should focus on the regulation of γ-carboxylation of osteocalcin, perhaps reawakening interest in vitamin K nutrition[13]. Warfarin treatment would be expected to reduce γ-carboxylation of osteocalcin, and it would be worthwhile to examine whether there are any metabolic effects associated with its use in cardiovascular disease.

The concept of the skeleton as a ductless gland, responding to environmental influences by producing a hormone that controls metabolism and energy requirements, is a revolutionary one. From the evolutionary point of view, it might not be so surprising. The mechanical support the skeleton provides to the musculature is energy dependent. The skeleton also senses environmental effects that require new bone formation or repair[14]—why should it not be a whole-body endocrine organ, assessing metabolic needs and responding accordingly?

COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.

1. Dennison, E.M. *et al. Diabetologia* **47**, 1963–1968 (2004).
2. de Liefde, I.I. *et al. Osteoporos. Int.* **16**, 1713–1720 (2005).
3. Ducy, P. *et al. Cell* **100**, 197–207 (2000).
4. Karsenty, G. *Cell Metab.* **4**, 341–348 (2006).
5. Lee, N.K. *et al. Cell* **130**, 456–469 (2007).
6. Morrison, D.F. & Mauro, L.J. *Gene* **257**, 195–208 (2000).
7. Hauschka, P.V. *et al. Physiol. Rev.* **69**, 990–1047 (1989).
8. Ducy, P. *et al. Nature* **382**, 448–452 (1996).
9. Schiller, K.R. & Mauro, L.J. *J. Cell. Biochem.* **96**, 262–277 (2005).
10. Miyawaki, K. *et al. Nat. Med.* **8**, 738–742 (2002).
11. Drucker, D.J. *J. Clin. Invest.* **117**, 24–32 (2007).
12. King, A. *et al. Diabetologia* **48**, 2074–2079 (2005).
13. Tsugawa, N. *et al. Am. J. Clin. Nutr.* **83**, 380–386 (2006).
14. Seeman, E. & Delmas, P.D. *N. Engl. J. Med.* **354**, 2250–2261 (2006).

Malaria's journey through the lymph node

Michael F Good & Denise L Doolan

T cells attack *Plasmodium*-infected hepatocytes when fighting malaria, and it was thought that T cells first encountered *Plasmodium* antigens in the liver. Instead, immediately after infection, small numbers of parasites drain to skin lymph nodes where they can prime T cells to mount a protective immune response (pages 1035–1041).

The causative agent of malaria, the *Plasmodium* sp. parasite, was shown to be transmitted by mosquitoes over 100 years ago. Since then, there have been many attempts to eradicate malaria by attacking either the mosquito or the parasite. These laudable efforts, which have involved the use of drugs, insecticides, mosquito nets and other methods of mosquito control, have saved millions of lives but have not eradicated the disease. Great hopes have been attached to a potential malaria vaccine, which could one day bring the ravages of malaria under control.

Despite intense research for decades, however, there is still no licensed malaria vaccine, and the mechanisms and antigenic targets of protective immunity to malaria remain poorly understood. Immunization with radiation-attenuated *Plasmodium* sp. sporozoites[1] remains the 'gold standard' for malaria vaccine development, as such a vaccine prevents both the development of the clinical symptoms of malaria and the transmission of the disease. In this issue of *Nature Medicine*, Chakravarty *et al.*[2] look at how and where sporozoite-induced immunity to malaria is initiated. Their findings elegantly implicate the draining lymph node as a key stop on the parasite's journey and suggest that antigens expressed during the early stages of infection can be crucial targets of protective immunity.

Plasmodium-infected mosquitoes inject sporozoites, which migrate to the liver, invade hepatocytes and develop into the blood forms that are responsible for the clinical symptoms of the disease. Parasites developing within the host hepatocyte are thought to be the targets of the sporozoite-induced immune responses mediated by effector $CD8^+$ T cells[3]. These T cells must recognize major histocompatibility complex (MHC)-associated parasite antigens that are presented on infected hepatocytes. One of the central mysteries of liver stage immunity, however, has been how and where the T cells are primed.

Two models of T-cell activation had been considered. In one model, hepatocytes could be the key antigen-presenting cells (APCs) in sporozoite-induced immunity; sporozoite-infected hepatocytes could present antigens to T cells directly. However, the hepatocyte is not considered a 'professional' APC. In an alternative model, dendritic cells (DCs), which are professional APCs, could be recruited to the liver, where they would phagocytose sporozoite-infected hepatocytes that have undergone apoptosis. The DCs could then 'cross-present' parasite antigens to T cells[4,5]. In both models, the T-cell population would subsequently expand and provide protection against future challenge by recognizing and destroying infected hepatocytes. But because the liver is a massive organ and very few sporozoites reach the liver after a mosquito bite, it is unclear how so little antigen could induce a protective response.

The relevance of those models has recently been questioned. In most experimental models of liver-stage immunity, large numbers (typically 50,000–100,000, ref. 1) of irradiated *Plasmodium* sporozoites are injected intravenously, whereas in nature mosquitoes inoculate small numbers of sporozoites (median <20 per mosquito[6]). After mosquito inoculation, sporozoites remain in the skin for up to 6 h[7]. Approximately one-third of these sporozoites leave the skin injection site and are drained to the regional lymph nodes[8]. There, they can be internalized by DCs[8], suggesting that these APCs could present sporozoite antigens to T cells. Nonetheless, it has been a reasonable assumption that sporozoites that enter the peripheral circulation from the inoculation site prime an immune response in a distant lymphatic organ or in the liver.

Chakravarty *et al.* used an immunization approach that mimicked natural infection to investigate how and where T-cell responses are primed for sporozoite-induced immunity[2] (**Fig. 1**). Using the *Plasmodium yoelii* rodent model of malaria, the authors transferred T cells specific for the immunodominant T-cell epitope of the sporozoite coat into

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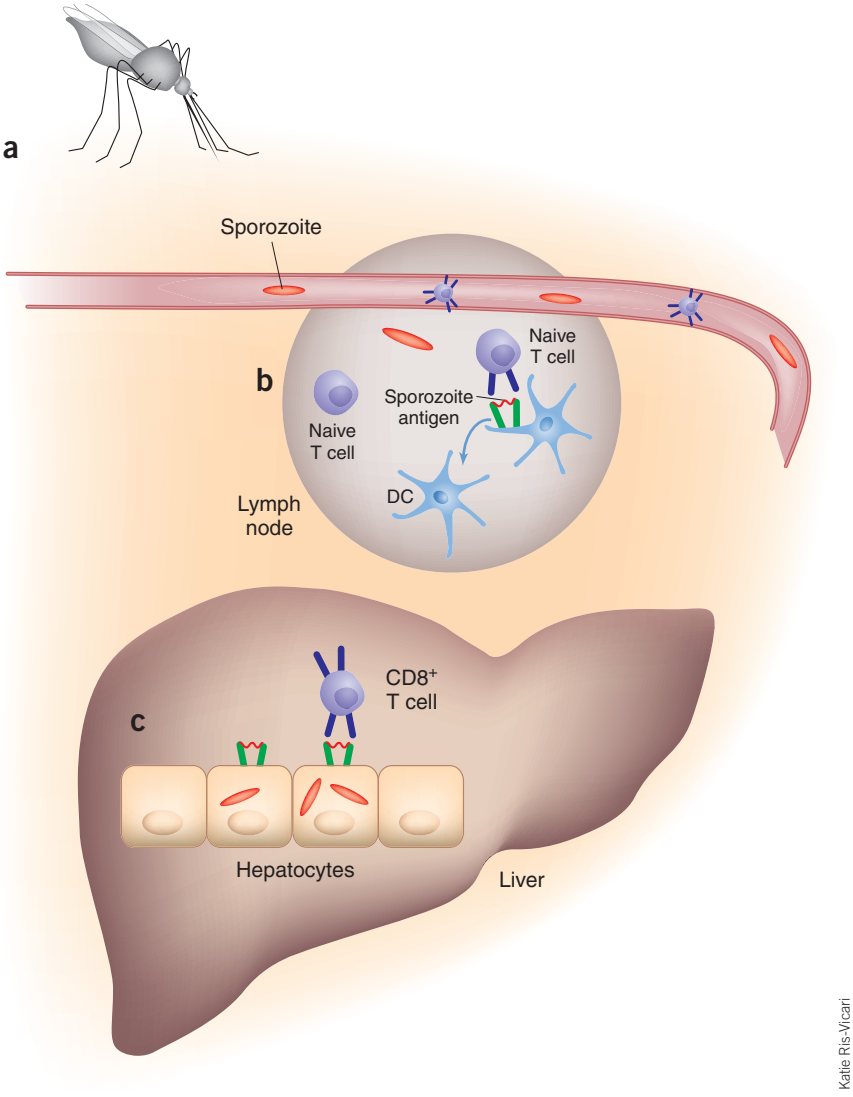

Katie Ris-Vicari

Figure 1 *Plasmodium* sporozoites are drained from the skin inoculation site to the lymph nodes to prime the T-cell response directed against the parasite-infected hepatocyte. (**a**) Mosquitoes inject small numbers of *Plasmodium* sporozoites into the skin. (**b**) Some of these parasites associate with DCs in the draining lymph nodes. These DCs can present *Plasmodium* sporozoite antigens to naive T cells, priming the T cells to recognize the parasites. These activated T cells enter the circulation and traffic to the liver. (**c**) Activated T cells can destroy the infected hepatocytes that display parasite antigen–MHC complexes on their surface, reducing liver-stage parasite load. Chakravarty *et al.* found that the draining lymph nodes are necessary and sufficient for the protective immune response to *Plasmodium* sporozoite infection.

malaria-naive mice. They then exposed the ear lobes of recipient mice to irradiated, sporozoite-infected mosquitoes. They found that the lymph nodes that drain from the ear lobe were necessary for T-cell priming against the parasite; if the draining lymph nodes were removed before sporozoite inoculation, the mice did not develop protective immunity. In contrast, other lymph nodes and the spleen were not necessary, suggesting that this primary response in the draining lymph node is sufficient to establish protective immunity. In bone marrow chimeras in which the APCs have a different MHC type than the hepatocyte target, Chakravarty *et al.* show, perhaps less surprisingly, that the primed and activated T cells recognize sporozoite antigens on parenchymal cells (presumably hepatocytes), and not on bone marrow–derived intermediaries such as DCs[2].

These data suggest that DCs cross-present sporozoite antigens to naive T cells in the draining lymph nodes. Sporozoite antigen presentation by DCs would prime T cells to recognize the parasite, and the activated T cells would then traffic to the liver to eliminate sporozoite-infected hepatocytes during subsequent infections. However, only antigens that are expressed by both the sporozoite and the liver-stage parasite, and not those that are expressed only in the liver stage, would be suitable targets. Additionally, Chakravarty *et al.* show that the immune response to the *Plasmodium* sp. parasite need not begin in the liver, as previously thought, but can be initiated in the draining lymph nodes[2]. This study does not exclude the possibility that T-cell priming can occur in sites other than the draining lymph nodes, but it does demonstrate that these nodes can effectively perform the task.

Strong immune responses were induced by the bites of 5–9 irradiated, *P. yoelii*-infected mosquitoes[2] (inoculating a median of <20 sporozoites each[6]) or by intradermal injection of 5,000 irradiated sporozoites[2]—contrasting with the much larger numbers required to elicit a similar response through intravenous injection[1]. These data support other observations that natural exposure to small numbers of parasites can result in anti-disease immunity[9] and that an ultra-low dose of infected red blood cells can induce potent T-cell–mediated immunity[10]. Although natural exposure to sporozoites does not induce complete immunity[11], the current study suggests that T-cell responses that reduce the parasite load in the liver could be induced in the field after a host has been bitten by an infected mosquito.

The real challenge will be to translate this new information on the site of immune induction, the route of inoculation and the relevance of parasite dose into new vaccine initiatives that use the intact parasite[12–15]. The demonstrated efficacy of immune induction with small amounts of whole parasites inoculated intradermally has clear implications for the logistics of developing a whole organismal vaccine.

COMPETING INTERESTS STATEMENT
The authors declare no competing financial interests.

1. Nussenzweig, R.S., Vanderberg, J., Most, H. & Orton, C. *Nature* **216**, 160–162 (1967).
2. Chakravarty, S. *et al. Nat. Med.* **13**,1033–1039 (2007).
3. Doolan, D.L. & Martinez-Alier, N. *Curr. Mol. Med.* **6**, 169–185 (2006).
4. Jung, S. *et al. Immunity* **17**, 211–220 (2002).
5. Leiriao, P., Mota, M.M. & Rodriguez, A. *J. Infect. Dis.* **191**, 1576–1581 (2005).
6. Medica, D.L. & Sinnis, P. *Infect. Immun.* **73**, 4363–4369 (2005).
7. Yamauchi, L.M., Coppi, A., Snounou, G. & Sinnis, P. *Cell. Microbiol.* **9**, 2093 (2007).
8. Amino, R. *et al. Nat. Med.* **12**, 220–224 (2006).
9. Baird, J.K. *Ann. Trop. Med. Parasitol.* **92**, 367–390 (1998).
10. Pombo, D.J. *et al. Lancet* **360**, 610–617 (2002).
11. Hoffman, S.L. *et al. Science* **237**, 639–642 (1987).
12. Luke, T.C. & Hoffman, S.L. *J. Exp. Biol.* **206**, 3803–3808 (2003).
13. Mueller, A.K. *et al. Proc. Natl. Acad. Sci. USA* **102**, 3022–3027 (2005).
14. van Dijk, M.R. *et al. Proc. Natl. Acad. Sci. USA* **102**, 12194–12199 (2005).
15. Mueller, A.K., Labaied, M., Kappe, S.H. & Matuschewski, K. *Nature* **433**, 164–167 (2005).