HHS Public Access

Author manuscript

Immunol Lett. Author manuscript; available in PMC 2019 May 03.

Published in final edited form as:

Immunol Lett. 2014 October ; 161(2): 189–195. doi:10.1016/j.imlet.2014.03.011.

Memory T cells maintain protracted protection against malaria

Urszula Krzych[1,*], **Stasya Zarling**[1,#], and **Alexander Pichugin**[1]

[1]Department of Cellular Immunology, Branch of Malaria Vaccine Development, Walter Reed Army Institute of Research, Silver Spring, MD 20910

Abstract

Immunologic memory is one of the cardinal features of antigen-specific immune responses, and the persistence of memory cells contributes to prophylactic immunizations against infectious agents. Adequately maintained memory T and B cell pools assure a fast, effective and specific response against re-infections. However, many aspects of immunologic memory are still poorly understood, particularly immunologic memory inducible by parasites, for example, *Plasmodium spp.*, the causative agents of malaria. For example, memory responses to *Plasmodium* antigens amongst residents of malaria endemic areas appear to be either inadequately developed or maintained, because persons who survive episodes of childhood malaria remain vulnerable to intermittent malaria infections. By contrast, multiple exposures of humans and laboratory rodents to radiation-attenuated *Plasmodium* sporozoites (γ-spz) induce sterile and long-lasting protection against experimental sporozoite challenge. Multifactorial immune mechanisms maintain this protracted and sterile protection. While the presence of memory CD4 T cell subsets has been associated with lasting protection in humans exposed to multiple bites from Anopheles mosquitoes infected with attenuated *P. falciparum*, memory CD8 T cells maintain protection induced with *P. yoelii* and *P. berghei* γ-spz in murine models. In this review, we discuss our observations that show memory CD8 T cells specific for antigens expressed by *P. berghei* liver stage parasites as an indispensable component for the maintenance of protracted protective immunity against experimental malaria infection; moreover, the provision of an Ag-depot assures a quick recall of memory T cells as IFN-γ−producing effector CD8 T cells and IL-4-producing CD4 T cells that collaborate with B cells for an effective antibody response.

Keywords

memory T cells; malaria; *Plasmodium*; liver; mouse model; CD8 T cells

[*]Correspondence to: Urszula Krzych, Department of Cellular Immunology, Walter Reed Army Institute of Research, 503 Robert Grant Ave, Silver Spring, MD 20910, Urszula.Krzych1.civ@mail.mil, Phone: 00-1-301-319-9558.

[#]NRC Fellow

1. Introduction

One of the cardinal features of adoptive, antigen (Ag)-specific immune responses is the persistence of memory T cells that are inextricably linked to long-lasting protection [1]. The availability of memory T cells assures a fast, effective and specific response against reoccurring infections. While many re-infections are prevented by optimally effective memory T and B cell responses, re-infections by *Plasmodium spp*. parasites amongst residents of malaria endemic areas occur frequently and it is still poorly understood as to why protection does not persist after malaria infection [2]. Recently conducted studies with the RTS,S vaccine, which is based on *Plasmodium falciparum* (Pf) circumsporozoite protein (CSP), indicate that protection is conferred to infants and small children but its duration is relatively short [3] and the reasons for the absence of sustained protection remain unknown. We hypothesized [4], that the absence of adequately developed immunologic memory stemming from the tolerant milieu of the liver [5], sequestration of liver-stage antigens (LS-Ags) within hepatocytes, and the relatively short duration of the liver phase of infection, are likely responsible for the lack of lasting protection. Additionally, the phenomenon known as altered peptide ligand, resulting from polymorphisms at CSP-inducing CD8 T cell epitope sites, interferes with the priming and survival of memory T cells [6] and poor immunogenicity, from inadequate immunizing doses or immunologic interferences from blood-stage parasites [7,8] may also play a factor in reducing the formation and persistence of memory responses.

In contrast to the immune responses observed in endemic areas or against experimental vaccination with RTS,S; immunization of laboratory rodents [9], monkeys [10] and humans [11] with radiation-attenuated (γ) *Plasmodium* sporozoites (γ-spz) induces sterile and long-lasting protection. *Plasmodium* γ-spz-induced protection is multifactorial [12], involving antibody [13], CD4 [14] and CD8 [15] T cell responses directed primarily to CSP. In other studies, blood-stage Ags also recall IL-4 producing memory CD4 T cells in protected subjects [16] and liver stage antigen-1-(LSA-1) specific proliferative T cell responses correlate with protection [17]. Studies aimed to investigate immunologic memory induced by the most promising of malaria vaccines, RTS,S, have also shown that memory CD4 T cells that produce IL-2 and TNF are more prevalent in protected than non-protected subjects exposed to experimental challenge [18].

CD8 T cells are considered key effectors against pre-erythrocytic (PE) stage infection. Evidence supporting the effector function of CD8 T cells is based on studies in human [19,20] and animal models of γ-spz- [21–23] and genetically-attenuated parasites(GAP)-induced protection [24–27]. According to more recent observations made in models of protection induced by infectious *Plasmodium* sporozoites administered under drug coverage [28] and by aseptically derived *P.falciparum* γ-spz administered i.v. to humans [29], CD8 T cells also play a crucial role. Studies of natural immunity acquired in malaria endemic areas also confirm the involvement of CD8 T cells in protection [30]. Responses of CD8 T cells are characterized mainly with the production of inflammatory cytokines such as IFN-γ or TNF-α that mediate elimination of the parasite within hepatocytes by the nitric oxide (NO) pathway [31]. CD8 T cells also exhibit direct cytolytic activity against targets that express antigens belonging to PE stage parasites [27,32–35]. In this review, we mainly focus on

memory CD8 T cells in the maintenance of protracted protection in the *P. berghei* (Pb) γ-spz mouse model; however, we also touch upon Pf γ-spz- and RTS,S-induced memory CD4 T cells that could be envisaged as helper T cells collaborating with B cells for antibody responses [16,18]. We hypothesize that long-term protection to malaria whether mediated by CD8 T cells or CD4 T cells can be induced and maintained. As such, it requires the formation and persistence of memory T cells with a reservoir of central memory cells, maintained in part by Ag-depot and by IL-15 and possibly by IL-4 and other cytokines that promote conscription of IFN-γ producing effector/effector memory cells during re-infections.

2. The mammalian liver as the venue for *Plasmodium* parasite development

Plasmodium sporozoites are inoculated into a mammalian host from the salivary glands of a female Anopheles mosquito during its blood meal. Sporozoites then quickly travel to the liver via the circulatory and/or lymphatic routes. In the liver, sporozoites continue to develop within the hepatocyte by undergoing nuclear division and expressing novel protein antigens before they emerge in the form of merosomes to infect red blood cells, causing clinical disease symptoms [36–38]. Hence, in the mammalian host the *Plasmodium* parasite exhibits three morphologically distinct phases of development: sporozoite-, liver-, and blood-stage. During each stage, the parasite expresses, to some extent, unique proteins. The sporozoite-associated proteins facilitate sporozoite traversal of the liver sinusoid cellular barrier [39–41]and invasion of hepatocytes [42–45] and some, for example, CSP and TRAP, have been shown to induce cellular and antibody responses that correlate with protection in humans [46] and mice [47,48]. Many of the novel proteins expressed during parasite development within the parasitopherous vacuole in hepatocytes have not yet been properly characterized and others not well defined [49,50]. Under certain conditions, these liver-stage (LS) proteins are the major inducers of protective cellular immune responses against the pre-erythrocytic stage parasites [51–53]. Proteins that characterize the erythrocytic stage play a role in the invasion of red blood cells [54]. Immune responses to a variety of proteins representing the blood stage development are also crucial for the resolution of infection and a necessary component of the total immunologic reactivity against the *Plasmodium* parasite [55,56].

The mammalian liver plays a key role in the life cycle of the *Plasmodium* parasite. The prevailing state of immune tolerance in the liver allows for infectious sporozoites to expand and continue their life cycle unnoticed by the immune system of the host. The liver provides a “filtration system” of blood carrying an abundance of toxins, food allergens, pathogens and other inflammation-inducing agents that clearly are the trigger to initiate the requisite innate responses for adoptive immune responses to ensue. However, the inherent liver tolerance mechanisms prevent the potentially excessive inflammation and immunologically destructive stimulation from occurring. For one, the non-parenchymal liver APCs such as Kupffer cells (KC), DC, and liver sinusoidal endothelial cells express very low concentrations of MHC class I and II and costimulatory molecules and as such they significantly limit the engagement of APCs with T cells [57]. Liver APCs also produce anti-inflammatory cytokines, IL-10 and TGF-β [58], even in responses to LPS [59], to down-regulate

immunologic functions and to preserve the general state of non-responsiveness in the liver. Hepatocytes as well as some liver APCs express elevated levels of PD-L1 [58], which combined with the presence of liver regulatory T cells suggests clonal T cells deletion as yet another mechanism of immune tolerance in this organ [60]. In fact, in the *P. berghei* system, we observed that MHC class I molecules are down-regulated on KC during infection of naïve ice and their antigen-presenting function is severely reduced [61]. Furthermore, we observed that infectious sporozoites not only fail to induce naïve KC to produce IL-12, they also downregulate IL-10 [61] and as shown by others, up-regulate anti-inflammatory responses [62].

The entry of infectious sporozoites into KC is mediated by membrane:membrane fusion and parasites localize in a vacuole that does not co-localize with lysosomes, thereby sporozoites avoid metabolic degradation before reaching hepatocytes [62,63]. However, *Plasmodium* γ −spz meet a different fate in the liver; the many liver cells that comprise the innate immune system respond to production of inflammatory cytokines, which in turn leads to the eventual activation of an spz meet a the attenuated form of *Plasmodium* conceivably by engaging in phagocytosis followed by a swift effector phase and the maintenance of immune memory responses [64]. Understanding immune events that occur in the liver in model systems of protective immunity, as well as during natural infection, will expand our knowledge of organ-specific immune responses and hence facilitate exploitation of these responses to expedite progress in vaccine development against malaria.

3. Sterile and lasting protection is induced by attenuated *Plasmodium* sporozoites

Plasmodium γ-spz-induced sterile and protracted protection is considered the gold standard of anti-malaria vaccines. Like infectious sporozoites, γ-spz carrying CSP and other sporozoite-associated proteins, invade the liver where they undergo aborted development and express LSAgs [65,66]. It is believed that antigens expressed by the underdeveloped liver schizonts remain in the liver forming a LS-Ag depot [67], which is critical for induction and persistence of Ag-specific protracted protective immunity [4]. Treatment of animals with primaquine, which disrupts liver-stage development, concurrently with the γ-spz immunizations abolishes lasting protection [21,67].

The initial immune responses induced by immunization with γ-spz have only partially been investigated. According to results from *in vitro* studies, before the invasion of hepatocytes, Pb γ-spz, like infectious sporozoites, pass through KC [68,69], a step that reverses the state of tolerance to inflammation. KC become active producers of IL-12 [61] and IL-15, cytokines needed for the induction as well as persistence of adaptive immune responses [24,70]. Inflammatory cytokines induce immune proteosomes for more efficient generation of antigenic peptides for entry into the ER and thereby provide a richer availability of peptides for loading onto MHC class I and increased expression of MHC class I-peptide complexes on the surface of APCs [71]. We observed a significant upregulation of MHC class I on KC after sporozoite challenge of γ-spz-immune mice and enhanced presentation of antigens by KC to specific T cells [61]. We propose that a cascade of pro-inflammatory

cytokines released during innate immunity induced by γ-spz leads to temporary local inflammation, which is perceived as a “danger signal” needed to trigger proper responses from the adaptive immune system and lead to long lasting immune memory [72].

4. The role of liver CD8 T cells in protective immunity against pre-erythrocytic stage parasitemia

In vivo depletion studies established CD8 T cells as key effectors in a rodent model of protection against malaria [23]. We confirmed these results in β_2 microglobulin knockout (β_2m KO) mice [73] and K^bD^b KO mice [74] immunized with Pb γ-spz. Pb GAP spz-induced protection is also CD8 T cell dependent [24,27]. We also established that effector CD8 T cells are MHC class I-restricted/dependent because protection is not transferred by γ-spz-immune wt cells into β_2m KO recipients as CD8 T cells must recognize LS-Ag peptides presented by MHC class I on APC in the liver [73]. Our observations were confirmed by results from experiments using MHC class I mismatched effector CD8 T cells and target hepatocytes [75]. The need for proximity between effector CD8 T cells and *Plasmodium*-infected target hepatocytes for an effective killing of LS parasites has been recently demonstrated by the IVM approach [32,76]. However, the details of the in vivo interactions between the hepatocytes and CD8 T cells will require further scrutiny before we fully understand the participation of both LS-specific and bystander CD8 T cells in this process [33].

Target LS-Ags that induce CD8 T cells are being currently defined by us as well as by other investigators [77], using the combination of genomic and proteomic approaches. For example, targeted gene deletion reveals that *Plasmodium* sporozoite low-complexity asparagine-rich protein is essential for early LS development [78], whereas fabf/f gene [79] is essential for late LS development. Interestingly, immunization with the late-arresting, genetically attenuated fabf/$f^{-/-}$ parasites allows for induction of durable protection in several mouse strains, presumably becasue the these parasites express a broader repertoire of potential antigens that activate a wider spectra of effector T cells [80].

The site of induction of liver resident CD8 T cells remains unclear. On the basis of results showing that the number of cCD8α$^+$ DC increases in the liver concurrently with the number of Pb γ-spz immunizations, while those in the spleen do not change, we hypothesized that CD8 T cells arise in the liver after interaction with liver APCs that present LS-Ags. Additionally, liver cDCs induce IFN-γ$^+$CD8 $T_{E/EM}$ cells, a process that is both MHC class I- and IL-12-dependent, and transfer protection [81]. Alternatively, CD8 T cells might be induced in a draining LN and during sporozoite challenge, migrate to the liver to undergo further expansion to increase their effector function. Support for the later scenario has been presented elegantly in studies using Tg TCR T cells specific for an epitope on *P. yoelii* (Py) CSP following infection by mosquito bite, [75] or using Tg Pb sporozoites expressing SIINFEKL OVA peptide within the Pb CSP sequence [82]. According to these results, CD8 T_E cells are generated in the draining LN near the infection (site of mosquito bite) [75], and then migrate to liver to kill infected hepatocytes, a process that has been shown to be TAP-dependent, but endosome independent [82]. It is possible that the induction of CD8 T_E cells

depends on their fine specificity and the site of parasite inoculation. Consequently, CSP-specific CD8 T cells would be induced in the skin draining LN, as sporozoites that are trapped in the skin shed CSP, which could be presented by DCs to T cells. In contrast, LS-Ags, being expressed exclusively in the liver, might activate T cells in the liver, or be transferred either directly or through liver-resident APCs to the liver-draining LNs to activate T cells there. Owing to the paucity of antigen-specific cells naturally present at the time of immunization or infection, it may not be possible to precisely determine the location of initial priming events. Although lack of firm evidence supporting either scenario favors the prevailing view that T cell activation occurs as a result of interaction with DC in the LN, the possibility of an organ-specific activation of CD8 T cells remains very attractive and should be explored further.

4.1 IFN-γ-producing effector CD8 T cells

Intrahepatic mononuclear cells (IHMC) induced by Pb γ-spz contain CD4 and CD8 T cells that express $CD44^{hi}$ $CD25^{hi}$ and $CD45RB^{lo/hi}$ phenotypic markers [83]. Multiple immunizations with Pb γ-spz also induce the shift to $CD62L^{lo}$ phenotype on effector CD8 T cells as well as other phenotypic and functional attributes, e.g., KLRG-$1^{hi}CD107^{+}$ and IFN-γ production, all of which coincide with the induction of sterile protection [84]. IFN-γ^{+}CD8 T cells peak around day 7 after challenge of Pb γ-spz-immunized mice [21] and these effector CD8 T cells might be physiologically relevant to the process of elimination of liver-stage parasites by nitric oxide synthetase (NOS) [85,86] as immunization with γ-spz fails to generate protection in IFN-γR KO mice [87]. Moreover, a reduced number of liver IFN-$\gamma^{+}CD44^{hi}$ CD8 T cells correlates with decreased protection in mice [28], an observation that is consistent with the more recent finding which shows that, in contrast to other infections, exceedingly high number of effector CD8 T cells is needed for protection in malaria [88]. In contrast, results from in vitro studies have shown that Pb sporozoite-induced CD8 T cells eliminate Pb- or Py-infected hepatocytes without the involvement of inflammatory cytokines [27]. Instead by recognizing CSP on the infected hepatocytes (84), they are able to eliminate them in a contact-dependent manner [27]. Collectively, these observations are in agreement about the importance of CD8 T cells as effectors and their transient expansion [1,89], however, the cellular and molecular requirements necessary for parasite killing are still poorly understood.

The release of IFN-γ, which coincides with the activation of CD8 T cells, is preceded by elevated production of IL-4, which declines when IFN-γ reaches its peak [4]. The reciprocal regulation between these two cytokines reflects the precise orchestration of functional activities among T cell subsets induced by Pb γ-spz. It is likely that IL-4 is produced in the liver by NK T cells, whereas IFN-γ is produced primarily by CD8 T cells [21]. This view is in agreement with the observation that CD8 T_E cells decline after inflammation has subsided [89], whereas memory CD8 T cells persist, if they are supported by lymphokine-secreting cells.

In our view, sustained protection requires various CD8 T cell specificities, particularly those belonging to proteins expressed during LS development. It could be envisaged that CSP-specific CD8 T cells initiate the effector stage of protection because they are the first cells to

produce IFN-γ upon encountering infectious sporozoites. Protracted protection might require the subsequent activation of a second wave of CD8 T cells specific for epitopes other than the CSP, as they would have to target hepatocytes by recognizing LS-Ags. Such concerted and functionally integrated activity provided by CD8 T_E cells with multiple specificities might be necessary to provide sustained protection. In their more recent study, Butler and colleagues have actually demonstrated that GAP parasites arrested during later LS development induce stronger CD8 T cell responses and durable protection presumably because these parasites contain a richer repertoire of antigens able to induce effector T cells [90]. Similar observations regarding the availability of more abundant level of late-LS antigens have been made in a model system of protection induced by *P. berghei* sporozoites delivered by the intravenous route under a drug coverage [28].

4.2. Memory T cell responses and lasting protection

The formation and maintenance of effective memory T cells is inextricably linked to long-lasting protective immunity [1]. Memory CD8 T cells that accumulate in the liver after immunization with Pb γ-spz represent at least two distinct but developmentally-related subsets: the IFN-γ-producing $CD44^{hi}CD45RB^{lo}CD122^{lo}CD62L^{lo}$ phenotype, or TE/EM cells that can be further subdivided into CD8 T_E ($CD127^{-}KLRG\text{-}1^{+}$) or CD8 T_{EM} ($CD127^{+}KLRG\text{-}1^{-}$); and the indolent IFN-γ producing $CD44^{hi}CD45RB^{hi}CD122^{hi}CD62L^{lo}$ phenotype, hence CD8 T_{CM} cells. A decay of protection is typically accompanied by the decline of IFN-γ-producing $KLRG\text{-}1^{+}CD107^{+}$ CD8 T cells [84].

The elevated expression of CD122 (IL-15Rα) on CD8 T_{CM} cells suggests that, in contrast to CD8 TE/EM cells, they are IL-15-dependent [21,70]. Because CD8 T_{CM} cells produce relatively low levels of IFN-γ, they do not appear to be directly involved in the elimination of the parasite. Instead, by acquiring the $CD122^{hi}$ phenotype, liver CD8 T_{CM} cells engage in homeostatic proliferation, which qualifies them to function as a reservoir to maintain the size of memory CD8 T cell pools [21,70,91]. The maintenance of memory pools is one of the prerequisites of a memory T cell response because attrition, particularly of CD8 T_E cells, is inevitable during any infection [89]. The co-presence of distinct subsets within the intrahepatic memory CD8 T cell pool in mice protected against malaria is consistent with an earlier view that virally induced memory CD8 T cells are organized into subsets on the basis of distinct functional activities and the maturation/activation status [92–94].

Results from our earlier work concerning recall responses of CD4 T cells specific for LS and blood stage antigens {parasitized red blood cells (pRBC)} showed that T cells from Pf γ-spz protected persons proliferate and produce IL-4, respectively [16]. Further analysis of the IL-4 producing cells revealed that this cytokine, which helps B cells for antibody production and activates effector T cells, is produced mainly by one of two discrete memory CD4 T cell subsets present in subjects protected by Pf γ-spz exposure. A CD4 T cell memory subset that expresses the $CD45RO^{+}CD27^{-}CD70^{+}$ phenotype and is considered a mature or late memory, produces IL-4, in contrast to a set expressing the $CD45RO^{+}CD27^{+}$ phenotype, designated as early memory, which does not produce this cytokine. Moreover, IL-4 is produced only in the presence of pRBC, which prompts the two memory sets to engage in an interaction mediated by CD27:CD70 coligation [16]. Similar to observations made in other

systems [95–97], this differentiation from the early $CD27^+$ to mature $CD27^-$ T cells occurs after prophylactic immunization with Pf γ-spz. Although the less mature $CD27^+$ were detectable in all persons exposed γ-spz, only the $CD27^-$ mature subset was present in persons protected against experimental sporozoite challenge. On the basis of these results we propose that persistence of memory CD4 T cells depends on a conscription of the less mature memory CD4 T cells into a fully effective memory set and that this process requires Ags presumably from the repositories of liver and blood stage antigens as well as the delivery of a second signal from the CD27:CD70 interaction.

We also observed the presence of memory CD4 T cells in subjects immunized with the RTS,S vaccine [18]. The Pf CSP-specific CD4 T cells form two distinct subsets, the long-lived self-renewing $CD45RO^+CCR7^+$ T central memory (T_{CM}) cells and the short-lived $CD45RO^+CCR7^-$ T effector/effector memory ($T_{E/EM}$) cell subset. While both memory T cell subsets produce IL-2 and TNF, subjects protected by experimental sporozoite challenge show higher number of both IL-2 producing T_{CM} and $T_{E/EM}$ cells than subjects who succumb to malaria. A strong association between CSP-specific antibody titers and the frequency of IL-2 producing CD4 T cells in the same subjects suggests that these CD4 T cells serve as memory helper cells.

Recently, various phenotypic and functional attributes have been evaluated in an effort to understand the differentiation of memory CD8 T cells [98] and CD4 T cells [97]. In addition, asymmetric division [99], duration and strength of the TCR signal [100] and inflammatory cytokines [101] have been examined as requirements for memory T cell development and differentiation. Nonetheless, many questions remain regarding the regulation of memory cell formation and in the case of organ-specific infections, like malaria, additional aspects of memory CD8 T cell development and differentiation need to be considered. We propose that these functionally and phenotypically unique subsets of liver memory CD8 T cells form an interactive network involving different phases of dynamic cell activation and differentiation [21].

5. Mechanisms for the persistence of memory T cells

5.1 Repositories of liver-stage antigens are required for the maintenance of protracted protection

The issue concerning the persistence of Ag for the maintenance of memory T cells remains controversial [102–105]. It appears that the type of infections, e.g., viral versus protozoan, dictate whether the presence of Ag is required for the persistent protection maintained by memory T cells. On the basis of results from our laboratory, the persistence of a threshold of accumulated LS-Ags is critical for the maintenance of protective immunity. Administration of primaquine at the time of immunization with Pb γ-spz does not affect protection at primary challenge, which is most likely mediated by the sporozoite-stage associated Ags, e.g., CSP, but results in a loss of protracted protection, which correlates with a decrease of CD8 $T_{E/EM}$ cells in the liver. The disruption of the intrahepatic-stage parasite development prevents the formation of a local Ag depot, which impedes the conscription of T_{CM} into $T_{E/EM}$ CD8 T cells upon re-challenge. In contrast, delayed administration of primaquine has no effect on lasting protective immunity [21]. These results are indeed expected, as the

primary primaquine action is directed against LS development, without affecting the sporozoite stage, represented by CSP-specific CD8 T cells [106,107]. Once the LS Ags have been expressed and accumulated in the liver, a delayed primaquine no longer affects their expression.

Although most of the results from viral systems argue against the need for antigen to maintain long-lived memory CD8 T cells [104,108], there is evidence that T cell memory persists if a protracted re-stimulation of effector T cells is maintained, either by persisting or by cross-reacting environmental antigens [103,109,110]. We suggest that antigen requirements might be quite different in malaria because the parasite exhibits tropism to the liver, which is characterized by immunologic tolerance. Although the precise location of the LS-Ag depot has not been established, hepatocytes might contain liver antigen repository. It could be envisaged that liver APCs internalize infected hepatocytes and engage in cross-presentation of LS-Ag that gained entry from phagosomes into the MHC class I pathway. Exogenous particulate Ags were shown to enter the MHC class I pathway via phagosome-ER fusion [111] or, as in the case of *Toxoplasma gondii*, fusion of the parasitophorous vacuole with the ER [112]. This scenario would provide a unique role for organ-specific maintenance of memory CD8 T cells by distinguishing the “locally” activated liver memory T cells from those found in the spleen or LN.

5.2 IL-15 is crucial for maintenance of protracted protection against pre-erythrocytic stage malaria

It has been established that IL-15 promotes the survival of long-term memory CD8 T cells by maintaining their homeostatic proliferation [113–116]. Although initially after the exposure to Pb γ-spz, the CD8 T_{CM} cell subset represents a much smaller fraction of the liver CD8 T cells, twice as many CD8 T_{CM} cells are $CD122^{hi}$ than $T_{E/EM}$ cells [21]. On the basis of results from *in vitro* studies, only CD8 T_{CM} cells proliferate in the presence of IL-15 and these cells are severely reduced in IL-15KO mice [70]. The enhanced sensitivity of the CD8 T_{CM} cells to reduced levels of IL-15 suggests that this subset preferentially expands to elevated levels of IL-15 in the liver.

Like wt mice, IL-15 KO mice are protected against a 1° challenge administered shortly after immunization with Pb γ-spz; however, at 2° challenge 2 months later, the IL-15KO mice become susceptible to malaria infection. In the absence of IL-15, the critical reservoir of CD8 T_{CM} cells is severely reduced and hence unable to differentiate into a sufficient number of CD8 T_E cells needed during re-infection. Severe attrition, as evidenced by the high level of apoptosis particularly within CD8 T_{CM} cells, seems to be responsible for the near absence of this subset in IL-15KO mice. Upon 2° challenge, the majority of $CD127^{hi}$CD8 T cells transitioned to $CD127^{lo}$ phenotype in wt mice, but in IL-15KO mice few cells became $CD127^{lo}$. These observations strongly support our hypothesis that CD8 $T_{E/EM}$ cells are conscripted from memory precursor effector cells or CD8 T_{CM} cells in a continuous, albeit slow, process that occurs in the liver as a result of an increased Ag load after repeated immunizations with γ-spz. The process also occurs during infection, when large numbers of CD8 T_{EM} cells would be most needed to combat the parasite. For example, it has been shown that a large number of Pb $CS_{252–260}$-specific CD8 T cells is needed to maintain

sterile protection in Balb/c mice [117]. The requirement for these large numbers of antigen-specific CD8 T cells may be, in part, due to the short time frame of the LS when the parasite is most vulnerable to immune intervention.

IL-15 is produced by a variety of cell types (although not by T cells) in response to signaling via TLRs or exposure to type I IFN [118]. Pb γ-spz induce upregulation of IL-15 mRNA in KC [70] and liver cDC [24]. Upon encounter with specific Ag from the liver repository or upon re-infections, IL-15 would drive CD8 T_{CM} cells to differentiate into the $CD62L^{lo}KLRG\text{-}1^{+}CD127^{-}CD122^{lo}$ phenotype that produces IFN-γ. Recently, it was demonstrated that CD8 T cell survival during influenza infection is promoted in the lung by trans-presentation of IL-15 by pulmonary $CD8\alpha^{+}$DCs [119]. There is evidence [120] that APC retain IL-15 bound to the IL-15Rα chain to trans-activate CD8 T cells expressing the IL-15Rβγc complex. On the basis of our previously published results that liver cDC that activate CD8 T cells in a MHC-class I dependent manner to express $CD44^{hi}$, up-regulate IL-15 mRNA [81] and express detectable IL-15 protein [121], together with KC they can function as APCs of LS-Ags and as trans-presenters of IL-15 that target only liver CD8 T_{CM} cells. Our hypothesis is supported by observations from *in vitro* conducted studies that only CD8 T_{CM} cells require trans-presentation of IL-15 in the context of a concurrent signaling via TCR for optimal recall response, as responses by CD8 T_{EM} cells are not augmented by IL-15 [122].

6. Summary

MHC class I-restricted CD8 T cells have been established as key effectors in protective immunity against pre-erythrocytic-stage malaria infection. Their effector function is associated mainly with the production of inflammatory cytokines such as IFN-γ or TNF-α that mediate elimination of the parasite within the hepatocytes by the NO pathway or by direct cytolytic activity on infected hepatocytes. The success of protection induced by γ-spz depends upon the long-lived intrahepatic memory CD8 T cells that consist of developmentally-related subsets as CD8 T_{CM} and CD8 T_{EM} cells. While the CD8 T_{EM} cells are maintained by antigen-driven conscription of CD8 T_{CM} cells, the latter, representing a very broad spectrum of antigen-specific T cells, is maintained by IL-15 and possibly the LS-Ag depot. This arrangement assures a steady availability of antigen-specific T cells should they be required to combat re-infection. The dependence on specific antigen essentially controls the balance between the two phenotypes and the differential expression of IL-15R prevents the CD8 T_{EM} cells from becoming activated in the event of sporadic co-infections. However, it is the activated status of the intrahepatic memory CD8 T cells that really distinguishes them from the memory CD8 T cells in the spleen and LN as it represents the sentinel of a local, organ-specific infection.

Acknowledgments

The authors would like to express their thanks to all the past and the present members of the Krzych lab. In particular, the authors would like to acknowledge Mr. Isaac Chalom, whose work and dedication during the past decade contributed significantly to the results presented in this review. This research was supported in part by a grant from the National Institutes of Health (UK) and the US Army Materiel Command. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense.

References

[1]. Ahmed R, Gray D. Immunological memory and protective immunity: understanding their relation. Science 1996;272:54–60. [PubMed: 8600537]

[2]. Langhorne J, Ndungu FM, Sponaas AM, Marsh K. Immunity to malaria: more questions than answers. Nature immunology 2008;9:725–732. [PubMed: 18563083]

[3]. Abdulla S, Oberholzer R, Juma O, Kubhoja S, Machera F, Membi C, et al. Safety and immunogenicity of RTS,S/AS02D malaria vaccine in infants. N Engl J Med 2008;359:2533–2544. [PubMed: 19064623]

[4]. Krzych U, Schwenk R, Guebre-Xabier M, Sun P, Palmer D, White K, et al. The role of intrahepatic lymphocytes in mediating protective immunity induced by attenuated Plasmodium berghei sporozoites. Immunol Rev 2000;174:123–134. [PubMed: 10807512]

[5]. Crispe IN. Liver antigen-presenting cells. J Hepatol 2011;54:357–365. [PubMed: 21084131]

[6]. Plebanski M, Lee EA, Hannan CM, Flanagan KL, Gilbert SC, Gravenor MB, et al. Altered peptide ligands narrow the repertoire of cellular immune responses by interfering with T-cell priming. Nat Med 1999;5:565–571. [PubMed: 10229235]

[7]. Good MF, Xu H, Wykes M, Engwerda CR. Development and regulation of cell-mediated immune responses to the blood stages of malaria: implications for vaccine research. Annu Rev Immunol 2005;23:69–99. [PubMed: 15771566]

[8]. Urban BC, Ing R, Stevenson MM. Early interactions between blood-stage plasmodium parasites and the immune system. Curr Top Microbiol Immunol 2005;297:25–70. [PubMed: 16265902]

[9]. Nussenzweig RS, Vanderberg J, Most H, Orton C. Protective immunity produced by the injection of x-irradiated sporozoites of Plasmodium berghei. Nature 1967;216:160–162. [PubMed: 6057225]

[10]. Nussenzweig RS, Vanderberg J, Most H, Orton C. Immunity in simian malaria induced by irradiated sporozoites. J Parasitol 1970;56:350–351. [PubMed: 5445830]

[11]. Clyde DF, Most H, McCarthy VC, Vanderberg JP. Immunization of man against sporozite-induced falciparum malaria. Am J Med Sci 1973;266:169–177. [PubMed: 4583408]

[12]. Nardin EH, Nussenzweig RS. T cell responses to pre-erythrocytic stages of malaria: role in protection and vaccine development against pre-erythrocytic stages. Annu Rev Immunol 1993;11:687–727. [PubMed: 8476576]

[13]. Egan JE, Hoffman SL, Haynes JD, Sadoff JC, Schneider I, Grau GE, et al. Humoral immune responses in volunteers immunized with irradiated Plasmodium falciparum sporozoites. Am J Trop Med Hyg 1993;49:166–173. [PubMed: 8357078]

[14]. Nardin EH, Herrington DA, Davis J, Levine M, Stuber D, Takacs B, et al. Conserved repetitive epitope recognized by CD4+ clones from a malaria-immunized volunteer. Science 1989;246:1603–1606. [PubMed: 2480642]

[15]. Wizel B, Houghten RA, Parker KC, Coligan JE, Church P, Gordon DM, et al. Irradiated sporozoite vaccine induces HLA-B8-restricted cytotoxic T lymphocyte responses against two overlapping epitopes of the Plasmodium falciparum sporozoite surface protein 2. J Exp Med 1995;182:1435–1445. [PubMed: 7595214]

[16]. Palmer DR, Krzych U. Cellular and molecular requirements for the recall of IL-4-producing memory CD4(+)CD45RO(+)CD27(−) T cells during protection induced by attenuated Plasmodium falciparum sporozoites. Eur J Immunol 2002;32:652–661. [PubMed: 11857339]

[17]. Krzych U, Lyon JA, Jareed T, Schneider I, Hollingdale MR, Gordon DM, et al. T lymphocytes from volunteers immunized with irradiated Plasmodium falciparum sporozoites recognize liver and blood stage malaria antigens. J Immunol 1995;155:4072–4077. [PubMed: 7561118]

[18]. Lumsden JM, Schwenk RJ, Rein LE, Moris P, Janssens M, Ofori-Anyinam O, et al. Protective immunity induced with the RTS,S/AS vaccine is associated with IL-2 and TNF-alpha producing effector and central memory CD4 T cells. PloS one 2011;6:e20775. [PubMed: 21779319]

[19]. Malik A, Egan JE, Houghten RA, Sadoff JC, Hoffman SL. Human cytotoxic T lymphocytes against the Plasmodium falciparum circumsporozoite protein. Proc Natl Acad Sci U S A 1991;88:3300–3304. [PubMed: 1707538]

[20]. Spring M, Murphy J, Nielsen R, Dowler M, Bennett JW, Zarling S, et al. First-in-human evaluation of genetically attenuated Plasmodium falciparum sporozoites administered by bite of Anopheles mosquitoes to adult volunteers. Vaccine 2013;31:4975–4983. [PubMed: 24029408]

[21]. Berenzon D, Schwenk RJ, Letellier L, Guebre-Xabier M, Williams J, Krzych U. Protracted protection to Plasmodium berghei malaria is linked to functionally and phenotypically heterogeneous liver memory CD8+ T cells. J Immunol 2003;171:2024–2034. [PubMed: 12902507]

[22]. 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]

[23]. Weiss WR, Sedegah M, Beaudoin RL, Miller LH, Good MF. CD8+ T cells (cytotoxic/suppressors) are required for protection in mice immunized with malaria sporozoites. Proc Natl Acad Sci U S A 1988;85:573–576. [PubMed: 2963334]

[24]. Jobe O, Lumsden J, Mueller AK, Williams J, Silva-Rivera H, Kappe SH, et al. Genetically attenuated Plasmodium berghei liver stages induce sterile protracted protection that is mediated by major histocompatibility complex Class I-dependent interferon-gamma-producing CD8+ T cells. J Infect Dis 2007;196:599–607. [PubMed: 17624847]

[25]. Mueller AK, Deckert M, Heiss K, Goetz K, Matuschewski K, Schluter D. Genetically attenuated Plasmodium berghei liver stages persist and elicit sterile protection primarily via CD8 T cells. Am J Pathol 2007;171:107–115. [PubMed: 17591958]

[26]. Tarun AS, Dumpit RF, Camargo N, Labaied M, Liu P, Takagi A, et al. Protracted sterile protection with Plasmodium yoelii pre-erythrocytic genetically attenuated parasite malaria vaccines is independent of significant liver-stage persistence and is mediated by CD8+ T cells. J Infect Dis 2007;196:608–616. [PubMed: 17624848]

[27]. Trimnell A, Takagi A, Gupta M, Richie TL, Kappe SH, Wang R. Genetically attenuated parasite vaccines induce contact-dependent CD8+ T cell killing of Plasmodium yoelii liver stage-infected hepatocytes. J Immunol 2009;183:5870–5878. [PubMed: 19812194]

[28]. Nganou-Makamdop K, van Gemert GJ, Arens T, Hermsen CC, Sauerwein RW. Long term protection after immunization with P. berghei sporozoites correlates with sustained IFNgamma responses of hepatic CD8+ memory T cells. PloS one 2012;7:e36508. [PubMed: 22563506]

[29]. Seder RA, Chang LJ, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, et al. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. Science 2013;341:1359–1365. [PubMed: 23929949]

[30]. Bejon P, Mwacharo J, Kai O, Todryk S, Keating S, Lowe B, et al. The induction and persistence of T cell IFN-gamma responses after vaccination or natural exposure is suppressed by Plasmodium falciparum. J Immunol 2007;179:4193–4201. [PubMed: 17785859]

[31]. Seguin MC, Klotz FW, Schneider I, Weir JP, Goodbary M, Slayter M, et al. Induction of nitric oxide synthase protects against malaria in mice exposed to irradiated Plasmodium berghei infected mosquitoes: involvement of interferon gamma and CD8+ T cells. J Exp Med 1994;180:353–358. [PubMed: 7516412]

[32]. Cockburn IA, Amino R, Kelemen RK, Kuo SC, Tse SW, Radtke A, et al. In vivo imaging of CD8+ T cell-mediated elimination of malaria liver stages. Proc Natl Acad Sci U S A 2013;110:9090–9095. [PubMed: 23674673]

[33]. Cockburn IA, Tse SW, Zavala F. CD8+ T cells eliminate liver stage Plasmodium parasites without detectable bystander effect. Infect Immun 2014.

[34]. Reyes-Sandoval A, Wyllie DH, Bauza K, Milicic A, Forbes EK, Rollier CS, et al. CD8+ T effector memory cells protect against liver-stage malaria. J Immunol 2011;187:1347–1357. [PubMed: 21715686]

[35]. Hill AV, Allsopp CE, Kwiatkowski D, Anstey NM, Twumasi P, Rowe PA, et al. Common west African HLA antigens are associated with protection from severe malaria. Nature 1991;352:595–600. [PubMed: 1865923]

[36]. Sturm A, Amino R, van de Sand C, Regen T, Retzlaff S, Rennenberg A, et al. Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids. Science 2006;313:1287–1290. [PubMed: 16888102]

[37]. Baer K, Klotz C, Kappe SH, Schnieder T, Frevert U. Release of hepatic Plasmodium yoelii merozoites into the pulmonary microvasculature. PLoS pathogens 2007;3:e171. [PubMed: 17997605]

[38]. Tarun AS, Baer K, Dumpit RF, Gray S, Lejarcegui N, Frevert U, et al. Quantitative isolation and in vivo imaging of malaria parasite liver stages. Int J Parasitol 2006;36:1283–1293. [PubMed: 16890231]

[39]. Ishino T, Chinzei Y, Yuda M. A Plasmodium sporozoite protein with a membrane attack complex domain is required for breaching the liver sinusoidal cell layer prior to hepatocyte infection. Cell Microbiol 2005;7:199–208. [PubMed: 15659064]

[40]. Kariu T, Ishino T, Yano K, Chinzei Y, Yuda M. CelTOS, a novel malarial protein that mediates transmission to mosquito and vertebrate hosts. Mol Microbiol 2006;59:1369–1379. [PubMed: 16468982]

[41]. Talman AM, Lacroix C, Marques SR, Blagborough AM, Carzaniga R, Menard R, et al. PbGEST mediates malaria transmission to both mosquito and vertebrate host. Mol Microbiol 2011;82:462–474. [PubMed: 21958024]

[42]. Fidock DA, Pasquetto V, Gras H, Badell E, Eling W, Ballou WR, et al. Plasmodium falciparum sporozoite invasion is inhibited by naturally acquired or experimentally induced polyclonal antibodies to the STARP antigen. Eur J Immunol 1997;27:2502–2513. [PubMed: 9368603]

[43]. Frevert U. Malaria sporozoite-hepatocyte interactions. Exp Parasitol 1994;79:206–210. [PubMed: 8056083]

[44]. Robson KJ, Frevert U, Reckmann I, Cowan G, Beier J, Scragg IG, et al. Thrombospondin-related adhesive protein (TRAP) of Plasmodium falciparum: expression during sporozoite ontogeny and binding to human hepatocytes. EMBO J 1995;14:3883–3894. [PubMed: 7664729]

[45]. Rogers WO, Malik A, Mellouk S, Nakamura K, Rogers MD, Szarfman A, et al. Characterization of Plasmodium falciparum sporozoite surface protein 2. Proc Natl Acad Sci U S A 1992;89:9176–9180. [PubMed: 1409621]

[46]. Hoffman SL, Goh LM, Luke TC, Schneider I, Le TP, Doolan DL, et al. Protection of humans against malaria by immunization with radiation-attenuated Plasmodium falciparum sporozoites. J Infect Dis 2002;185:1155–1164. [PubMed: 11930326]

[47]. Schmidt NW, Butler NS, Badovinac VP, Harty JT. Extreme CD8 T cell requirements for anti-malarial liver-stage immunity following immunization with radiation attenuated sporozoites. PLoS pathogens 2010;6:e1000998. [PubMed: 20657824]

[48]. Hafalla JC, Bauza K, Friesen J, Gonzalez-Aseguinolaza G, Hill AV, Matuschewski K. Identification of targets of CD8(+) T cell responses to malaria liver stages by genome-wide epitope profiling. PLoS pathogens 2013;9:e1003303. [PubMed: 23675294]

[49]. Mueller AK, Camargo N, Kaiser K, Andorfer C, Frevert U, Matuschewski K, et al. Plasmodium liver stage developmental arrest by depletion of a protein at the parasite-host interface. Proc Natl Acad Sci U S A 2005;102:3022–3027. [PubMed: 15699336]

[50]. Prudencio M, Rodriguez A, Mota MM. The silent path to thousands of merozoites: the Plasmodium liver stage. Nat Rev Microbiol 2006;4:849–856. [PubMed: 17041632]

[51]. Bucci K, Kastens W, Hollingdale MR, Shankar A, Alpers MP, King CL, et al. Influence of age and HLA type on interferon-gamma (IFN-gamma) responses to a naturally occurring polymorphic epitope of Plasmodium falciparum liver stage antigen-1 (LSA-1). Clin Exp Immunol 2000;122:94–100. [PubMed: 11012624]

[52]. Fidock DA, Gras-Masse H, Lepers JP, Brahimi K, Benmohamed L, Mellouk S, et al. Plasmodium falciparum liver stage antigen-1 is well conserved and contains potent B and T cell determinants. J Immunol 1994;153:190–204. [PubMed: 7515922]

[53]. Speake C, Duffy PE. Antigens for pre-erythrocytic malaria vaccines: building on success. Parasite Immunol 2009;31:539–546. [PubMed: 19691557]

[54]. Holder AA. Preventing merozoite invasion of erythrocytes In: Hoffman SL, editor. Malaria vaccine development: a multi-immune response approach. Washington, DC: ASM Press, 1996: 77–92.

[55]. Achtman AH, Stephens R, Cadman ET, Harrison V, Langhorne J. Malaria-specific antibody responses and parasite persistence after infection of mice with Plasmodium chabaudi chabaudi. Parasite Immunol 2007;29:435–444. [PubMed: 17727567]

[56]. Stephens R, Albano FR, Quin S, Pascal BJ, Harrison V, Stockinger B, et al. Malaria-specific transgenic CD4(+) T cells protect immunodeficient mice from lethal infection and demonstrate requirement for a protective threshold of antibody production for parasite clearance. Blood 2005;106:1676–1684. [PubMed: 15890689]

[57]. Jenne CN, Kubes P. Immune surveillance by the liver. Nature immunology 2013;14:996–1006. [PubMed: 24048121]

[58]. Lau AH, de Creus A, Lu L, Thomson AW. Liver tolerance mediated by antigen presenting cells: fact or fiction? Gut 2003;52:1075–1078. [PubMed: 12865260]

[59]. Knolle PA, Germann T, Treichel U, Uhrig A, Schmitt E, Hegenbarth S, et al. Endotoxin down-regulates T cell activation by antigen-presenting liver sinusoidal endothelial cells. J Immunol 1999;162:1401–1407. [PubMed: 9973395]

[60]. Crispe IN, Giannandrea M, Klein I, John B, Sampson B, Wuensch S. Cellular and molecular mechanisms of liver tolerance. Immunol Rev 2006;213:101–118. [PubMed: 16972899]

[61]. Steers N, Schwenk R, Bacon DJ, Berenzon D, Williams J, Krzych U. The immune status of Kupffer cells profoundly influences their responses to infectious Plasmodium berghei sporozoites. Eur J Immunol 2005;35:2335–2346. [PubMed: 15997465]

[62]. Frevert U, Nacer A, Cabrera M, Movila A, Leberl M. Imaging Plasmodium immunobiology in the liver, brain, and lung. Parasitology international 2014;63:171–186. [PubMed: 24076429]

[63]. Tavares J, Formaglio P, Thiberge S, Mordelet E, Van Rooijen N, Medvinsky A, et al. Role of host cell traversal by the malaria sporozoite during liver infection. J Exp Med 2013;210:905–915. [PubMed: 23610126]

[64]. Krzych U. Malaria and the liver: tolerance and immunity to attenuated Plasmodia sporozoites In: Crispe IN, editor. T lymphocytes in the liver: immunobiology, pathology and host defense. New York: Wiley-Liss, 1999: 163–195.

[65]. Hollingdale MR, Krzych U. Immune responses to liver-stage parasites: implications for vaccine development. Chem Immunol 2002;80:97–124. [PubMed: 12058653]

[66]. Zechini B, Cordier L, Ngonseu E, D'Alessandro U, Wery M, Chatterjee S. Plasmodium berghei development in irradiated sporozoite-immunized C57BL6 mice. Parasitology 1999;118 (Pt 4): 335–338. [PubMed: 10340322]

[67]. Scheller LF, Azad AF. Maintenance of protective immunity against malaria by persistent hepatic parasites derived from irradiated sporozoites. Proc Natl Acad Sci U S A 1995;92:4066–4068. [PubMed: 7732032]

[68]. Pradel G, Frevert U. Malaria sporozoites actively enter and pass through rat Kupffer cells prior to hepatocyte invasion. Hepatology 2001;33:1154–1165. [PubMed: 11343244]

[69]. Silvie O A role for apical membrane antigen 1 during invasion of hepatocytes by Plasmodium falciparum sporozoites. J Biol Chem 2004;279:9490–9496. [PubMed: 14676185]

[70]. Zarling S, Berenzon D, Dalai S, Liepinsh D, Steers N, Krzych U. The survival of memory CD8 T cells that is mediated by IL-15 correlates with sustained protection against malaria. J Immunol 2013;190:5128–5141. [PubMed: 23589611]

[71]. Khan S, van den Broek M, Schwarz K, de Giuli R, Diener PA, Groettrup M. Immunoproteasomes largely replace constitutive proteasomes during an antiviral and antibacterial immune response in the liver. J Immunol 2001;167:6859–6868. [PubMed: 11739503]

[72]. Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol 1994;12:991–1045. [PubMed: 8011301]

[73]. White KL, Snyder HL, Krzych U. MHC class I-dependent presentation of exoerythrocytic antigens to CD8+ T lymphocytes is required for protective immunity against Plasmodium berghei. J Immunol 1996;156:3374–3381. [PubMed: 8617963]

[74]. Krzych U, Schwenk J. The dissection of CD8 T cells during liver-stage infection. Curr Top Microbiol Immunol 2005;297:1–24. [PubMed: 16265901]

[75]. Chakravarty S, Cockburn IA, Kuk S, Overstreet MG, Sacci JB, Zavala F. CD8+ T lymphocytes protective against malaria liver stages are primed in skin-draining lymph nodes. Nat Med 2007;13:1035–1041. [PubMed: 17704784]

[76]. Cabrera M, Pewe LL, Harty JT, Frevert U. In vivo CD8+ T cell dynamics in the liver of Plasmodium yoelii immunized and infected mice. PloS one 2013;8:e70842. [PubMed: 23967119]

[77]. Doolan DL, Southwood S, Freilich DA, Sidney J, Graber NL, Shatney L, et al. Identification of Plasmodium falciparum antigens by antigenic analysis of genomic and proteomic data. Proc Natl Acad Sci U S A 2003;100:9952–9957. [PubMed: 12886016]

[78]. Aly AS, Lindner SE, MacKellar DC, Peng X, Kappe SH. SAP1 is a critical post-transcriptional regulator of infectivity in malaria parasite sporozoite stages. Mol Microbiol 2011;79:929–939. [PubMed: 21299648]

[79]. Vaughan AM, O'Neill MT, Tarun AS, Camargo N, Phuong TM, Aly AS, et al. Type II fatty acid synthesis is essential only for malaria parasite late liver stage development. Cell Microbiol 2009;11:506–520. [PubMed: 19068099]

[80]. Butler NS, Schmidt NW, Vaughan AM, Aly AS, Kappe SH, Harty JT. Superior antimalarial immunity after vaccination with late liver stage-arresting genetically attenuated parasites. Cell host & microbe 2011;9:451–462. [PubMed: 21669394]

[81]. Jobe O, Donofrio G, Sun G, Liepinsh D, Schwenk R, Krzych U. Immunization with radiation-attenuated Plasmodium berghei sporozoites induces liver cCD8alpha+DC that activate CD8+T cells against liver-stage malaria. PloS one 2009;4:e5075. [PubMed: 19347042]

[82]. Cockburn IA, Tse SW, Radtke AJ, Srinivasan P, Chen YC, Sinnis P, et al. Dendritic cells and hepatocytes use distinct pathways to process protective antigen from plasmodium in vivo. PLoS pathogens 2011;7:e1001318. [PubMed: 21445239]

[83]. Guebre-Xabier M, Schwenk R, Krzych U. Memory phenotype CD8(+) T cells persist in livers of mice protected against malaria by immunization with attenuated Plasmodium berghei sporozoites. Eur J Immunol 1999;29:3978–3986. [PubMed: 10602007]

[84]. Krzych U, Dalai S, Ehrler L, Jobe O, Egner L Infectious challenge of Plasmodium berghei γ-spz immunized mice rescues effector CD8+ T cells, thus assuring protracted protection. J Immunol 2010;184:52.59.

[85]. Doolan DL, Hoffman SL. IL-12 and NK cells are required for antigen-specific adaptive immunity against malaria initiated by CD8+ T cells in the Plasmodium yoelii model. J Immunol 1999;163:884–892. [PubMed: 10395683]

[86]. Klotz FW, Scheller LF, Seguin MC, Kumar N, Marletta MA, Green SJ, et al. Co-localization of inducible-nitric oxide synthase and Plasmodium berghei in hepatocytes from rats immunized with irradiated sporozoites. J Immunol 1995;154:3391–3395. [PubMed: 7534796]

[87]. Tsuji M, Miyahira Y, Nussenzweig RS, Aguet M, Reichel M, Zavala F. Development of antimalaria immunity in mice lacking IFN-gamma receptor. J Immunol 1995;154:5338–5344. [PubMed: 7537305]

[88]. Schmidt NW, Podyminogin RL, Butler NS, Badovinac VP, Tucker BJ, Bahjat KS, et al. Memory CD8 T cell responses exceeding a large but definable threshold provide long-term immunity to malaria. Proc Natl Acad Sci U S A 2008;105:14017–14022. [PubMed: 18780790]

[89]. Badovinac VP, Porter BB, Harty JT. Programmed contraction of CD8(+) T cells after infection. Nature immunology 2002;3:619–626. [PubMed: 12055624]

[90]. Butler NS, Vaughan AM, Harty JT, Kappe SH. Whole parasite vaccination approaches for prevention of malaria infection. Trends in immunology 2012;33:247–254. [PubMed: 22405559]

[91]. Judge AD, Zhang X, Fujii H, Surh CD, Sprent J. Interleukin 15 controls both proliferation and survival of a subset of memory-phenotype CD8(+) T cells. J Exp Med 2002;196:935–946. [PubMed: 12370255]

[92]. Kaech SM, Wherry EJ, Ahmed R. Effector and memory T-cell differentiation: implications for vaccine development. Nature reviews Immunology 2002;2:251–262.

[93]. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 1999;401:708–712. [PubMed: 10537110]

[94]. Wherry EJ, Teichgraber V, Becker TC, Masopust D, Kaech SM, Antia R, et al. Lineage relationship and protective immunity of memory CD8 T cell subsets. Nature immunology 2003;4:225–234. [PubMed: 12563257]

[95]. De Jong R, Brouwer M, Hooibrink B, Van der Pouw-Kraan T, Miedema F, Van Lier RA. The CD27- subset of peripheral blood memory CD4+ lymphocytes contains functionally differentiated T lymphocytes that develop by persistent antigenic stimulation in vivo. Eur J Immunol 1992;22:993–999. [PubMed: 1348033]

[96]. Morimoto C, Schlossman SF. P. Rambotti Lecture. Human naive and memory T cells revisited: new markers (CD31 and CD27) that help define CD4+ T cell subsets. Clin Exp Rheumatol 1993;11:241–247. [PubMed: 8394793]

[97]. Hintzen RQ, Lens SM, Lammers K, Kuiper H, Beckmann MP, van Lier RA. Engagement of CD27 with its ligand CD70 provides a second signal for T cell activation. J Immunol 1995;154:2612–2623. [PubMed: 7876536]

[98]. Joshi NS, Cui W, Dominguez CX, Chen JH, Hand TW, Kaech SM. Increased numbers of preexisting memory CD8 T cells and decreased T-bet expression can restrain terminal differentiation of secondary effector and memory CD8 T cells. J Immunol 2011;187:4068–4076. [PubMed: 21930973]

[99]. Ciocca ML, Barnett BE, Burkhardt JK, Chang JT, Reiner SL. Cutting edge: Asymmetric memory T cell division in response to rechallenge. J Immunol 2012;188:4145–4148. [PubMed: 22467651]

[100]. D'Souza WN, Hedrick SM. Cutting edge: latecomer CD8 T cells are imprinted with a unique differentiation program. J Immunol 2006;177:777–781. [PubMed: 16818730]

[101]. Obar JJ, Lefrancois L. Early signals during CD8 T cell priming regulate the generation of central memory cells. J Immunol 2010;185:263–272. [PubMed: 20519649]

[102]. Gray D, Matzinger P. T cell memory is short-lived in the absence of antigen. J Exp Med 1991;174:969–974. [PubMed: 1834764]

[103]. Jelley-Gibbs DM, Brown DM, Dibble JP, Haynes L, Eaton SM, Swain SL. Unexpected prolonged presentation of influenza antigens promotes CD4 T cell memory generation. J Exp Med 2005;202:697–706. [PubMed: 16147980]

[104]. Murali-Krishna K, Lau LL, Sambhara S, Lemonnier F, Altman J, Ahmed R. Persistence of memory CD8 T cells in MHC class I-deficient mice. Science 1999;286:1377–1381. [PubMed: 10558996]

[105]. Zammit DJ, Turner DL, Klonowski KD, Lefrancois L, Cauley LS. Residual antigen presentation after influenza virus infection affects CD8 T cell activation and migration. Immunity 2006;24:439–449. [PubMed: 16618602]

[106]. Cockburn IA, Chen YC, Overstreet MG, Lees JR, van Rooijen N, Farber DL, et al. Prolonged antigen presentation is required for optimal CD8+ T cell responses against malaria liver stage parasites. PLoS pathogens 2010;6:e1000877. [PubMed: 20463809]

[107]. Hafalla JC, Sano G, Carvalho LH, Morrot A, Zavala F. Short-term antigen presentation and single clonal burst limit the magnitude of the CD8(+) T cell responses to malaria liver stages. Proc Natl Acad Sci U S A 2002;99:11819–11824. [PubMed: 12185251]

[108]. Lau LL, Jamieson BD, Somasundaram T, Ahmed R. Cytotoxic T-cell memory without antigen. Nature 1994;369:648–652. [PubMed: 7516038]

[109]. Martin DL, Tarleton RL. Antigen-specific T cells maintain an effector memory phenotype during persistent Trypanosoma cruzi infection. J Immunol 2005;174:1594–1601. [PubMed: 15661921]

[110]. Zinkernagel RM, Bachmann MF, Kundig TM, Oehen S, Pirchet H, Hengartner H. On immunological memory. Annu Rev Immunol 1996;14:333–367. [PubMed: 8717518]

[111]. Gagnon E, Duclos S, Rondeau C, Chevet E, Cameron PH, Steele-Mortimer O, et al. Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. Cell 2002;110:119–131. [PubMed: 12151002]

[112]. Goldszmid RS, Coppens I, Lev A, Caspar P, Mellman I, Sher A. Host ER-parasitophorous vacuole interaction provides a route of entry for antigen cross-presentation in Toxoplasma gondii-infected dendritic cells. J Exp Med 2009;206:399–410. [PubMed: 19153244]

[113]. Ku CC, Murakami M, Sakamoto A, Kappler J, Marrack P. Control of homeostasis of CD8+ memory T cells by opposing cytokines. Science 2000;288:675–678. [PubMed: 10784451]

[114]. Li XC, Demirci G, Ferrari-Lacraz S, Groves C, Coyle A, Malek TR, et al. IL-15 and IL-2: a matter of life and death for T cells in vivo. Nat Med 2001;7:114–118. [PubMed: 11135625]

[115]. Sprent J, Surh CD. T cell memory. Annu Rev Immunol 2002;20:551–579. [PubMed: 11861612]

[116]. Waldmann TA, Dubois S, Tagaya Y. Contrasting roles of IL-2 and IL-15 in the life and death of lymphocytes: implications for immunotherapy. Immunity 2001;14:105–110. [PubMed: 11239443]

[117]. Schmidt NW, Butler NS, Harty JT. Plasmodium-host interactions directly influence the threshold of memory CD8 T cells required for protective immunity. J Immunol 2011;186:5873–5884. [PubMed: 21460205]

[118]. Mattei F, Schiavoni G, Belardelli F, Tough DF. IL-15 is expressed by dendritic cells in response to type I IFN, double-stranded RNA, or lipopolysaccharide and promotes dendritic cell activation. J Immunol 2001;167:1179–1187. [PubMed: 11466332]

[119]. McGill J, Van Rooijen N, Legge KL. IL-15 trans-presentation by pulmonary dendritic cells promotes effector CD8 T cell survival during influenza virus infection. J Exp Med 2010;207:521–534. [PubMed: 20212069]

[120]. Dubois S, Mariner J, Waldmann TA, Tagaya Y. IL-15Ralpha recycles and presents IL-15 In trans to neighboring cells. Immunity 2002;17:537–547. [PubMed: 12433361]

[121]. Krzych U, Dalai S, Zarling S, Pichugin A. Memory CD8 T cells specific for plasmodia liver-stage antigens maintain protracted protection against malaria. Front Immunol 2012;3:370. [PubMed: 23233854]

[122]. Kokaji AI, Hockley DL, Kane KP. IL-15 transpresentation augments CD8+ T cell activation and is required for optimal recall responses by central memory CD8+ T cells. J Immunol 2008;180:4391–4401. [PubMed: 18354159]

1. The mammalian liver serves as the venue for Plasmodium parasite development
2. Sterile and lasting protection is inducible by exposure to attenuated Plasmodium sporozoites
3. Liver CD8 T cells are the key effectors in protective immunity against pre-erythrocytic stage parasitemia
4. Memory CD4 T cells and CD8 T cells maintain protracted protection against malaria
5. Antigen depot and cytokines, e.g., IL-15, are crucial for the for maintenance of protracted protection against pre-erythrocytic stage malaria