Priming of CD8⁺ T cell responses to liver stage malaria parasite antigens

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INTRODUCTION

It is currently accepted that priming of CD8⁺ T lymphocytes by antigen-presenting cells (APCs) takes place in the secondary lymphoid organs such as spleen and lymph nodes [reviewed in Ref. (1)]. Multi-photon-based intravital microscopy revealed that the first contact between naïve CD8⁺ T cells and APC takes place in the periphery of draining lymph nodes (DLN) shortly after infection and mainly occurs in the subcapsular sinus or the interfollicular regions enriched with pathogen-derived antigens (2, 3). Depending on the pathogen’s nature, the rapid relocation of naïve T cells to the periphery of the draining lymph node can be either antigen-specific (2) or antigen-independent, associated with decreased local levels of chemokines and the drastic alteration of the lymph node architecture by the pathogen (3, 4). Data from mice infected with vesicular stomatitis virus demonstrated that, though CD169⁺ macrophages (5) residing in the subcapsular sinus were the major cell population bearing virus-derived antigens (2), dendritic cells (DCs) served as the primary APC triggering antigen-specific naïve CD8⁺ T cells. The ability of immature dendritic cells to acquire exogenous antigens followed by their proteolytic processing and presentation on the MHC class I molecules, commonly referred to as “cross-presentation,” is believed to be the major requirement for the generation of primary antigen-specific CD8⁺ T cell responses against pathogens (6–9).

Upon the initial encounter of naïve T cells with APC, a heterogeneous progeny of antigen-specific CD8⁺ T cells including short-lived effector cells (SLEC) and memory precursor effector cells (MPEC) [reviewed in Ref. (10, 11)] is generated. It is still not clear whether the SLEC versus MPEC differentiation is enforced by the asymmetric segregation of transcription factors and protein degradation machinery already at the first cell division (12–14) or it reflects the differential exposure to inflammatory and co-stimulatory “help” signals received from APCs by antigen-specific CD8⁺ T cells during the expansion phase [reviewed in Ref. (10, 11)]. While generation of primary CD8⁺ T cell responses to non-inflammatory antigens requires CD4⁺ T cell help, induction of primary CD8⁺ T cells responses to Listeria, LCMV, and influenza virus is CD4⁺ T cell-independent and results from direct activation of APCs by the pathogen (15–17). Moreover, CD4⁺ T cell help can be replaced by the CD40 triggering on the DCs, which prime antigen-specific naive CD8⁺ T cells (18, 19). Thus, the exact nature and requirements for “help” signals necessary for the initial triggering and subsequent expansion of primary antigen-specific CD8⁺ T cell responses vary among different pathogens and sites of primary infection. In this report, our objective is to present and discuss the published data regarding CD8⁺ T cell activation in Plasmodium infection, and suggest experiments to better understand the antigen presentation process.

Malaria infection is initiated through the bites by Plasmodium-carrying female Anopheles searching for blood to support egg development. As the mosquito probes the host environment under the skin for the presence of blood vessels, it injects salivary gland proteins both prior and during blood feeding to inhibit blood coagulation. Parasites deposited into the skin can also traverse surrounding cells and enter the circulation with subsequent infection of liver cells. Studies performed with parasites injected intradermally or intravenously show that the resulting liver parasite load is similar (20). In addition, transfer of parasites from the skin sites to DLN occurs (21).

Identification of the anatomical site and the type of APC, which orchestrate the induction of primary CD8⁺ T cell responses against a particular antigen, represents an essential step in rational design of CD8⁺ T cell-based vaccination strategies. Whereas the research on the effector phase of CD8⁺ T cell response against malaria has been quite extensive (22–25), a rather limited number
of studies attempted to dissect the issue of liver stage-specific CD8+ T cell priming in the infected host.

ROLE OF DIFFERENT ORGANS IN ANTIGEN PRESENTATION

In this respect, the study by Chakravarty et al. (21) appears to be one of the most comprehensive and systematic up to date. The authors concluded that intrahepatic lymphoid tissues, in particular the DLN and spleen are the most important sites contributing to the generation of the effector T-cell pool in the liver. In agreement with these data, Obeid and colleagues demonstrated that strictly subcutaneous immunization with irradiated sporozoites led to induction of sterile immunity against pre-erythrocytic malaria with T cell priming occurring in skin-draining lymph node (26). It was proposed that parasite-specific CD8+ T cell priming depends on cross-presentation of malaria antigens (21). This indicates that professional APC, rather than infected hepatocytes, trigger priming of naïve CD8+ T cells directed to liver stage antigens.

Several lines of experimental evidence were presented in support of these conclusions. Thus, IFNγ production by adoptively transferred circumsporozoite protein (CSP)-specific naïve transgenic T cells was first detected in the skin-DLN as early as on day 2 after mouse immunization by microinjection or mosquito bites, whereas no detectable T cell activation was detected in other organs including spleen. Hence, Chakravarty and co-authors suggested that these temporal differences in the onset of parasite-specific T cell activation could reflect the hierarchical order of T cell priming initiated in the DLN that could be followed by migration of primed CD8+ T cells to other organs, including the spleen and the liver. However, removal of lymph nodes draining the site of parasite injection prior to the adoptive transfer of parasite-specific CD8+ T cells, though resulted in a 60% reduction of activated CD8+ T cells in the liver, did not affect the frequencies of primed CD8+ T cells in the spleen where the first signs of T cells activation were documented only 24 h later than in DLN and at the same time point as in the liver. These data indicate that temporal differences in the onset of T cell activation used as a parameter for same time point as in the liver. These data indicate that temporal differences in the onset of parasite-specific T cell priming could reflect the hierarchical order of T cell priming occurring in skin-draining lymph node (26). It was proposed that parasite-specific CD8+ T cell priming depends on cross-presentation of malaria antigens (21). This indicates that professional APC, rather than infected hepatocytes, trigger priming of naïve CD8+ T cells directed to liver stage antigens.

At the same time, several lines of evidence presented by Chakravarty and colleagues (21) do not firmly support the essential role of the spleen in the parasite-specific CD8+ T cell priming.

First, DCs isolated from the spleens 60 h after injection of sporozoites were unable to trigger proliferation of parasite-specific CD8+ T cells, whereas DCs isolated from the DLN efficiently induced T cell proliferation and, presumably, presented the antigen. Since no data with liver-resident DCs were generated, a direct role of intrahepatic professional APC in priming of parasite-specific CD8+ T cells still needs to be addressed. In addition, as the first signs of activation of parasite-specific T cells in DLN were detected at day 2 post immunization, it is not completely clear whether DCs from DLN had a greater capacity to prime CD8+ T cells as compared to spleen and liver-resident DCs at time points earlier than 60 h.

Second, animals subjected to simultaneous lymphadenectomy and splenectomy prior to the adoptive transfer of CSP-specific CD8+ T cells followed by immunization with sporozoites and subsequent challenge with viable parasites 10 days later had similar load of parasites in the liver as non-immunized mice, indicating that either DLN or/splenic are required for CSP-specific CD8+ T cell priming. At the same time, splenectomy alone did not affect inhibition of parasite development in the liver, prompting the authors to conclude, that DLNs are the priming site of protective CD8+ T cell responses.

Interestingly, as shown by Chakravarty and co-authors (21), removal of both DLNs and the spleen prior to immunization with sporozoites, though drastically reduced the activated T cell pool in the liver, failed to abrogate it completely, suggesting that at least a proportion of parasite-specific CD8+ T cells found in the liver had been primed outside the DLN and the spleen. These findings could reflect the process of parasite-specific CD8+ T cell triggering in the liver and define it as the organ essential for the parasite development. On the other hand, animals treated with FTY720, a drug, which inhibits lymphocyte egress from lymph nodes (28, 29), had substantially less IFN gamma producing parasite-specific CD8+ T cells at day 7 post injection with irradiated sporozoites. Based on this observation, the authors concluded that systemic distribution of CD8+ T cells, at least in part, contributes to the intrahepatic pool of parasite-specific CD8+ T cells (21). It still needs to be seen, if treatment with FTY720 (30) inhibits the development of “early-primed” parasite-specific CD8+ T cells in the liver and spleen, previously noted by the authors already 72 h after mosquito bite. In addition, effect of FTY720 on the protection of animals from subsequent challenge with live sporozoites has to be addressed in this model. Noteworthy, the time course of the parasite-specific clonal T cell activation in the lymph nodes, liver, and other organs is only slightly delayed (by 24 h) while it is known that activated T cells egress from the lymph nodes 4–5 days after antigen encounter (31, 32). The latter suggests that either activation of parasite-specific T cells may take place simultaneously in various organs, or unusually rapid egress from the lymph node after priming is an intrinsic feature of T cells in this specific experimental model.

ROLE OF INFECTED HEPATOCYTES IN ANTIGEN PRESENTATION

The role of infected hepatocytes in direct priming of naïve parasite-specific CD8+ T cells is still a subject of controversy. Early study by Renia et al. demonstrated that intrasplenic injection of infected hepatocytes induced protective T cell-mediated immunity against infection with Plasmodium yoelii and P. berghei sporozoites (33). Leiriao et al. demonstrated that apoptotic hepatocytes infected with irradiated sporozoites are phagocytosed by DCs and merely serve as a source of Plasmodium antigens for the initiation of the protective immune responses via cross-priming (34). In contrast, Renia and collaborators argued against apoptotic infected hepatocytes as a source of antigens and suggested that liver DCs could be activated upon uptake of parasite antigens directly from...
viable infected hepatocytes (35) as previously seen in other experimental models (36, 37). However, data from Chakravarty et al. implied that though cross-priming is required, it takes place in the DLNs and not in the liver (21). In agreement with these data, Jung et al. demonstrated that mice subjected to chemical depletion of CD11c+ DCs fail to induce CD8+ T cell responses to infection with *Plasmodium yoelii* (38). Neither of these studies considered hepatocytes as an APC subset capable of initiating the primary parasite-specific T cell responses.

A recent study by Balam et al. (39) focused on two questions: can infected hepatocytes directly prime naïve parasite-specific T cells and does stimulation of already primed CD8+ T cells protect mice against parasite challenge? Administration of CD8+ CSP-specific T cells but not an irrelevant T cell clone injected into TAP-deficient MHC class I mismatched recipient mice, simultaneously with infected hepatocytes bearing MHC haplotype relevant for parasite-specific T cells, resulted in 100% protection of mice from subsequent challenge with live sporozoites (39). As the observed protection was not due to a bystander effect or a continuous cytokine secretion by parasite-specific CD8+ T cells, these data demonstrate that infected hepatocytes are capable of presenting the antigen to CD8+ T cells, reactivating resting CSP-specific CD8+ T cells and inducing protection.

Importantly, more than 60% of naïve BALB/c mice injected with irradiated sporozoite-infected hepatocytes were also protected from subsequent live parasite challenge, suggesting that infected hepatocytes could contribute to the priming of endogenous naïve T cell. However, T cell depletion experiments are required to confirm that protection is T cell-mediated. Finally, to formally exclude contamination with other APC potentially present in the hepatocyte preparations and capable of presenting CSP and priming the naïve CD8+ T cells, isolation of pure hepatocyte population devoid of cells bearing markers of DCs, macrophages, and stellate cells should be done by flow cytometry using fluorescent transgenic parasites. On the other hand, arguing against the sole role of professional APC in priming of naïve immune responses to malaria parasites, mice depleted of DCs by treatment with cytochrome c were still protected from the challenge with live sporozoites in spite of significantly lower frequencies of endogenous parasite-specific T cells primed by the immunization with irradiated sporozoites (39). These data do not fully support the previously discussed role of dendritic cell function in induction of primary malaria liver stage-specific T cell responses (21, 38).

**OTHER CONSIDERATIONS**

The quality of hepatocytes as APCs capable of triggering T cells responses had been recently dissected by Ma et al. (40). It had been demonstrated that *P. berghei* and *P. falciparum* infected human hepatocytes retain largely unaltered expression of multiple molecules of the MHC class I pathway until very late stages of parasite development (40). Moreover, infected cells exhibited no obvious defects in the capacity to upregulate expression of different molecular components of the MHC class I machinery in response to pro-inflammatory lymphokines or trigger direct activation of allo-specific as well as peptide-specific human CD8+ T cells (40). At the same time, it is not known whether or not the characteristic features of professional APC believed to be important for efficient T-cell priming, i.e., co-stimulatory molecules B7.1 and B7.2 ("signal 2"), as well as production of cytokines essential for the survival and maintenance of primed T cells ("signal 3") are possessed by the primary human hepatocytes in vivo and/or induced upon infection.

Current literature dissecting the ability of primary hepatocytes to specifically prime naïve CD8+ T cells is scarce. Bertolino et al. demonstrated that purified primary murine hepatocytes were able to induce activation and proliferation of antigen-specific naïve CD8+ T cells in vitro, even in the absence of exogenously added cytokines as well as CD80 and CD86 co-stimulatory molecules (41). Moreover, the magnitude of T cell proliferation induced by primary hepatocytes was comparable to that induced by DCs. Naïve T cell priming by hepatocytes did not require CD4+ T cell help and induced expression of early T cell activation markers and transient CD8+ T cell effector activity followed by rapid cell death of activated T cells. Thus, primary hepatocytes were able to prime naïve T cells but failed to sustain productive antigen-specific CD8+ T cell responses (41). In agreement with these data, in vivo experiments using endogenous expression of alloantigens under hepatocyte-specific promoters demonstrated that activation of primary T cells by hepatocytes as antigen-presenting cells leads to T cell apoptosis rather than formation of antigen-specific memory T cell pool (42–44). It was further demonstrated that T cells activated by hepatocytes died "by neglect" and lack of IL-2 and low expression of pro-survival genes due to insufficient co-stimulation during the priming phase (45). Hence, taking into account the inability of primary hepatocytes to provide appropriate co-stimulation during T cells priming along with the immunosuppressive microenvironment created by multiple subsets of the liver-resident APC [reviewed in Ref. (46, 47)], it may appear unlikely that hepatocytes infected with malaria parasites play a major role in the generation of effective parasite-specific CD8+ T cell memory responses. However, it does not preclude the possibility that CD8+ T cells specific to malaria antigens could be primed and activated, at least shortly, by hepatocytes supporting development of exo-erythrocytic forms. Indeed, given proper stimuli, such T cells can be rescued to full immunological competence and longer survival (48, 49). In the case of malaria, proper activation stimuli could be induced by *Plasmodium* infection leading to activation of numerous genes in hepatocytes (50, 51) including those involved in native immunity and antigen presentation. Since no transcriptional analysis has been performed in Kupffer cells traversed by sporozoites so far, it would be important to understand whether or not liver-resident macrophages change their immunomodulatory properties in the site of malaria infection.

At this point, a word of caution should be expressed to the fact that all animal studies discussed above were based on a single mouse strain, BALB/c, as well as a single CD8+ T cell epitope derived from the CSP. Future studies on the induction of primary T cell responses to exo-erythrocytic forms of malaria need to be extended to other protective CD8+ T cell epitopes including responses, which appear later in the liver stage by using either radiation attenuated (RAS) or genetically attenuated (GAS) sporozoites or sporozoites combined with chloroquine chemoprophylaxis (CPS) (52).
FIELD STUDIES
It is still unclear to what extent animal models dissecting induction of primary T cell responses to malaria as well as human studies involving vaccinated volunteers reflect the acquisition of natural cellular immune responses in malaria endemic areas. An acquisition of a sterile immune protection following immunization with RAS, GAS, or using CPS regime in animals and humans sharply contrasts with the situation in the field, where, in spite of frequent (up to 30 per month in certain areas) biting by infected mosquitoes (53, 54), no sterile protection is usually obtained in both adults and children in response to natural infection under drug treatment or intermittent preventive treatment (IPT).
Several hypotheses could be proposed to explain this discrepancy: (1) sporozoite “charge” (and, as a result, supply of parasite antigens) is too small in the field as compared to that given under experimental conditions; (2) down-regulation of the parasite-specific CD8+ T cell responses by the content of mosquito salivary glands delivered together with sporozoites to the site of T cell priming; and (3) excessive and/or preceding induction of immune responses to salivary gland proteins. As for the latter, given the fact that only a fraction (0–25% depending on the seasons and location) of mosquitoes are infected (54–56), a memory T-cell pool specific for salivary gland antigens is most likely established prior to parasite infection. As a result, secondary T cell responses directed to mosquito antigens could be preferentially activated at the expense of parasite-specific T-cell activation via, for example, competition for IL-2, homeostatic niche, or by active secretion of inhibitory molecules (57–64). If this is the case, the efficacy of sporozoite-based pre-erythrocytic vaccines may turn out to be low in endemic regions, as well as the general failure [with the exception for a single donor so far (65)] to obtain stable human T-cell clones specific to malaria liver stage antigens.

FINAL REMARKS
In conclusion, existing experimental data obtained from animal models suggest that: (1) both DCs and hepatocytes can prime naïve malaria parasite-specific CD8+ T cells, at least those directed to epitopes derived from CSP and (2) either DCs or hepatocytes are sufficient to induce protective CSP-specific T cell responses if the parasite load is not excessive. Identification of the essential site for priming of malaria liver stage-directed CD8+ T cell found in humans from malaria endemic regions, as well as the general failure [with the exception for a single donor so far (65)] to obtain stable human T-cell clones specific to malaria liver stage antigens.

REFERENCES
1. Zhang N, Bevan MJ. CD8+ T cells: foot soldiers of the immune system. Immunity (2011) 35:161–8. doi:10.1016/j.immuni.2011.07.010
2. Hickman HD, Takeda K, Skon CN, Murray FR, Hensley SE, Loomis J, et al. Direct priming of antiviral CD8+ T cells in the peripheral interfollicular region of lymph nodes. Nat Immunol (2008) 9:155–65. doi:10.1038/ni.1557
3. John B, Harris TH, Tait ED, Wilson EH, Gregg B, Ng LG, et al. Dynamic Imaging of CD8+ T cells and dendritic cells during infection with Toxoplasma gondii. PLoS Pathog (2009) 5:e1000505. doi:10.1371/journal.ppat.1000505
4. Mueller SN, Hosiawa-Meagher KA, Konieczyk BT, Sullivan BM, Bachmann MF, Locksley RM, et al. Regulation of homeostatic chemokine expression and cell trafficking during immune responses. Science (2007) 317:670–4. doi:10.1126/science.1144830
5. Kuka M, Iannacone M. The role of lymph node sinus macrophages in host defense. Ann N Y Acad Sci (2014) 1319:38–46. doi:10.1111/nyas.12387
6. Wei J, Waitthin J, Xiao K, Oveisii S, Chen W. Optimal conditions required for influenza A infection-enhanced cross-priming of CD8+ T cells: specific to cell-associated antigens. Immunol Cell Biol (2013) 91:576–82. doi:10.1038/icb.2013.46
7. Helft J, Manicasamy B, Guermounpaz B, Hashimoto D, Silvin A, Agudo J, et al. Cross-presenting CD103+ dendritic cells are protected from influenza virus infection. J Clin Invest (2012) 122:4037–47. doi:10.1172/JCI60695
8. Reinicke AT, Omland KS, Basha G, Jeffries WA. Dendritic cell cross-priming is essential for protective responses to Listeria monocytogenes. PLoS One (2009) 4:e2710. doi:10.1371/journal.pone.0002710
9. Munz C. Dendritic cells during Epstein Barr virus infection. Front Microbiol (2014) 5:308. doi:10.3389/fmicb.2014.00308
10. Parish IA, Kaech SM. Diversity in CD8+ T cell differentiation. Curr Opin Immunol (2009) 21:291–7. doi:10.1016/j.coi.2009.05.008
11. Mescher MF, Curtisinger JM, Agarwal P, Casey KA, Germer M, Hammerbeck CD, et al. Signals required for programming effector and memory development by CD8+ T cells. Immunol Rev (2006) 211:81–92. doi:10.1111/j.0038-0260.2006.00382.x
12. Joshi NS, Cui W, Chandele A, Lee HK, Urso DR, Hagman J, et al. Inflammation directs memory precursor and short-lived effector CD8+ T cell fates via the graded expression of T-bet transcription factor. Immunity (2007) 27:281–95. doi:10.1016/j.immuni.2007.07.010
13. Smith-Garvin JE, Burns JC, Gohil M, Zou T, Kim JS, Maltzman JS, et al. T-cell receptor signals direct the composition and function of the memory CD8+ T-cell pool. Blood (2010) 116:3548–59. doi:10.1182/blood-2010-06-292748
14. Chang JT, Ciocoa MC, Kinjyo I, Palamivel VR, McClurkin CE, Dejong CS, et al. Asymmetric proteasome segregation as a mechanism for unequal partitioning of the transcription factor T-bet during T lymphocyte division. Immunity (2011) 34:492–504. doi:10.1016/j.immuni.2011.03.017
15. Buller RM, Holmes RL, Hugan A, Frederickson TN, Morse HC III. Induction of cytotoxic T-cell responses in vivo in the absence of CD4 helper cells. Nature (1991) 353:180–4. doi:10.1038/353180a0
16. Rahemtulla A, Fung-Leung WP, Silhalm MW, Kundig TM, Sambhara SR, Narendran A, et al. Normal development and function of CD8+ cells but markedly decreased helper cell activity in mice lacking CD4. J Immunol (2002) 169:4401–4407. doi:10.4049/jimmunol.169.7.4401
17. Wu Y, Liu Y. Viral induction of co-stimulatory activity on antigen-presenting cells bypasses the need for CD4+ T-cell help in CD8+ T-cell responses. Curr Biol (1994) 4:499–505. doi:10.1016/S0960-9822(00)00110-X
18. Ridge JP, Di Rosa F, Matzingr P. A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. Nature (1998) 393:474–8. doi:10.1038/30989
19. Bennett SR, Carbone FR, Karamalis F, Flavell RA, Miller JE, Heath WR. Help for cytotoxic T-cell responses is mediated by CD40 signalling. Nature (1998) 393:478–80. doi:10.1038/30996
20. Yamauchi LM, Coppi A, Snounou G, Sinnis P. Plasmodium sporozoites trickle out of the injection site. Cell Microbiol (2007) 9:1215–22. doi:10.1111/j.1462-5822.2006.00881.x
21. Chakraverty S, Cookburn IA, Kuk S, Overstreet MG, Sacci IB, Zavala F. CD8+ T lymphocytes protective against malaria liver stages are primed in skin-draining lymph nodes. Nat Med (2007) 13:1035–41. doi:10.1038/nm1628
22. 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 (2008) 217:123–34. doi:10.1111/j.1600-0528.2008.00733.x
23. Overstreet MG, Chen YC, Cockburn IA, Tse SW, Zavala F. CD4+ T cells modulate expansion and survival but not functional properties of effector and memory CD8+ T cells induced by malaria sporozoites. PLoS One (2011) 6:e15948. doi:10.1371/journal.pone.0015948

24. Tse SW, Cockburn IA, Zhang H, Scott AL, Zavala F. Unique transcriptional profile of liver-resident memory CD8+ T cells induced by malaria sporozoites. *Genes Immun* (2013) 14:302–9. doi:10.1038/genes.2013.20

25. Bengen SE, Torger R, Romero JF, Renia L, Corradin G. Plasmodium berghei-infected primary hepatocytes process and present the circumsporozoite protein to specific CD8+ T cells in vitro. *J Immunol* (2007) 178:7054–63. doi:10.4049/jimmunol.178.11.7054

26. Obeid M, Franetich JF, Lorthiois A, Gego A, Grüner AC, Test M, et al. Skin-draining lymph node priming is sufficient to induce sterile immunity against pre-erythrocytic malaria. *EMBO Mol Med* (2013) 5:250–63. doi:10.1002/emmm.201206177

27. Sano G, Hafalla JC, Morrot A, Abe R, Lafaille JI, Zavala F. Swift development of protective effector functions in naive CD8+ T cells against malaria liver stages. *J Exp Med* (2001) 194:173–80. doi:10.1084/jem.194.2.173

28. Brinkmann V, Cyster JG, Hi A T. FTY720: sphingosine 1-phosphate receptor-1 in the control of lymphocyte egress and endothelial barrier function. *Am J Transplant* (2004) 4:2019–25. doi:10.1111/j.1600-6143.2004.00476.x

29. Pinscher UD, Ochsenbein AF, Odermatt B, Brinkmann V, Hengartner H, Zinkernagel RM. FTY720 immunosuppression impairs effecter T cell peripheral homing without affecting induction, expansion, and memory. *J Immunol* (2000) 164:5761–70. doi:10.4049/jimmunol.164.11.5761

30. Masberg S, von Andrian UH. Fingolimod and sphingosine-1-phosphate – modulators of lymphocyte migration. *N Engl J Med* (2006) 355:1088–91. doi:10.1056/NEJMop068159

31. Mempel TR, Henrickson SE, Von Andrian UH. T-cell priming by dendritic cells in lymph node oases occurs in three distinct phases. *Nature* (2004) 427:154–9. doi:10.1038/nature02238

32. Henrickson SE, von Andrian UH. Single-cell dynamics of T-cell priming. *Curr Opin Immunol* (2007) 19:249–58. doi:10.1016/j.coi.2007.04.013

33. Renia L, Rodrigues MM, Nussenzweig V. Intravascular immunization with infected hepatocytes: a mouse model for studying protective immunity against malaria pre-erythrocytic stage. *Immunology* (1994) 82:164–8.

34. Leiriao P, Mota MM, Rodrigues A. Apoptotic Plasmodium-infected hepatocytes provide antigen to liver dendritic cells. *J Infect Dis* (2005) 191:1576–81. doi:10.1086/426835

35. Renia L, Maranon C, Hosmalin A, Grunen AC, Silvie O, Soumou G. Do apoptotic Plasmodium-infected hepatocytes initiate protective immune responses? *J Infect Dis* (2006) 193:163–4. doi:10.1086/498536

36. Marachón C, Desoutert JF, Hoefl G, Cohen W, Hanau D, Hosmalin A. Dendritic cells cross-reactive HIV antigens from live as well as apoptotic infected CD8+ T lymphocytes. *Proc Natl Acad Sci U S A* (2004) 101:6092–7. doi:10.1073/pnas.030487101

37. Harshyne LA, Watkins SC, Gambotto A, Barratt-Boyes SM. Dendritic cells acquire antigens from live cells for cross-presentation to CTL. *Science* (2004) 303:2509–36.

38. Collins FH, Zavala F, Graves PM, Cochrane AH, Gowed BW, Akoh J, et al. First field trial of an immunoradiometric assay for the detection of malaria sporozoites in mosquitoes. *Am J Trop Med Hyg* (1994) 50:529–36.

39. Balam S, Romero JF, Bongfen SE, Guillaume P, Corradin G. CSP – a model for acquisition of naive CD8+ T cells from PD-1-mediated exhaustion. *PLoS Pathog* (2009) 5:e1000349. doi:10.1371/journal.ppat.1000349

40. Gehring AI, Haniffa M, Kennedy PT, Ho ZZ, Boni C, Shin A, et al. Mobilizing monocytes to cross-present circulating viral antigen in chronic infection. *J Clin Invest* (2013) 123:3766–76. doi:10.1172/JCI66043

41. Albuquerque SS, Carret C, Grosso AR, Tarun AS, Peng X, Kappe SH, et al. Host cell transcriptional profiling during malaria liver stage infection reveals a coordinated and sequential set of biological events. *BMCGenomics* (2009) 10:270. doi:10.1186/1471-2164-10-270

42. Chattopadhyay R, de la VegaP, Paik SH, Chakraborty S, Vainio T, et al. Early transcriptional responses of HepG2-A16 liver cells to infection by *Plasmodium falciparum* sporozoites. *J Biol Chem* (2011) 286:26396–405. doi:10.1074/jbc.M111.240879

43. Nganou-Makamado K, Sauerwein RW. Liver or blood-stage arrest during malaria sporozoite immunization: the later the better? *Trends Parasitol* (2013) 29:304–10. doi:10.1016/j.pt.2013.03.008

44. Ouédraogo AL, de Vlas SJ, Nébié I, Ilboudo-Sanogo E, Bousema JT, Ouattara T, et al. Seasonal patterns of *Plasmodium falciparum* malaria pre-erythrocytic stage. *Parasite Immunol* (2014) 36:371–23. doi:10.1111/j.1365-2915.2014.00935.x

45. Cross ML, Cupp EW, Enriquez FI. Differential modulation of murine cellular immune responses by salivary gland extract of *Aedes aegypti*. *Am J Trop Med Hyg* (1994) 51:969–90.

46. Zedlner NS, Higgs S, Happ CM, Beatty BJ, Miller BR. Mosquito feeding modulates TH1 and TH2 cytokines in flavivirus susceptible mice: an effect mimicked by injection of salivainius, but not demonstrated in flavivirus resistant mice. *Parasite Immunol* (1999) 21:35–44. doi:10.1046/j.1042-1130.1999.00199.x

47. Vanensan N, Nussenzweig RH, Champagne DE, Soong L, Higgs S. Differential modulation of murine host immune response by salivary gland extracts from the mosquitoes *Aedes aegypti* and *Culex quinquefasciatus*. *Med Vet Entomol* (2004) 18:191–9. doi:10.1111/j.1365-2915.2004.00498.x

48. Wasserman HA, Singh S, Champagne DE. Saliva of the yellow fever mosquito, *Aedes aegypti*, modulates murine lymphocyte function. *Parasite Immunol* (2004) 26:295–306. doi:10.1046/j.1042-1130.2004.00712.x

49. Depinay N, Hacini F, Beghdadi W, Peronet R, Mercheri S. Mast cell-dependent down-regulation of antigen-specific immune responses by mosquito bites. *J Immunol* (2006) 176:4114–6. doi:10.4049/jimmunol.176.7.4114

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64. Schneider BS, Soong L, Zeidner NS, Higgs S. Aedes aegypti salivary gland extracts modulate anti-viral and TH1/TH2 cytokine responses to sindbis virus infection. *Viral Immunol* (2004) 17:565–73. doi:10.1089/vim.2004.17.565

65. Bonelo A, Valmori D, Triponez F, Tiercy JM, Menthia G, Oberholser J, et al. Generation and characterization of malaria-specific human CD8(+) lymphocyte clones: effect of natural polymorphism on T cell recognition and endogenous cognate antigen presentation by liver cells. *Eur J Immunol* (2000) 30:3079–88. doi:10.1002/1521-4141(200011)30:11<3079::AID-IMMU3079>3.0.CO;2-7

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