



Universiteit
Leiden
The Netherlands

Wiles and wanderings: immune-evasive maneuvers of skin-penetrating parasites

Winkel, B.M.F.

Citation

Winkel, B. M. F. (2021, October 5). *Wiles and wanderings: immune-evasive maneuvers of skin-penetrating parasites*. Retrieved from <https://hdl.handle.net/1887/3214576>

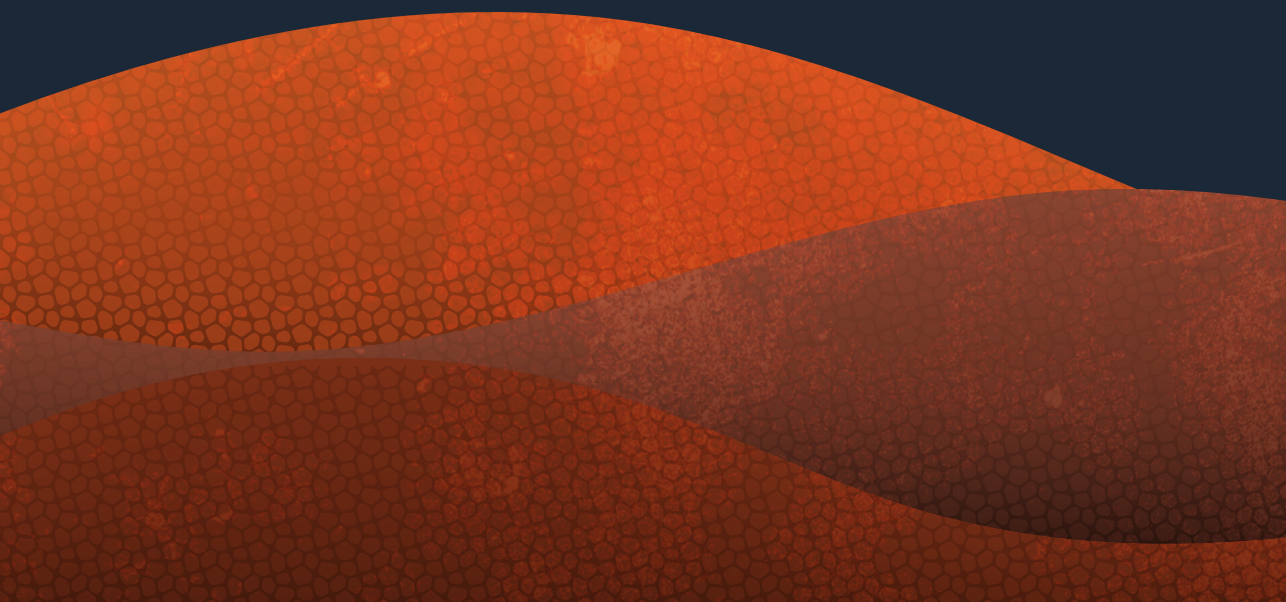
Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

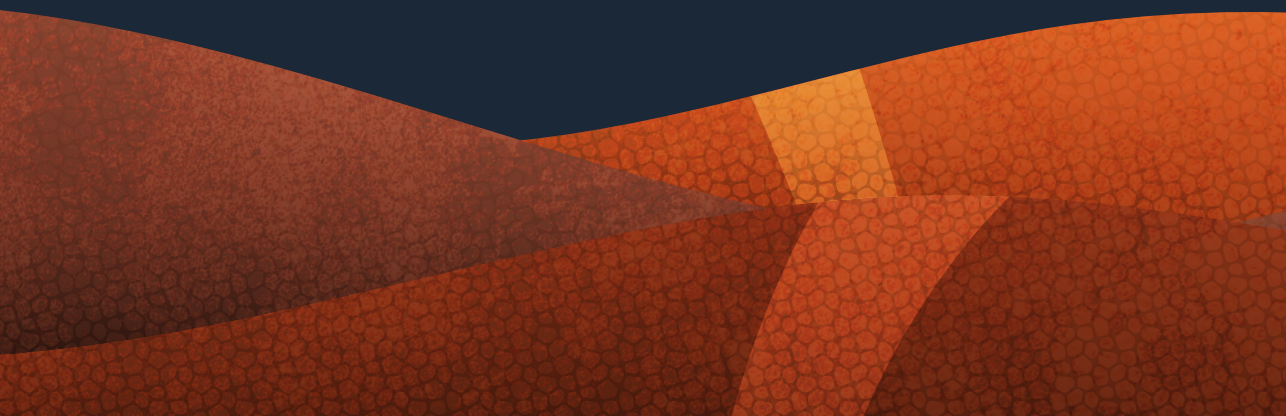
Downloaded from: <https://hdl.handle.net/1887/3214576>

Note: To cite this publication please use the final published version (if applicable).

1



General introduction and thesis outline



GENERAL INTRODUCTION

Skin-penetrating parasites such as those causing malaria and schistosomiasis infect hundreds of millions of people annually. Over half of the world's population is at risk for malaria, and in 2017 alone, an estimated 216 million people were infected, eventually leading to the death of 435 thousand individuals, 61% of whom were children under the age of five¹. Schistosomiasis is a less deadly disease yet causes substantial morbidity worldwide as patients suffer from chronic illness. Over 240 million people are infected worldwide and several million suffer from severe morbidity. It is estimated that around 200 thousand patients die yearly due to the disease and it is still thought that the burden of disease due to schistosomiasis is underestimated as it often goes unrecognized². Given the global burden of these diseases, the need for an effective vaccine is evident^{1,2}. Yet to date, no such vaccine exists for any parasitic disease. Naturally acquired protection following parasite exposure is a slow process that may take years or decades to develop and does not result in sterile immunity³. Currently, the most promising vaccine candidates are live-attenuated parasites, either yielding sterile immunity (in the case of malaria)^{4,5} or decreasing parasite burden after subsequent infection (in the case of *Schistosoma*)⁶⁻⁸.

Migration and kinetics

Malaria infection starts when the skin-penetrating form of the parasite, motile protozoans called sporozoites, are injected into the dermal stroma via the bite of infected mosquitoes while they search for blood⁹. Although the majority of parasites remain in the skin, within a matter of hours approximately 15% make their way through the dermis to reach the hosts vasculature which transports them to the liver, where they continue development intracellularly in hepatocytes¹⁰⁻¹². *Schistosoma cercariae*, on the other hand, are released from freshwater snails and are aquatic multicellular organisms that bore their way into skin tissue upon contact. Subsequently, the tail detaches and the head of the cercaria continues as a schistosome; slowly making its way out of the skin in the span of days before gaining entry to the vascular space by entering venules or lymphatic vessels. Eventually, the schistosomes mature in the hepatic portal vein¹³⁻¹⁵. The dermal stage of both malaria and schistosomiasis is clinically silent, although the initial skin penetration is accompanied by a mosquito bite or a cercarial rash¹⁶, showing that at least a mild form of immune activation at the site of parasite entry does exist.

Immunity to malaria and schistosomiasis

Immunity to malaria is complex and parasite-stage specific. When natural acquired immunity develops, it is primarily against blood-stage and does not appear to protect

against re-infection¹⁷. Vaccines against malaria are aimed to mediate protection at the pre-erythrocytic stage of disease, as up to this stage the disease is symptom free, parasites numbers are still low, parasites are located extracellularly making them vulnerable to antibody attack and blood-stage malaria does not yet activate immunoregulatory effects. RTS,S is currently the only licensed malaria vaccine and yields only approximately 30% protection over the course of the study period¹⁸⁻²⁰. It is based on the circumsporozoite protein (CSP) which coats sporozoites and aims to increase anti-CSP antibodies in order to prevent migration of parasites from the skin to the liver. However, failure in blocking just one sporozoite from migrating into the vasculature would already render the vaccine inadequate as RTS,S initiates only modest T cell immunity.

The rationale for developing a live-attenuated parasite vaccine which confers sterile protection stems from findings by Nussenzweig and colleagues in the 1960's. They were the first to prove complete protection against pre-erythrocytic stages of malaria in mice vaccinated with radiation-attenuated sporozoites (RAS) that terminate development in the liver²¹. In the 70's it was then shown that exposure to over one thousand attenuated-parasite infected mosquitoes could indeed also protect human volunteers^{22,23}. Currently, controlled human malaria infection studies using intravenously injected RAS show protective efficacy of up to 100%^{24,25}. The immunological basis of protection by RAS has been characterized in murine malaria models. In mice, RAS vaccination results in antibody generation, which block migration of sporozoites during subsequent infections, and CD8⁺ T cell activation, which eliminate infected hepatocytes²⁶⁻²⁸. Although antibodies significantly contribute to protection, CD8⁺ T cells alone are capable of conferring sterile protection in mice^{29,30}. In addition, more recently important roles for $\gamma\delta$ -T cells as well as tissue resident memory (T(RM)) cells were suggested^{5,31}. Overall, antibodies appear to play a role in sporozoite stage (anti-CSP antibodies) and blood-stage infection³², and T cells have a dominant role in liver stage immunity, when parasites are localized intracellularly.

In the case of schistosomiasis, mouse studies have characterized immune responses to the various parasite stages. In the first 5 weeks of infection Th1 responses develop which are primarily associated with IFN- γ production. Subsequently starting at 6 weeks post infection, Th2 responses are initiated when parasites mature and start to generate eggs. Lastly, later stages of disease are characterized by immunomodulatory responses such as activation of alternatively-activated (M2) macrophages and regulatory T cells (Tregs), as well as IL-10 production as a regulatory feedback to proinflammatory immune activation³³⁻³⁵. Nonetheless, exposure to wild-type cercariae does not result in protective immunity. This has been suggested to result from the early increase in dermal

immunoregulatory mechanisms in the first few days post infection³⁶. However, in the early 1960's mice were immunized using irradiated cercariae resulting in a reduction of worm burden post challenge³⁷. Although immune responses to these diseases are slowly unraveled, none of the vaccination approaches have thus far resulted in long-term sterile protection to either parasitic disease.

Why the skin matters

The common ground for all skin-penetrating parasites is the route they must navigate from their site of entry through a densely packed immune organ: the skin³⁸. The skin is the largest organ in the human body, and it is a specialized barrier to the outside world, both by forming a physiological barrier as well as functioning as an important immunological organ. Its most important function is to maintain the immunological balance between tolerance to commensal microbes and inflammation in response to pathogens. Within the skin reside a wide variety of immune cells such as dermal T cells, Natural Killer cells, innate lymphoid cells and mast cells. Importantly, the human dermis contains antigen presenting cells (APCs), dendritic cells (DCs) and macrophages (MΦ), that sample the surroundings and present antigens to the adaptive immune system³⁹⁻⁴². These cells coordinate the following adaptive immune responses by polarizing lymphocytes towards regulation or inflammation depending on the type of antigen encountered⁴². In addition, the dermis is rich in blood and lymphatic vessels which allow quick access to blood-derived immune cells such as neutrophils, monocytes, monocyte-derived macrophages and additional lymphocytes. Antigens are transferred continuously from the skin to the skin-draining lymph nodes either actively by migrating APCs, or passively through lymphatic vessels. It is here, in the skin draining lymph node, that an adaptive response is launched. For skin-penetrating parasites, the skin is the first site of interaction with the host's immune response.

In the case of malaria, the importance of the skin stage in vaccine development against parasitic infections is demonstrated by the importance of the route of administration of live-attenuated parasite vaccines on the protectivity of the response. In vaccination protocols against a variety of diseases dose reduction can be achieved by administering the vaccine not subcutaneously or intramuscularly, but directly into the skin⁴³⁻⁴⁵. In contrast, early clinical studies show that inoculation with malaria sporozoites results in a strong protective immune response when delivered by the bite of an infected mosquito or after intravenous administration of purified parasites⁴. However, the intradermal route of administration, often preferred due to practicalities, results in inferior protective immunity in both rodent and human models of malaria^{46,47}. In addition to this, the skin may well be an important effector site for antibody responses against pre-erythrocytic

stages of malaria. Blocking dermal parasite migration retains sporozoites in the skin, preventing them from reaching the liver and initiating infection^{48,49}. For schistosomiasis it was shown that irradiated cercariae could induce an APC-mediated IFN- γ response in the skin draining lymph node (sdLN) of mice. Additionally, non-attenuated larvae were demonstrated to induce the production of IL-10 in mouse skin⁶. These findings suggest that the skin stage of disease may be critical to the initiation of tolerance for both malaria and *Schistosoma*.

Could pathogens hijack existing immune pathways to avoid clearance?

Some pathogens, such as viruses and bacteria but also parasites, are capable of exploiting APC mechanisms for regulation in order to evade degradation by the immune system⁵⁰⁻⁵⁵. To convey a signal to the adaptive immune system, APCs present antigens in a context of co-stimulation and cytokine signals⁴². The human body contains a variety of mechanisms to down-modulate immune responses, in order to prevent continuous inflammation and subsequent tissue destruction. This can be achieved for example by secretion of regulatory cytokines such as interleukin 10 (IL-10)⁵⁶ or by co-stimulatory signaling through immune checkpoint molecules such as the PD-1/PD-L1 pathway^{57,58}. Although immune modulation by parasites has widely been described during the blood stadia of these diseases⁵⁹⁻⁶¹, early immunoregulatory responses to skin stage parasites have not been investigated in the human host to date. Some murine models have begun to look into the skin-stage of skin-penetrating parasitic disease, nonetheless human responses have remained wholly uncharacterized. As murine skin differs drastically from human skin, both anatomically as well as functionally, looking into the human counterpart of the skin stage could prove critical in order to investigate some of the pitfalls in vaccine-induced immunity. In this thesis we aimed to test human responses to both malaria and *Schistosoma*, by characterizing responses of human monocyte-derived APCs as well as primary dermal APCs freshly isolated from human skin. In addition, we use a human skin explant model in order to expose skin to parasites in its natural three-dimensional state.

Parasite motility, prerequisite for immune responses

A critical feature in both parasite infectivity and subsequent responses is their motility. For malaria, parasites deficient in motility proteins do not establish an infection. In the case of *Schistosoma* parasite motility equally plays an important role; irradiated cercariae have been shown to persist in the skin much longer than their non-irradiated counterparts, increasing their time in the dermis from a few days up to a week^{62,63}. In addition, over-irradiation of cercariae led to a much decreased number of parasites in the hepatic veins and a reduction of protective immunity³⁷. Although the significance

of parasite movement has been widely accepted, motility analysis of live-attenuated sporozoite vaccines is routinely performed *in vitro*⁶⁴, and in-host motility of *Schistosoma* parasites has not been characterized at all. However, *in vitro* modelling passes over the consequential effect of the tissue environment on motility^{65,66}. Therefore visualization and quantification of parasite motility in the three-dimensional setting may prove a crucial factor in the detailed comprehension of parasite-skin interactions. We dedicated the second part of this thesis to the development and implementation of (molecular) imaging techniques in the characterization of dermal parasite movement.

Understanding the details of dermal immune regulation by skin-penetrating parasites could significantly aid in the development and optimization of live-attenuated parasite vaccines. Bypassing or counteracting the regulatory effects of these parasites on dermal immune cells may optimize their protective effect. This thesis aims to unravel the pivotal role of the skin stage of skin-penetrating parasites in the potential polarization towards immune tolerance, both by investigating the kinetics of parasite migration, as well as the immune responses after exposure.

THESIS OUTLINE

The first part of this thesis describes the host immune-regulatory mechanisms that are exploited by two different skin-penetrating parasites immediately after their entry into the skin. In **chapter 2** we investigated APC responses to whole *Plasmodium falciparum* (Pf) sporozoite stimulation and show that malaria sporozoites induce regulatory MΦs that can suppress subsequent adaptive T cell responses. **Chapter 3** investigates the effect of the route of administration of whole sporozoites on their ability to skew towards dermal immune regulation. In **chapter 4** we show that a different skin-penetrating parasite, *Schistosoma mansoni*, is similarly capable of inducing regulatory immune responses in human skin and that its ability to do so is decreased upon radiation attenuation, the most commonly used mode of attenuation in parasite vaccines.

The second part of this thesis focusses on the motility behavior of malaria parasites in the human dermis. In **chapter 5**, we show that radiation attenuation impairs sporozoite movement in the human skin and reverts sporozoite motility back to “default”, non-directional movement. **Chapter 6** describes a novel method for targeted molecular imaging of genetically wild-type Pf sporozoites, allowing for imaging and subsequent motility analysis of non-GMO Pf sporozoites in human skin tissue.

Finally, in **chapter 7** the results are summarized and discussed in the broader context of the current literature and regarding potential new lines of research necessary for

refinement and development of novel vaccines. We draw parallels between the two species of skin-penetrating parasites investigated and discuss the effect of radiation attenuation of parasites.

REFERENCES

- 1 [Internet] WHO: Geneva Switzerland. 19 November 2018, World Malaria report 2018. <https://www.who.int/malaria/media/world-malaria-report-2018/en/>.
- 2 [Internet] WHO: Geneva Switzerland. 17 April 2019, Schistosomiasis fact sheet. <https://www.who.int/news-room/fact-sheets/detail/schistosomiasis> (2019).
- 3 Doolan, D. L., Dobano, C. & Baird, J. K. Acquired immunity to malaria. *Clin Microbiol Rev* 22, 13-36, Table of Contents, doi:10.1128/CMR.00025-08 (2009).
- 4 Seder, R. A. et al. Protection against malaria by intravenous immunization with a nonreplicating sporozoite vaccine. *Science* 341, 1359-1365, doi:10.1126/science.1241800 (2013).
- 5 Ishizuka, A. S. et al. Protection against malaria at 1 year and immune correlates following PfSPZ vaccination. *Nat Med* 22, 614-623, doi:10.1038/nm.4110 (2016).
- 6 Hogg, K. G., Kumkate, S., Anderson, S. & Mountford, A. P. Interleukin-12 p40 secretion by cutaneous CD11c+ and F4/80+ cells is a major feature of the innate immune response in mice that develop Th1-mediated protective immunity to *Schistosoma mansoni*. *Infect Immun* 71, 3563-3571 (2003).
- 7 Hogg, K. G., Kumkate, S. & Mountford, A. P. IL-10 regulates early IL-12-mediated immune responses induced by the radiation-attenuated schistosome vaccine. *Int Immunol* 15, 1451-1459 (2003).
- 8 Hewitson, J. P., Hamblin, P. A. & Mountford, A. P. Immunity induced by the radiation-attenuated schistosome vaccine. *Parasite Immunol* 27, 271-280, doi:10.1111/j.1365-3024.2005.00764.x (2005).
- 9 Menard, R. et al. Looking under the skin: the first steps in malarial infection and immunity. *Nat Rev Microbiol* 11, 701-712, doi:10.1038/nrmicro3111 (2013).
- 10 Amino, R. et al. Quantitative imaging of *Plasmodium* transmission from mosquito to mammal. *Nat Med* 12, 220-224, doi:10.1038/nm1350 (2006).
- 11 Amino, R. et al. Imaging malaria sporozoites in the dermis of the mammalian host. *Nat Protoc* 2, 1705-1712, doi:10.1038/nprot.2007.120 (2007).
- 12 Yamauchi, L. M., Coppi, A., Snounou, G. & Sinnis, P. *Plasmodium* sporozoites trickle out of the injection site. *Cell Microbiol* 9, 1215-1222, doi:10.1111/j.1462-5822.2006.00861.x (2007).
- 13 Wheeler, P. R. & Wilson, R. A. *Schistosoma mansoni*: a histological study of migration in the laboratory mouse. *Parasitology* 79, 49-62 (1979).
- 14 Mangold, B. L. & Dean, D. A. Autoradiographic analysis of *Schistosoma mansoni* migration from skin to lungs in naive mice. Evidence that most attrition occurs after the skin phase. *Am J Trop Med Hyg* 32, 785-789 (1983).
- 15 Wilson, R. A., Coulson, P. S., Sturrock, R. F. & Reid, G. D. Schistosome migration in primates: a study in the olive baboon (*Papio anubis*). *Trans R Soc Trop Med Hyg* 84, 80-83 (1990).
- 16 Gray, D. J., Ross, A. G., Li, Y. S. & McManus, D. P. Diagnosis and management of schistosomiasis. *BMJ* 342, d2651, doi:10.1136/bmj.d2651 (2011).
- 17 Tran, T. M. et al. An intensive longitudinal cohort study of Malian children and adults reveals no evidence of acquired immunity to *Plasmodium falciparum* infection. *Clin Infect Dis* 57, 40-47, doi:10.1093/cid/cit174 (2013).
- 18 Rts, S. C. T. P. Efficacy and safety of the RTS,S/AS01 malaria vaccine during 18 months after vaccination: a phase 3 randomized, controlled trial in children and young infants at 11 African sites. *PLoS Med* 11, e1001685, doi:10.1371/journal.pmed.1001685 (2014).
- 19 Rts, S. C. T. P. et al. A phase 3 trial of RTS,S/AS01 malaria vaccine in African infants. *N*

- Engl J Med 367, 2284-2295, doi:10.1056/NEJMoa1208394 (2012).
- 20 Rts, S. C. T. P. Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial. *Lancet* 386, 31-45, doi:10.1016/S0140-6736(15)60721-8 (2015).
- 21 Nussenzweig, R. S., Vanderberg, J., Most, H. & Orton, C. Protective immunity produced by the injection of x-irradiated sporozoites of plasmodium berghei. *Nature* 216, 160-162, doi:10.1038/216160a0 (1967).
- 22 Clyde, D. F. Immunization of man against falciparum and vivax malaria by use of attenuated sporozoites. *Am J Trop Med Hyg* 24, 397-401, doi:10.4269/ajtmh.1975.24.397 (1975).
- 23 Luke, T. C. & Hoffman, S. L. Rationale and plans for developing a non-replicating, metabolically active, radiation-attenuated Plasmodium falciparum sporozoite vaccine. *J Exp Biol* 206, 3803-3808, doi:10.1242/jeb.00644 (2003).
- 24 Roestenberg, M. et al. Protection against a malaria challenge by sporozoite inoculation. *N Engl J Med* 361, 468-477, doi:10.1056/NEJMoa0805832 (2009).
- 25 Mordmuller, B. et al. Sterile protection against human malaria by chemo attenuated PfSPZ vaccine. *Nature* 542, 445-449, doi:10.1038/nature21060 (2017).
- 26 Chakravarty, S. et al. CD8+ T lymphocytes protective against malaria liver stages are primed in skin-draining lymph nodes. *Nat Med* 13, 1035-1041, doi:10.1038/nm1628 (2007).
- 27 Schofield, L. et al. Gamma interferon, CD8+ T cells and antibodies required for immunity to malaria sporozoites. *Nature* 330, 664-666, doi:10.1038/330664a0 (1987).
- 28 Rodrigues, M., Nussenzweig, R. S. & Zavala, F. The relative contribution of antibodies, CD4+ and CD8+ T cells to sporozoite-induced protection against malaria. *Immunology* 80, 1-5 (1993).
- 29 Cockburn, I. A., Tse, S. W. & Zavala, F. CD8+ T cells eliminate liver-stage Plasmodium berghei parasites without detectable bystander effect. *Infect Immun* 82, 1460-1464, doi:10.1128/IAI.01500-13 (2014).
- 30 Van Braeckel-Budimir, N. & Harty, J. T. CD8 T-cell-mediated protection against liver-stage malaria: lessons from a mouse model. *Front Microbiol* 5, 272, doi:10.3389/fmicb.2014.00272 (2014).
- 31 Lyke, K. E. et al. Attenuated PfSPZ Vaccine induces strain-transcending T cells and durable protection against heterologous controlled human malaria infection. *Proc Natl Acad Sci U S A* 114, 2711-2716, doi:10.1073/pnas.1615324114 (2017).
- 32 Teo, A., Feng, G., Brown, G. V., Beeson, J. G. & Rogerson, S. J. Functional Antibodies and Protection against Blood-stage Malaria. *Trends Parasitol* 32, 887-898, doi:10.1016/j.pt.2016.07.003 (2016).
- 33 Dunne, D. W. & Cooke, A. A worm's eye view of the immune system: consequences for evolution of human autoimmune disease. *Nat Rev Immunol* 5, 420-426, doi:10.1038/nri1601 (2005).
- 34 Colley, D. G. & Secor, W. E. Immunology of human schistosomiasis. *Parasite Immunol* 36, 347-357, doi:10.1111/pim.12087 (2014).
- 35 Grogan, J. L., Kremsner, P. G., Deelder, A. M. & Yazdanbakhsh, M. The effect of anti-IL-10 on proliferation and cytokine production in human schistosomiasis: fresh versus cryopreserved cells. *Parasite Immunol* 20, 345-349, doi:10.1046/j.1365-3024.1998.00157.x (1998).
- 36 Mountford, A. P. & Trottein, F. Schistosomes in the skin: a balance between immune priming and regulation. *Trends Parasitol* 20, 221-226, doi:10.1016/j.pt.2004.03.003 (2004).
- 37 Vilella, J. B., Gomberg, H. J. & Gould, S. E. Immunization to Schistosoma mansoni in mice inoculated with radiated cercariae.

- Science 134, 1073-1075, doi:10.1126/science.134.3485.1073 (1961).
- 38 Di Meglio, P., Perera, G. K. & Nestle, F. O. The multitasking organ: recent insights into skin immune function. *Immunity* 35, 857-869, doi:10.1016/j.immuni.2011.12.003 (2011).
- 39 Tay, S. S., Roediger, B., Tong, P. L., Tikoo, S. & Weninger, W. The Skin-Resident Immune Network. *Curr Dermatol Rep* 3, 13-22, doi:10.1007/s13671-013-0063-9 (2014).
- 40 Wang, X. N. et al. A three-dimensional atlas of human dermal leukocytes, lymphatics, and blood vessels. *J Invest Dermatol* 134, 965-974, doi:10.1038/jid.2013.481 (2014).
- 41 Richmond, J. M. & Harris, J. E. Immunology and skin in health and disease. *Cold Spring Harb Perspect Med* 4, a015339, doi:10.1101/cshperspect.a015339 (2014).
- 42 de Jong, E. C., Smits, H. H. & Kapsenberg, M. L. Dendritic cell-mediated T cell polarization. *Springer Semin Immunopathol* 26, 289-307, doi:10.1007/s00281-004-0167-1 (2005).
- 43 Denis, M. et al. An overview of the immunogenicity and effectiveness of current human rabies vaccines administered by intradermal route. *Vaccine* 37 Suppl 1, A99-A106, doi:10.1016/j.vaccine.2018.11.072 (2019).
- 44 Kenney, R. T., Frech, S. A., Muenz, L. R., Villar, C. P. & Glenn, G. M. Dose sparing with intradermal injection of influenza vaccine. *N Engl J Med* 351, 2295-2301, doi:10.1056/NEJMoa043540 (2004).
- 45 Okayasu, H. et al. Intradermal Administration of Fractional Doses of Inactivated Poliovirus Vaccine: A Dose-Sparing Option for Polio Immunization. *J Infect Dis* 216, S161-S167, doi:10.1093/infdis/jix038 (2017).
- 46 Epstein, J. E. et al. Live attenuated malaria vaccine designed to protect through hepatic CD8(+) T cell immunity. *Science* 334, 475-480, doi:10.1126/science.1211548 (2011).
- 47 Haeberlein, S. et al. Protective immunity differs between routes of administration of attenuated malaria parasites independent of parasite liver load. *Sci Rep* 7, 10372, doi:10.1038/s41598-017-10480-1 (2017).
- 48 Vanderberg, J. P. & Frevert, U. Intravital microscopy demonstrating antibody-mediated immobilisation of *Plasmodium berghei* sporozoites injected into skin by mosquitoes. *Int J Parasitol* 34, 991-996, doi:10.1016/j.ijpara.2004.05.005 (2004).
- 49 Stewart, M. J., Nawrot, R. J., Schulman, S. & Vanderberg, J. P. *Plasmodium berghei* sporozoite invasion is blocked in vitro by sporozoite-immobilizing antibodies. *Infect Immun* 51, 859-864 (1986).
- 50 Horne-Debets, J. M. et al. PD-1 dependent exhaustion of CD8+ T cells drives chronic malaria. *Cell Rep* 5, 1204-1213, doi:10.1016/j.celrep.2013.11.002 (2013).
- 51 Huang, X. et al. PD-1 expression by macrophages plays a pathologic role in altering microbial clearance and the innate inflammatory response to sepsis. *Proc Natl Acad Sci U S A* 106, 6303-6308, doi:10.1073/pnas.0809422106 (2009).
- 52 Illingworth, J. et al. Chronic exposure to *Plasmodium falciparum* is associated with phenotypic evidence of B and T cell exhaustion. *J Immunol* 190, 1038-1047, doi:10.4049/jimmunol.1202438 (2013).
- 53 Narasimhan, P. B. et al. Similarities and differences between helminth parasites and cancer cell lines in shaping human monocytes: Insights into parallel mechanisms of immune evasion. *PLoS Negl Trop Dis* 12, e0006404, doi:10.1371/journal.pntd.0006404 (2018).
- 54 Winkel, B. M. F. et al. Early Induction of Human Regulatory Dermal Antigen Presenting Cells by Skin-Penetrating *Schistosoma Mansoni* Cercariae. *Front Immunol* 9, 2510, doi:10.3389/fimmu.2018.02510 (2018).
- 55 Xiao, J., Li, Y., Yolken, R. H. & Viscidi, R. P. PD-1 immune checkpoint blockade promotes brain leukocyte infiltration and diminishes cyst burden in a mouse model of *Toxoplasma* infection. *J Neuroimmunol* 319, 55-62, doi:10.1016/j.jneuroim.2018.03.013 (2018).

- 56 Couper, K. N., Blount, D. G. & Riley, E. M. IL-10: the master regulator of immunity to infection. *J Immunol* 180, 5771-5777, doi:10.4049/jimmunol.180.9.5771 (2008).
- 57 Riella, L. V., Paterson, A. M., Sharpe, A. H. & Chandraker, A. Role of the PD-1 pathway in the immune response. *Am J Transplant* 12, 2575-2587, doi:10.1111/j.1600-6143.2012.04224.x (2012).
- 58 Francisco, L. M., Sage, P. T. & Sharpe, A. H. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev* 236, 219-242, doi:10.1111/j.1600-065X.2010.00923.x (2010).
- 59 Ocana-Morgner, C., Mota, M. M. & Rodriguez, A. Malaria blood stage suppression of liver stage immunity by dendritic cells. *J Exp Med* 197, 143-151, doi:10.1084/jem.20021072 (2003).
- 60 Coban, C., Ishii, K. J., Horii, T. & Akira, S. Manipulation of host innate immune responses by the malaria parasite. *Trends Microbiol* 15, 271-278, doi:10.1016/j.tim.2007.04.003 (2007).
- 61 Maizels, R. M., Smits, H. H. & McSorley, H. J. Modulation of Host Immunity by Helminths: The Expanding Repertoire of Parasite Effector Molecules. *Immunity* 49, 801-818, doi:10.1016/j.immuni.2018.10.016 (2018).
- 62 Wilson, R. A., Coulson, P. S. & Dixon, B. Migration of the schistosomula of *Schistosoma mansoni* in mice vaccinated with radiation-attenuated cercariae, and normal mice: an attempt to identify the timing and site of parasite death. *Parasitology* 92 (Pt 1), 101-116, doi:10.1017/s0031182000063484 (1986).
- 63 Richter, D., Harn, D. A. & Matuschka, F. R. The irradiated cercariae vaccine model: looking on the bright side of radiation. *Parasitol Today* 11, 288-293, doi:10.1016/0169-4758(95)80041-7 (1995).
- 64 Prinz, H. L., Sattler, J. M. & Frischknecht, F. Plasmodium Sporozoite Motility on Flat Substrates. *Bio-protocol* 7, e2395, doi:10.21769/BioProtoc.2395. (2017).
- 65 Battista, A., Frischknecht, F. & Schwarz, U. S. Geometrical model for malaria parasite migration in structured environments. *Phys Rev E Stat Nonlin Soft Matter Phys* 90, 042720, doi:10.1103/PhysRevE.90.042720 (2014).
- 66 Hellmann, J. K. et al. Environmental constraints guide migration of malaria parasites during transmission. *PLoS Pathog* 7, e1002080, doi:10.1371/journal.ppat.1002080 (2011).

