLRG1^{lo}) from splenic memory cells that are
269 CD69⁺ KLRG1^{hi} [52••]. Detailed phenotypic analysis revealed that liver Trm cells also lacked
270 CD103 expression and differentially expressed a number of surface markers, with higher
271 levels of CXCR3, CXCR6, CD101, BTLA, FR4, Ly6ae, CD25, CD31, CD93, IL-4R, CD127,
272 gp130, CD200R, and CD43, but lower levels of CX3CR1 and NKG2D as compared to
273 circulating effector memory T cells [52••]. Induced following Pb RAS immunisation, these
274 tissue-resident memory T (Trm) cells have the core gene signature of Trm cells from gut, skin
275 and lung. Parabiosis experiments in mice showed that these cells do not recirculate,
276 confirming their liver-resident status [52••]. Intravital imaging revealed that liver-resident
277 cells depend on LFA-ICAM-1 interactions [74]. An immunisation strategy, which involves
278 systemic DC-targeted priming followed by the expression of the antigen on hepatocytes to
279 trap circulating primed CD8⁺ T cells in the liver, enabled conversion to Trm cells [52••]. In
280 this study, DC-targeted priming was achieved by conjugating the peptide antigen to a
281 monoclonal antibody that targets the surface receptor Clec9A, which is expressed by CD8α⁺
282 DCs, and a recombinant adeno-associated virus that targets hepatocytes was used to express
283 the antigen in the liver. This prime-trap vaccination strategy was shown to protect against
284 normal *Pb* sporozoite challenge [52••].

285

286 **Conclusion**

287 Despite the strong evidence for the role of CD8⁺ T cells in sterile protection against
288 malaria, critical qualitative and quantitative characteristics of the protective response and
289 effector mechanisms engaged by CD8⁺ T cells have only started to emerge recently. Whilst
290 multiple immune mechanisms appear to contribute to protection against pre-erythrocytic
291 stages, the generation of liver-resident parasite-specific memory CD8⁺ T cells is emerging as
292 a key determinant of protective immunity. The design of strategies inducing this type of

293 response and the identification of protective target antigens will be instrumental for the
294 development of an efficacious malaria vaccine.

295

296

297 **Acknowledgements**

298 OS is supported by grants from the European Union (FP7 PathCo Collaborative Project
299 HEALTH-F3-2012-305578), the French Government's Investissement d'Avenir program,
300 Laboratoire d'Excellence "ParaFrap" (ANR-11- LABX-0024), and the French National
301 Research Agency (MALINV project, ANR-16-CE15-0004-01). RA is supported by grants
302 from the Institut Pasteur – Paris, the French National Research Agency (ANR-14-CE16-
303 Im3alaria) and the French Government's Investissement d'Avenir program, Laboratoire
304 d'Excellence "Integrative Biology of Emerging Infectious Diseases" (ANR-10-LABX-62-
305 IBEID). JCH is supported by university research fellowships (UF0762736 and UF120026)
306 and grants (RG130034, IE131078, IE14111 and CH160018) from the Royal Society (United
307 Kingdom), as well as a project grant (NC/L000601/1) from the National Centre for the
308 Replacement, Refinement & Reduction of Animals in Research (NC3Rs).

309

310

311 **References and recommended reading**

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575 **Figure legends**

576 **Figure 1. Potential factors contributing to the lack of protective immunity during**
577 **natural *Plasmodium* infection.** Under natural transmission conditions, only a few
578 sporozoites are injected by an infected mosquito into the host skin. The motile sporozoites
579 enter the blood stream by traversing a dermal capillary, are transported to the liver and
580 traverse across liver sinusoidal endothelial cells (LSEC) or Kupffer cells (KC) to reach
581 hepatocytes. Sporozoites invade hepatocytes inside a vacuole, where they replicate into
582 thousands of merozoites, which once released into the bloodstream invade erythrocytes and
583 initiate the blood stage infection. A combination of factors concurs to the lack of protective
584 immunity in naturally exposed individuals. Infected mosquitoes inject very low numbers of
585 sporozoites (1). Dermal inoculation is associated with immune regulatory mechanisms (2).
586 The liver environment is prone to immune tolerance (3). The membrane of the
587 parasitophorous vacuole limits diffusion of parasite liver stage antigens and exposure to the
588 immune system (4). The blood stage infection that follows complete parasite development in
589 the liver has immunosuppressive effects on liver stage immunity (5).

590

591 **Figure 2. Potential factors contributing to protective immunity against liver stages after**
592 **immunisation with live attenuated sporozoites administered intravenously.** Immunisation
593 with live attenuated sporozoites administered intravenously is the most efficient approach to
594 confer full protection against normal sporozoite challenge. Under these conditions, high
595 numbers of sporozoites can be inoculated (1), allowing systemic delivery of antigens (2).
596 Alteration of the parasitophorous vacuole integrity in arrested liver stage parasites likely
597 favours exposure of liver stage antigens to the immune system (3). Sporozoite and liver stage
598 antigens can be captured and presented by CD8 α ⁺ DCs in the spleen and/or the liver draining
599 lymph nodes (DLN) for priming of naïve CD8⁺ T cells (4). Exposure of parasite antigens in

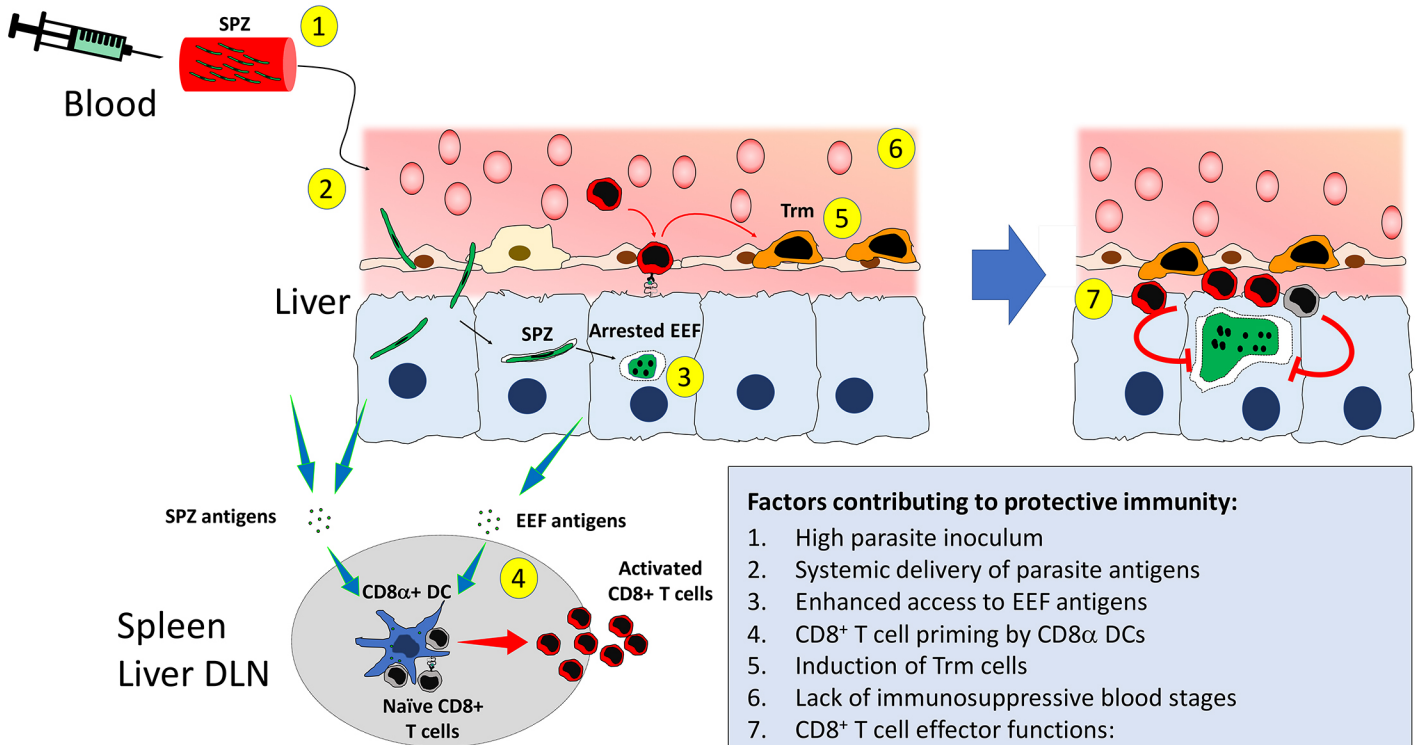
600 the liver leads to the differentiation of activated CD8⁺ T cells into tissue-resident memory T
601 (Trm) cells that patrol the liver sinusoids (5). Aborted liver stage development prevents the
602 appearance of an immunosuppressive blood stage infection (6). Upon reinfection or
603 challenge, effector CD8⁺ T cells form clusters around infected hepatocytes and can eliminate
604 parasites through direct killing of the infected cell and/or through the release of cytokines that
605 inhibit parasite development (7).

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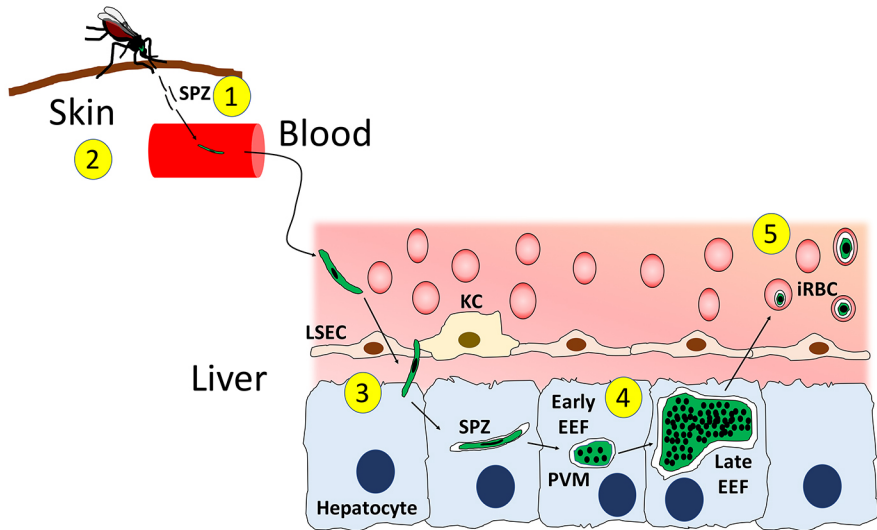
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Factors contributing to protective immunity:

1. High parasite inoculum
2. Systemic delivery of parasite antigens
3. Enhanced access to EEF antigens
4. CD8 $^+$ T cell priming by CD8 α DCs
5. Induction of Trm cells
6. Lack of immunosuppressive blood stages
7. CD8 $^+$ T cell effector functions:
 - direct killing of infected cells
 - parasite inhibition through cytokine secretion



Lack of protective immunity:

1. Low parasite inoculum
2. Regulatory mechanisms induced in the skin
3. Immune tolerance in the liver
4. Limited access to EEF antigens
5. Regulatory mechanisms induced by blood stage infection