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

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Human dermal APC responses to needle- and mosquito bite-injected *Plasmodium falciparum* sporozoites

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ABSTRACT

Whole sporozoite vaccines can yield sterile protection against malaria, however their efficacy critically depends on their route of administration. Early clinical trials demonstrated high levels of protection with mosquito bite administered parasites, whereas protection after needle injection was much reduced. Previously, we have shown that *Plasmodium* sporozoites induce a regulatory immune response in the dermis. To investigate whether differences in dermal immune regulation could underlie the differences in protection, we studied early dermal APC responses to mosquito bite and needle injected *Plasmodium falciparum* sporozoites directly in human skin explants. We analyzed subset distribution of the four main dermal APC subsets ($CD14^+$, $CD1a^+$, $CD141^+$ dermal dendritic cells (DDCs) and Langerhans cells (LCs)), their surface expression of activation markers, their cytokine production and the subsequent effect on priming naïve $CD4^+$ T cells. We found a small increase in the $CD14^+$ DDC subset when sporozoites were administered by mosquito compared to needle. We did not detect changes in activation markers, cytokine production or polarization of naïve $CD4^+$ T cells. In this study we take the first steps to elucidate the dermal immune responses during the uncharacterized human skin stage of malaria. Understanding the initial interaction of SPZ with the innate immune system and the subsequent effect on adaptive immunity may aid in the development of effective attenuated sporozoite malaria vaccines.

Keywords*: Plasmodium falciparum*, sporozoite, malaria, skin, innate immunity, dendritic cells

INTRODUCTION

Malaria infection starts with the bite of an infected *anopheles* mosquito injecting a motile form of the *Plasmodium* parasite, sporozoites (SPZ), into the dermis of the host. It is often thought that mosquitoes inoculate sporozoites directly into the blood stream. However, sporozoites are ejected when a probing mosquito salivates. Salivation stops when the mosquito has located a blood vessel[1,2]. Interrupted feeding and bite site removal experiments[2], as well as video microscopic analysis[3] have demonstrated a predominantly intradermal delivery of sporozoites by mosquito bite. These SPZ subsequently migrate through the dermal tissue and make their way into the vasculature with which they are transported to the liver, where they continue development in hepatocytes. Because the SPZ are still low in number and located extracellularly, where they are vulnerable to immune attack, SPZ antigens have been used as malaria vaccines.

The inoculation of live, radiation-attenuated malaria parasites is one of the most promising vaccine approaches. These vaccines are based on the whole sporozoite instead of its immunodominant antigen CSP, and are currently undergoing field efficacy trials. Remarkably, these parasites induce high levels of protection when administered by mosquito bite[4,5], but intradermal needle injection results in greatly reduced protective efficacy, both in clinical trials and in rodent models of malaria[6-8]. Given the inferiority of ID administration, we hypothesize that the interaction between SPZ and skin immune cells might downmodulate the ensuing immune responses.

Contrasting initial assumptions that the SPZ stay within the skin is only brief, experiments in both primates and rodents revealed that SPZ exit from the dermis can take up to many hours. Approximately half of injected sporozoites trickle out of the injection site over the course of several hours, the majority of these reaching the blood stream. A small portion ends up in lymphatic vessels where they are eliminated by immune cells in the skin draining lymph nodes. Surprisingly, a high amount of sporozoites remain inside the dermis, where they can be detected up to 7h after inoculation[9,10]. During this dermal stage of the infection, malaria sporozoites are extracellularly located and secrete various proteins as they migrate[11]. Combined, this results in significant antigen exposure at the skin site.

The skin is an important immunological organ containing different types of immune cells, including antigen-presenting cells (APCs) of the innate immune system such as dendritic cells (DCs) and macrophages (MΦ) that are capable of orchestrating subsequent innate immune responses. The dermal stage of disease is the first site of interaction of SPZ with the immune system. In the human skin, four APC subsets have

been identified based on their surface marker expression: $CD14^+$, $CD141^+$, and $CD1a^+$ dermal dendritic cells (DDCs), and epidermal Langerhans cells (LCs). In general the different DDC subsets can be ascribed certain functionalities however, these depend on the type of antigen encountered. The functions of the various skin APC subsets in response to malaria sporozoites are currently unknown.

In order to understand why immune responses to malarial sporozoites are poor, it is essential to unravel the role of skin resident APCs in the activation or hindering of subsequent adaptive immune responses. To date research has focused solely on rodent models in order to elucidate the dermal stage. Indeed, in a murine model using *Plasmodium berghei* parasites, the lower levels of protection after ID vaccination were associated with an increase in lymphocytes producing IL-10 in the skin draining lymph node[8]. However, although murine models may provide valuable clues, using rodent skin poses obstacles for translation of the findings. Murine skin differs drastically from human skin both anatomically as well as immunologically[12-15]. Therefore, in order to address the immunological basis for the difference in protective efficacy between the two routes of administration we used human skin explants to study the phenotypic and functional effects of SPZ on human dermal APCs. It will be essential to increase our understanding of the skin immune response to sporozoites, in order to exploit the skin as an immunological priming site for the development and/or optimization of vaccines against malaria.

MATERIALS AND METHODS

Parasite materials

Sporozoites were obtained from the human parasite *Plasmodium falciparum* (*Pf*; NF5416, WT or GFP-luciferase expressing under the *pf*CS promotor) kindly provided by Radboudumc or TropIQ health sciences respectively, both Nijmegen, The Netherlands). Mosquitoes were infected by standard membrane feeding as previously described[17]. Salivary glands of infected and uninfected mosquitoes were manually dissected at day 14-21 post infection. Salivary glands were kept on ice until use within 1 hours. Immediately prior to their use, glands were homogenized to extract parasites. Parasites were counted using a Bürker chamber.

Animal use for this study was approved by the Animal Experiments Committee of the Leiden University Medical Center (DEC 14307). The Dutch Experiments on Animal

Act is established under European guidelines (EU directive no. 86/609/EEC regarding the Protection of Animals used for Experimental and Other Scientific Purposes). All experiments were performed in accordance with relevant guidelines and regulations.

Dermal APC emigration assay

Human skin explants were obtained from collaborating hospitals immediately after abdominal skin reduction surgery (IRB B18.009 see ethics statement) and kept at 4°C until use (within 6 hours). Subcutaneous fat was removed, and the epidermal side thoroughly cleaned with 70% ethanol. For injections, sporozoite solutions were diluted in RPMI (42401-042; Invitrogen, Carlsbad, CA, USA) to a concentration of 2x105 sporozoites/ml (104 sporozoites per 50µl injection). Additionally, uninfected salivary gland extract (SGE) was diluted to match salivary glands injected with the sporozoite solutions. 50µl solutions were injected intradermally using an insulin syringe (BD biosciences, Franklin lakes, NJ, USA). For mosquito bite conditions, skin pieces were wrapped around an electrically heated pad and placed upon mesh cages containing 100 infected Anopheles mosquitoes or uninfected control mosquitoes. Mosquitoes were allowed to probe the skin in an interrupted feeding schedule (2-5 min on, 30 seconds off for 15 total feeding minutes) in the dark, after which the sample was removed. The injection site or mosquito exposed area was cleaned and biopsied using 6mm punch biopsies (12-24 biopsies per condition). As controls, the injection site of 50µl intradermal injections of RMPI, lipopolysaccharide (LPS; 20µg/ml; Invivogen, San Diego, CA, USA) or 1,25-dihydroxyvitamin D3 (Vitamin D; 25µM; Invivogen) was biopsied. All controls were diluted in RMPI (42401-042; Invitrogen). Biopsies were rinsed in RPMI supplemented with 0.1% fetal calf serum (FCS, Bodinco, Alkmaar, The Netherlands) and transferred to a 48 wells plate containing 1ml RPMI 10% FCS per well supplemented with 500U/ml GM-CSF. Plates were incubated for 3 days at 37˚C, 5% CO2.

Biopsy supernatant was collected after 3 days. Supernatant was spun down to collect emigrated immune cells. And the supernatant was stored at -20 degrees for subsequent Luminex analysis. Emigrated cells were washed and stained for Flow Cytometric analysis or irradiated to a total dose of 3000 rad and brought into culture for co-culture assays.

Flow Cytometric analysis of emigrated cells

Cells were stained with 7AAD live/dead dye (Abcam, Cambridge, UK; uptake) or with Aqua fixable live/dead dye (Thermo Fischer Scientific, Waltham, MA, USA; phenotyping) antibodies against HLA-DR, CD11c, CD1a, CD14, CD141 and CD80 and analyzed by Flow

Cytometry using a BD FACSCanto II (BD Biosciences). Data was analyzed in FlowJoTM version 9.9.6 (FlowJo LLC, Ashlan, OR, USA). Gates were set using 'fluorescence minus one' (FMO) stained control samples.

Cytokine measurement

Biopsy supernatants were harvested 3 days after exposure to SPZ or controls. Supernatants were analyzed by commercially available custom Luminex kit including IFNγ, IL1β, IL-10, IL-2, IL-23, IP10, MIP1α, MIP1β and TNFα (Affymetrix, Santa Clara, CA, USA).

Naïve CD4$^+$ T cell co-culture

Naïve T cell co-culture analysis of T cell polarization was performed as described previously[24]. In brief: 5x103 emigrated APCs were irradiated (3000 rad) and co-cultured with 2x104 allogeneic naïve CD4+ T cells isolated from buffy coat (Sanquin, Amsterdam, The Netherlands). Co-cultures were performed in the presence of staphylococcal enterotoxin B (10pg/ml). On days 6 and 8, recombinant human IL2 (10U/ml; R&D Systems) was added and the T cells were expanded until day 11. Intracellular cytokine production was analyzed after polyclonal restimulation with 100ng/ml phorbol myristate acetate (PMA) and 1ug/ml ionomycin (Sigma Aldrich) for 6 hours. Brefaldin A (10ug/ml; Sigma Aldrich) was added for the last 4 hours of restimulation. Cells were fixed in 3.7% paraformaldehyde (Sigma Aldrich), permeabilized with permeabilization buffer (Affymetrix, Santa Clara, CA, USA), stained with antibodies against IL-4 and IFNγ (BD bioscience) and analyzed with flow cytometry. In addition, 105 expanded CD4 T cells were restimulated with antibodies against CD3 and CD28 for 24 hours in a 96-wells plate. Supernatants were harvested and analyzed for IL-10 secretion using standard ELISA (Sanquin, Amsterdam, The Netherlands).

Statistical analysis

Data was analyzed using GraphPad Prism (La Jolla, CA, USA) version 7. Comparisons between two or more independent data groups were made by student's T test or analysis of variance test (ANOVA; respectively). Luminex cytokine analysis was performed using the Kruskall-Wallis test for comparison of non-parametric data from multiple groups. $P<0.05$ was considered statistically significant.

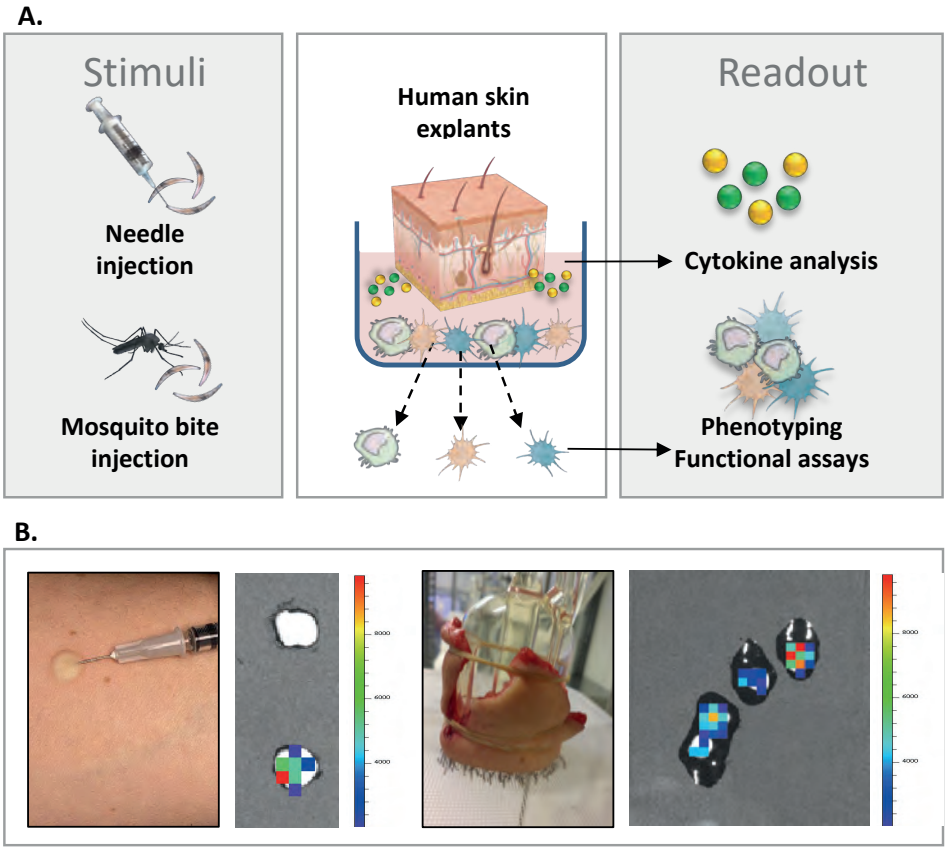

Figure 1. Experimental setup. A. Human skin explants were exposed to *Pf* infected mosquitoes or injected with *Pf* SPZ or controls (SGE, Medium, Vitamin D and LPS). The exposed or injected areas were then biopsied and cultured to allow emigration of migratory dermal APCs. We analysed the biopsy cytokine environment and the phenotype and function of emigrated APCs. **B.** Sporozoite luciferin signal seen after intradermal needle (left, bottom) or mosquito bite (right) injection of sporozoites confirms sporozoites are deposited in skin explants by mosquito bite. Uninjected biopsy as a control in top left.

RESULTS

In order to assess the responses of dermal immune cells to SPZ, we exposed full thickness human abdominal skin explants to SPZ injected by needle or by mosquito bite. We confirmed dermal location of 10 000 luciferase-expressing SPZ by IVIS (Figure 1B, left). Delivery of SPZ by the bites of 100 mosquitoes yielded lower signals, however still clearly detectable (right). As mosquitoes inject approximately 100 sporozoites per bite[5,18], we estimated approximately 85-fold fewer parasites in the mosquito bite delivered biopsies

(±118 sporozoites per biopsy; 100 mosquitoes*100 sporozoites=10.000 sporozoites in the total surface area of 23.8 cm2, compared to 10.000 injected sporozoites per single biopsy surface area of 0.28 cm2).

Emigrating CD14$^+$ DDCs are increased after mosquito bite administration of SPZ and decreased after needle injection.

We subsequently tested the phenotype and function of dermal APCs (Figure 1A). We measured approximately three to seven thousand emigrated HLA-DR$^+$, CD11c$^+$ dermal APCs per biopsy, and found a trend of decreased total APC emigration after exposure to SPZ both by mosquito bite and needle injection (Figure 2A). Examination of DDC subset distribution (CD14$^+$, CD1a$^+$, CD141$^+$ DDCs and LCs), revealed only very small differences after exposure to salivary gland extract of uninfected (SGE) or Plasmodium infected mosquitoes (SPZ) with respect to the medium control (Figure 2B). These small differences were especially evident compared to the effects of LPS and Vitamin D controls (Supplementary Figure S1). We did find increased emigration of one subset of DDCs, the CD14$^+$ subset, after SPZ exposure by infected mosquito bite, although this trend was not statistically significant (P=0.09). This effect was primarily due to an increase in the CD14+, Auto fluorescence$^+$ population, which have previously been reported as "macrophage-like"[29] (Figure 2C). In contrast, emigration of the CD14$^+$ subset was suppressed after needle injection of both SGE and SPZ (Figure 2B). We did not detect differences in the other DDC subsets. Additionally, analysis of surface expression of activation markers CD80, CD86 and HLA-DR showed that the changes in CD14$^+$ DDCs were not accompanied by differences in activation marker expression with either administration method in either subset (Figure 3, Supplementary figure S2). We concluded that, although dermal APCs do not readily respond to intradermal SPZs administered either via mosquito bite or needle injection, emigration of total dermal APCs is reduced after SPZ exposure. In addition, the CD14$^+$ DDC subset may be the subset of interest with regards to the route of administration of SPZ.

ID SPZ delivery does not result in changes in the dermal cytokine environment

Luminex analysis of 8 different cytokines and chemokines which can be produced by APCs in response to antigen encounter yielded no changes upon administration of SPZ. We tested proinflammatory cytokines IFN-γ, IL-1β, TNF-α and IL-23, as well as proinflammatory chemokines MIP-1α and MIP-1β. In addition we measured regulatory cytokine IL-10 and chemokine IP-10. Although control injections using LPS showed clear increases in the production of both pro- and anti-inflammatory cytokines and chemokines (Supplementary figure S3), we did not detect any responses to mosquito

bite or needle injection of SPZ or SGE (Figure 4). Thus, we concluded that dermal APCs do not readily respond to intradermal SPZ or uninfected SGE by alteration of cytokine production.

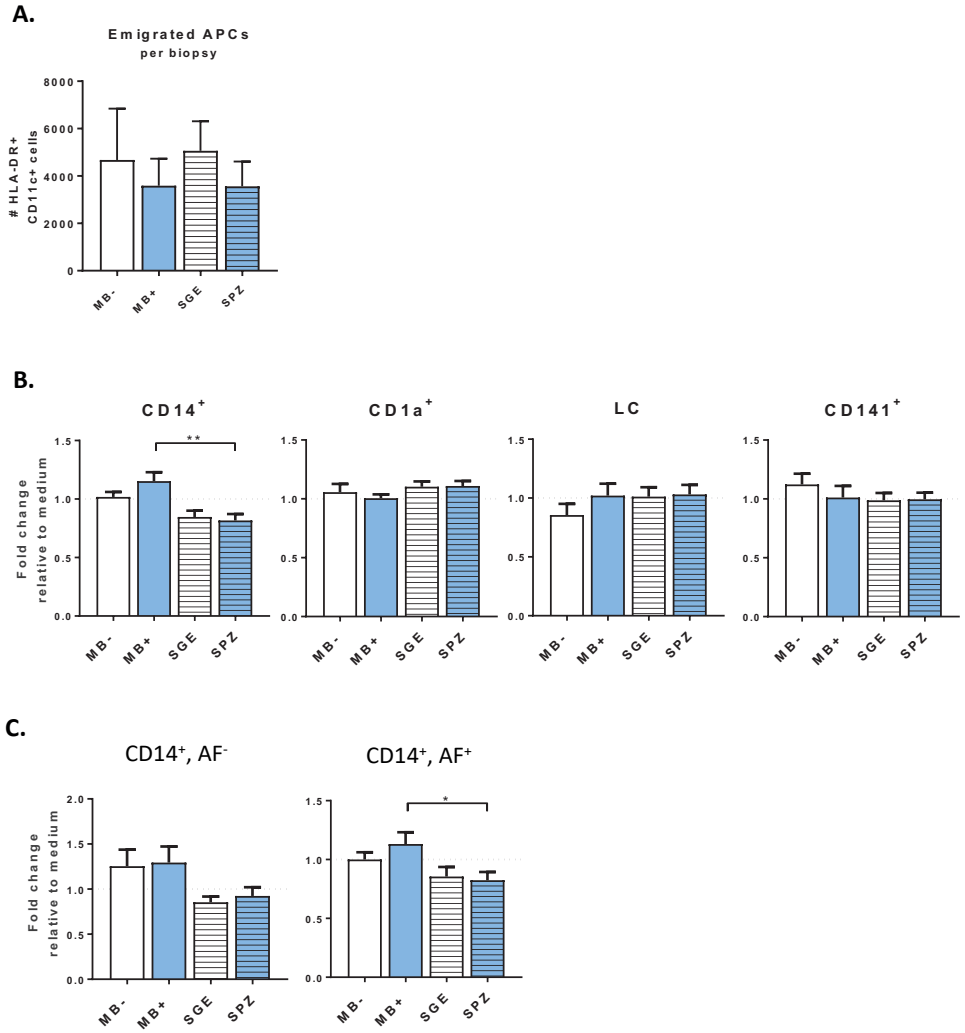

Figure 2. DDC subset distribution after ID SPZ inoculation via MB or needle. A. Total number of emigrated APCs per biopsy. Trend of a decrease in APC emigration upon exposure to SPZ, both with MB and needle injection. **B.** Subset distribution of emigrated DDCs. Statistical testing using paired student's T test. **C.** $CD14^+$ subset consists of $CD14^+$ DDCs (CD14+ $Autofluorescence^-$) and Macrophage-like APCs ($CD14^+$, $Autofluorescence^+$).

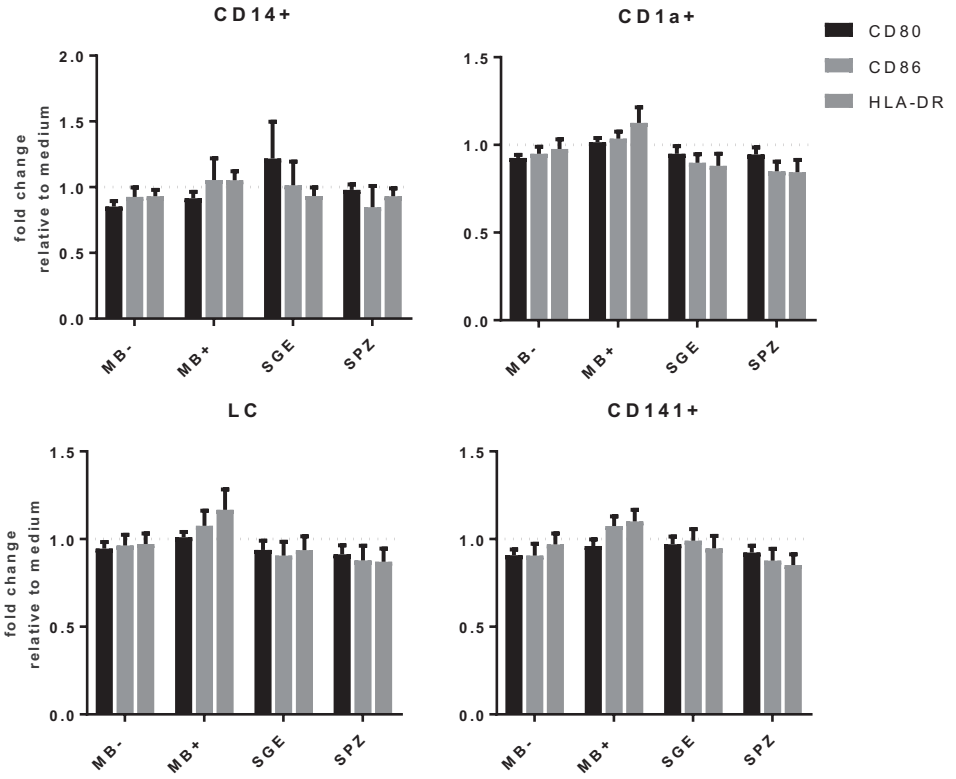

Figure 3. Activation status of emigrated DDCs. No changes in activation marker expression after exposure to SPZ either via mosquito bite (MB+), needle (SPZ) or uninfected controls (MB- and SGE respectively). Data shown as CD80, CD86 and HLA-DR expression over the four main DDC subsets. Statistical testing using one way ANOVA.

No altered T cell polarization after dermal APC exposure to mosquito bite or needle injected SPZ

Next we investigated whether dermal APCs exposed to SPZ or SGE could affect naïve $CD4^+$ T cell polarization. We co-cultured heterologous T cells from anonymous donors with emigrated DDCs from the previous setup, in the presence of staphylococcal enterotoxin B (SEB) to ligate the discordant MHC receptors and measured IFN-γ, IL-4 and IL-10 production by T cells. Using this, method we did not detect altered $CD4^+$ T cell polarization towards either Th1, Th2 or regulatory responses after DDC exposure to SPZ or SGE (Figure 5). In conclusion, dermal APC exposure to SPZ or uninfected SGE does not affect subsequent T cell polarization.

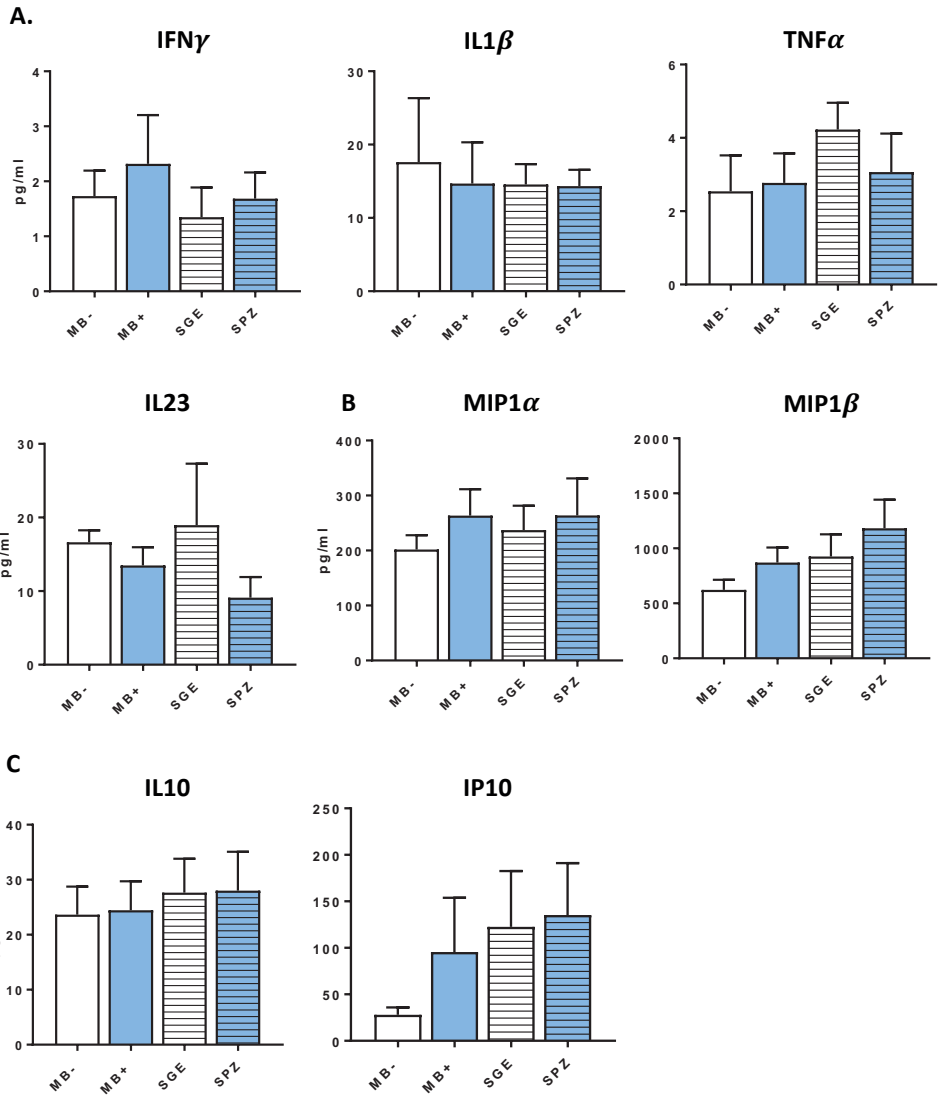

Figure 4. No changes in whole biopsy cytokine and chemokine environment after SPZ inoculation. A. Pro-inflammatory cytokines. **B.** Proinflammatory chemokines. **C.** Regulary cytokine IL10 and chemokine IP10. Testing using Kruskal-Wallis test (unpaired one way ANOVA non-parametric)

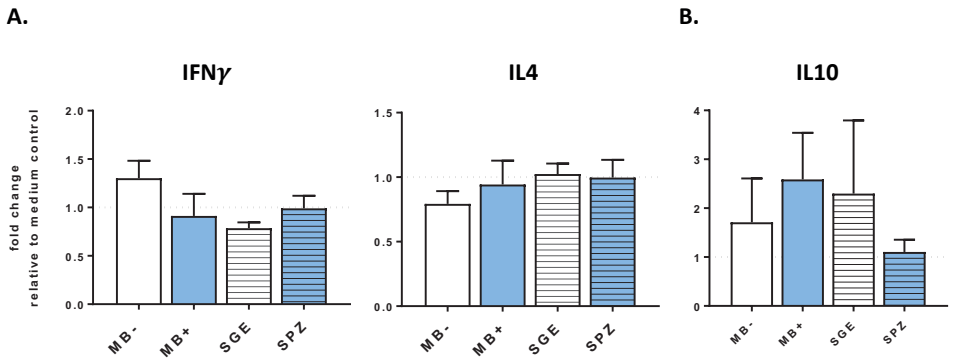

Figure 5. No skewing of naïve CD4^{+} T cells by stimulated DDCs. A. Naïve CD4 T cell polarization. IFNγ or IL-4 producing CD4+ T cells (Th1 and Th2 polarization respectively). **B.** IL-10 production by naïve CD4 T cells (after anti CD3/28 stimulation)

DISCUSSION

After sporozoite delivery, skin resident immune cells have been found capable of priming a protective immune response against infected hepatocytes. This response is initiated in the skin draining lymph node[19,20]. However, we have recently shown that *Plasmodium falciparum* (*Pf*) SPZ are able to induce regulatory immune responses mediated through contact with APCs *in vitro* (Winkel 2020). These findings underline the importance of the skin stage in malaria, and could prove especially important in the field of vaccine development, where intradermal immunization has readily been shown to enhance vaccine potency for a great many diseases[21-23]. In this study, we take the first steps to characterize human dermal immune responses after intradermal delivery of *Pf* SPZ. In order to address the immunological basis for the reduced protectivity of needle administered SPZ we compared needle injected and mosquito bite administered parasites. To the best of our knowledge this is the first study examining dermal immune responses to SPZ using human skin explants.

Overall, we see very few differences in the APC responses of our *ex vivo* skin explant model. A possible explanation could be that the explant emigration assay is not sensitive enough to pick up clear differences after three days of APC emigration. Another explanation is that the lack of vascularization in our setup excludes recruited blood-resident immune cells such as granulocytes and monocytes (including monocyte derived APCs). In spite of these limitations, a similar setup has been used to study APC responses to other pathogens such as Schistosoma mansoni cercariae and candida[24,25]

as well as in vaccine delivery studies[26]. Nevertheless, SPZ are single cell protozoa measuring 1x10 µm. Much smaller than the multicellular cercariae reaching 500 µm length or hyphae-forming candida. With a roughly similar number of parasites per biopsy (~118 for SPZ, ~140 for cercaria), the antigen load using cercariae is much higher than that of SPZ. This low SPZ antigen load however, is representative for both natural infection (mosquito bite) as well as attenuated SPZ vaccine trials (needle delivery). The discrepancy between responses to other pathogens and our findings with SPZ leads us to hypothesize the regulatory, suppressive propensity of *Pf* SPZ in the skin.

Previously, we demonstrated that SPZ are capable to induce regulatory macrophages *in vitro*, which can suppress subsequent antigen-specific $CD8^+$ T cell responses (winkel 2020). These macrophages express high levels of activation marker CD80 as well as regulatory marker PD-L1. Additionally, they produce IL-10 and display reduced motility. These features align them with regulatory macrophages described in the context of tumor immunology[27,28]. In our current study, we show decreased $CD14^+$ APC emigration after needle injection of compounds. $CD14^+$ APCs have been transcriptionally aligned to human monocytes and macrophages and have been postulated to be (related to) monocyte-derived macrophages[29-31]. We therefore suggest that needle injection results in increased immunoregulation compared to mosquito bite. This effect is already seen in SGE delivery; however, it becomes more evident with SPZ inoculation.

We hypothesize that immune regulation starts at the earliest interaction of SPZ with the immune system of the host, where we propose a central role for regulatory macrophages. When SPZ are administered via needle injection, these regulatory macrophages are induced and remain inside the dermis due to their decreased motility. Here they can locally exert their immuno-suppressive effect, eventually resulting in the hindering of the adaptive immune response. We theorize that this regulation depends on the route of administration of SPZ due to a number of differences:

Firstly, the total SPZ load administered by needle injection greatly exceeds that of MB injection per biopsy. A higher SPZ load may proportionally result in increased immune-regulation within the skin. Especially the ratio between migrating (pro-inflammatory) and dermally-residing (regulatory) SPZ might be critical. Mosquito bite delivery deposits SPZ in close proximity to blood vessels as the mosquito probes, increasing their emigrating potential. In addition, it has been postulated that mosquito delivery of SPZ could select for the most mature and most motile SPZ to be injected. SPZ maturing inside the mosquito migrate to the salivary gland ducts and it is thought that the most mature SPZ are the first to be expelled in salivation[32]. In contrast, by extracting whole salivary glands from mosquitoes and injecting the homogenized product, this

selection is lost. Arguably, immature SPZ showing reduced migratory capacity are maintained within the skin longer and increase antigen exposure resulting in increased regulation. Secondly, there are large differences in the volumes used. Mosquitoes inject less than 1ul of saliva whereas standard intradermal injections used in vaccinations are 50µl[33]. The larger volumes may result in flooding in the skin which hampers SPZ (and potentially also DDC) migration[34], resulting in increased antigen exposure at the inoculation site and decreased migration to either the skin draining lymph node or the liver. Corroborating this, 50µl needle injection of SGE also resulted in suppressed emigration of DDCs. Thirdly, the differences in depth of injection between mosquito bite and needle may bring SPZ in contact with different DDC subsets[30]. As DDC subsets can respond differently to antigens, activation or bypassing of certain subsets may influence the final immunological outcome[31,35-37].

In summary, we took the first steps in characterizing human dermal immune responses after SPZ delivery directly in human skin explants. Although overall responses to SPZ were very limited, the decreased $CD14^+$ emigration after needle injection of SPZ corresponds with our previous *in vitro* findings and could indicate a similarly regulatory effect within skin. Our study revealed differences in DDC migration after antigen delivery via two main routes of administration, with suppression most evident comparing needle to mosquito bite delivery of SPZ. More studies are needed to determine the nature and role of dermal macrophages in the route of administration of SPZ vaccines. Future studies will include biopsy lysis and analysis of cellular markers within the skin biopsy. Ideally, *in vivo* studies should be conducted to include vascularization and cellular influx. Alternatives for dermal immunity studies that can be translated to human infection could be the use of non-human primates[38,39] or controlled human malaria infections[40]. Using advanced imaging techniques on skin biopsies as well as flow cytometric or Cytof analysis, these models could elucidate the human skin stage of malaria in the *in vivo* setting.

SUPPLEMENTARY NOTES

Author contributions: The methodology was developed by BW, , EJ and MR. Experiments were performed and interpreted by BW, LP, RS, EB, MG, HG, GG, RvS, ML, SC, BF and MR and supervised by MY, BE, BF and MR. BW and MR drafted the manuscript. All authors reviewed and contributed to finalizing the manuscript.

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Ethics statement: The use of human skin explants (obtained as waste material after abdominal reduction surgery) for this research was approved by the Commission Medical Ethics (CME) of the LUMC, Leiden. Approval number CME: B18-009.

Conflict of interest statement: The authors declare no conflict of interest.

REFERENCES

1 Matsuoka, H., Yoshida, S., Hirai, M. & Ishii, A. A rodent malaria, Plasmodium berghei, is experimentally transmitted to mice by merely probing of infective mosquito, Anopheles stephensi. Parasitol Int 51, 17-23, doi:10.1016/s1383-5769(01)00095-2 (2002).

2 Sidjanski, S. & Vanderberg, J. P. Delayed migration of Plasmodium sporozoites from the mosquito bite site to the blood. Am J Trop Med Hyg 57, 426-429, doi:10.4269/ajtmh.1997.57.426 (1997).

3 Choumet, V. et al. Visualizing non infectious and infectious Anopheles gambiae blood feedings in naive and saliva-immunized mice. PLoS One 7, e50464, doi:10.1371/journal.pone.0050464 (2012).

4 Roestenberg, M. et al. Protection against a malaria challenge by sporozoite inoculation. N Engl J Med 361, 468-477, doi:10.1056/NEJMoa0805832 (2009).

5 Medica, D. L. & Sinnis, P. Quantitative dynamics of Plasmodium yoelii sporozoite transmission by infected anopheline mosquitoes. Infect Immun 73, 4363-4369, doi:10.1128/IAI.73.7.4363-4369.2005 (2005).

6 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).

7 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).

8 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).

9 Amino, R., Thiberge, S., Shorte, S., Frischknecht, F. & Menard, R. Quantitative imaging of Plasmodium sporozoites in the mammalian host. C R Biol 329, 858-862, doi:10.1016/j.crvi.2006.04.003 (2006).

10 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).

11 Garcia, J. E., Puentes, A. & Patarroyo, M. E. Developmental biology of sporozoite-host interactions in Plasmodium falciparum malaria: implications for vaccine design. Clin Microbiol Rev 19, 686-707, doi:10.1128/CMR.00063-05 (2006).

12 Pasparakis, M., Haase, I. & Nestle, F. O. Mechanisms regulating skin immunity and inflammation. Nat Rev Immunol 14, 289-301, doi:10.1038/nri3646 (2014).

13 Kashem, S. W., Haniffa, M. & Kaplan, D. H. Antigen-Presenting Cells in the Skin. Annu Rev Immunol 35, 469-499, doi:10.1146/annurev-immunol-051116-052215 (2017).

14 Mestas, J. & Hughes, C. C. Of mice and not men: differences between mouse and human immunology. J Immunol 172, 2731-2738 (2004).

15 Treuting, P. M., Dintzis, S. M. & Montine, K. S. Comparative Anatomy and Histology: A Mouse and Human Atlas. Academic Press, Elsevier Chapter 24, 433-441 (2017).

16 Ponnudurai, T., Leeuwenberg, A. D. & Meuwissen, J. H. Chloroquine sensitivity of isolates of Plasmodium falciparum adapted to in vitro culture. Trop Geogr Med 33, 50-54 (1981).

17 Ponnudurai, T. et al. Infectivity of cultured Plasmodium falciparum gametocytes to mosquitoes. Parasitology 98 Pt 2, 165-173 (1989).

18 Jin, Y., Kebaier, C. & Vanderberg, J. Direct microscopic quantification of dynamics of Plasmodium berghei sporozoite transmission from mosquitoes to mice. Infect Immun 75, 5532-5539, doi:10.1128/IAI.00600-07 (2007).

19 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).

20 Obeid, M. et al. Skin-draining lymph node priming is sufficient to induce sterile immunity against pre-erythrocytic malaria. EMBO Mol Med 5, 250-263, doi:10.1002/emmm.201201677 (2013).

21 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).

22 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).

23 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).

24 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).

25 Maboni, G. et al. A Novel 3D Skin Explant Model to Study Anaerobic Bacterial Infection. Front Cell Infect Microbiol 7, 404, doi:10.3389/fcimb.2017.00404 (2017).

26 Duinkerken, S. L., J; Stolk, D; Vree, J; Ambrosini, M; Kalay, H; van Kooyk, Y. Comparison of intradermal injection and epicutaneous laser microporation for antitumor vaccine delivery in a human skin explant model. BioRxiv, doi:https://doi.org/10.1101/861930 (2019).

27 Noy, R. & Pollard, J. W. Tumor-associated macrophages: from mechanisms to therapy. Immunity 41, 49-61, doi:10.1016/j.immuni.2014.06.010 (2014).

28 Wynn, T. A. & Vannella, K. M. Macrophages in Tissue Repair, Regeneration, and Fibrosis. Immunity 44, 450-462, doi:10.1016/j.immuni.2016.02.015 (2016).

29 McGovern, N. et al. Human dermal CD14(+) cells are a transient population of monocyte-derived macrophages. Immunity 41, 465-477, doi:10.1016/j.immuni.2014.08.006 (2014).

30 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).

31 Haniffa, M. et al. Differential rates of replacement of human dermal dendritic cells and macrophages during hematopoietic stem cell transplantation. J Exp Med 206, 371-385, doi:10.1084/jem.20081633 (2009).

32 Vanderberg, J. Development of infectivity by the Plasmodium berghei sporozoite. J Parasitol 61, 43-50 (1975).

33 Roestenberg, M. et al. Controlled human malaria infections by intradermal injection of cryopreserved Plasmodium falciparum sporozoites. Am J Trop Med Hyg 88, 5-13, doi:10.4269/ajtmh.2012.12-0613 (2013).

34 Vanderberg, J. P. Imaging mosquito transmission of Plasmodium sporozoites into the mammalian host: immunological implications. Parasitol Int 63, 150-164, doi:10.1016/j.parint.2013.09.010 (2014).

35 Klechevsky, E. et al. Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. Immunity 29, 497-510, doi:10.1016/j.immuni.2008.07.013 (2008).

36 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).

37 Chu, C. C. et al. Resident CD141 (BDCA3)+ dendritic cells in human skin produce IL-10 and induce regulatory T cells that suppress skin inflammation. J Exp Med 209, 935-945, doi:10.1084/jem.20112583 (2012).

38 Martinelli, A. & Culleton, R. Non-human primate malaria parasites: out of the forest and into the laboratory. Parasitology 145, 41-54, doi:10.1017/S0031182016001335 (2018).

39 Avci, P. et al. Animal models of skin disease for drug discovery. Expert Opin Drug Discov 8, 331-355, doi:10.1517/17460441.2013.761202 (2013).

40 Roestenberg, M., Kamerling, I. M. C. & de Visser, S. J. Corrigendum: Controlled Human Infections As a Tool to Reduce Uncertainty in Clinical Vaccine Development. Front Med (Lausanne) 6, 23, doi:10.3389/fmed.2019.00023 (2019).

SUPPLEMENTARY INFORMATION

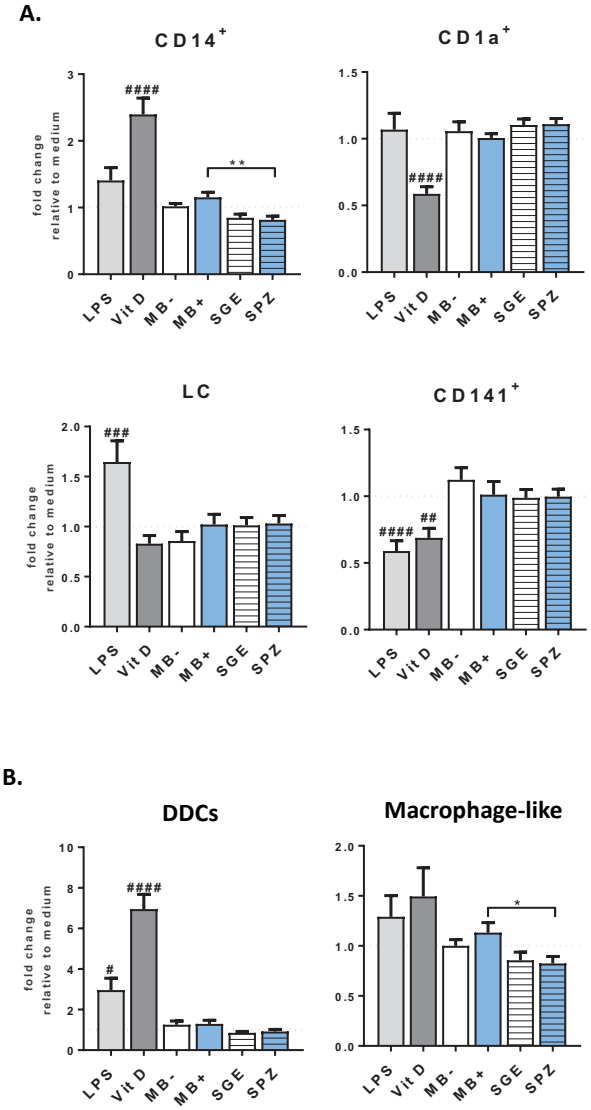

Figure S1. Small increase in macrophage like emigrated APCs after MB injection of SPZ compared needle injection. A. Subset distribution of emigrated DDCs. Statistical testing using one way ANOVA. **B.** $CD14^+$ subset consists of $CD14^+$ DDCs (CD14+ $Autofluorescence^-$) and Macrophage-like APCs ($CD14^+$, $Autofluorescence^+$).

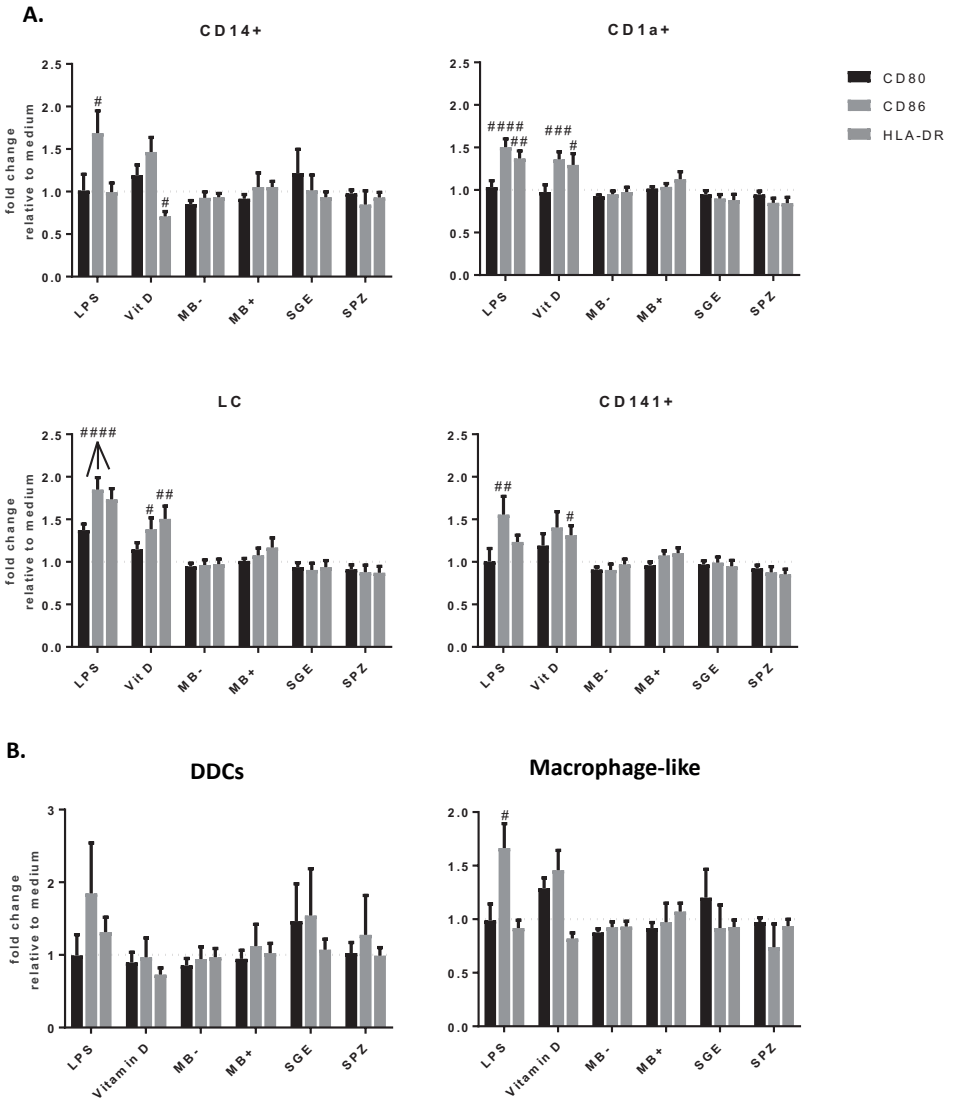

Figure S2. Activation status of emigrated DDCs. A. Changes in activation marker expression after exposure to SPZ delivered either via mosquito bite (MB+)or needle (SPZ), or controls (LPS, Vitamin D and uninfected controls (MB- and SGE respectively). Data shown as CD80, CD86 and HLA-DR expression over the four main DDC subsets. **B.** Activation markers of all DDC subsets combined (left) and on the macrophage-like, auto fluorescent CD14+ population alone (right). Statistical testing using one way ANOVA.

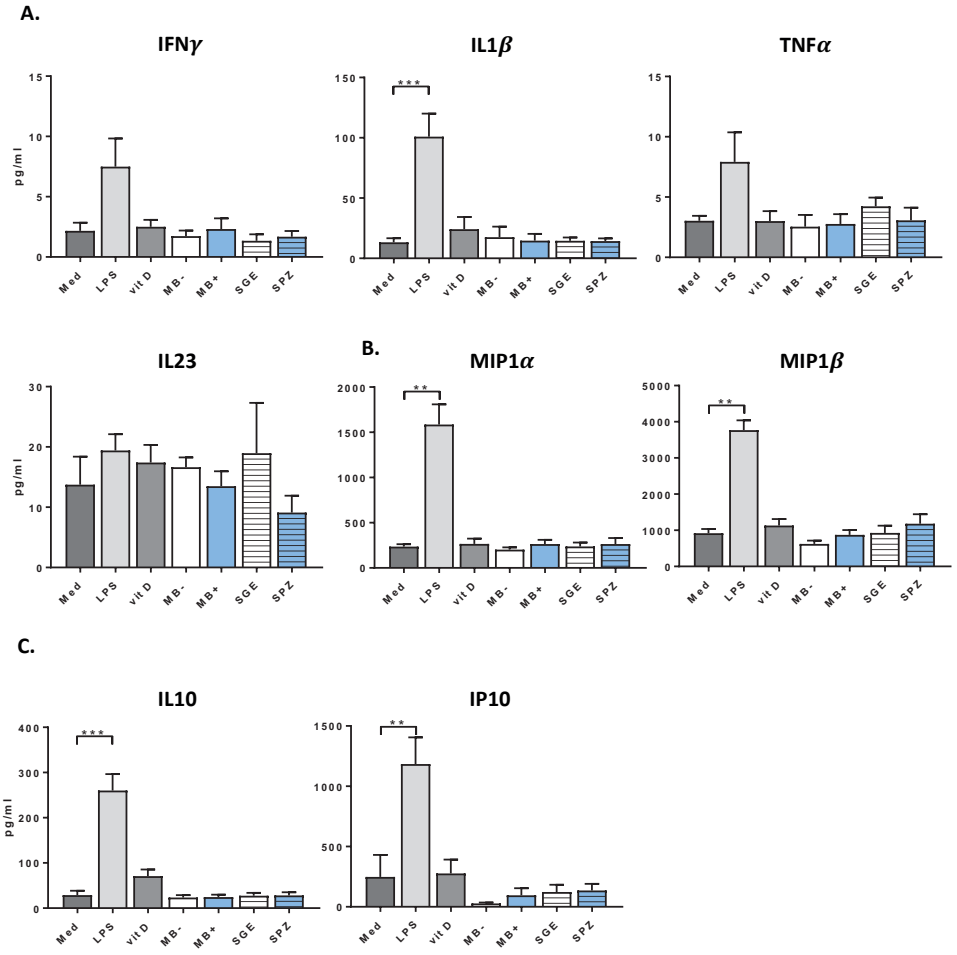

Figure S3. No changes in whole biopsy cytokine and chemokine environment after SPZ inoculation. A. Pro-inflammatory cytokines. **B.** Proinflammatory chemokines. **C.** Regulary cytokine IL10 and chemokine IP10. Testing using Kruskal-Wallis test (unpaired one way ANOVA non-parametric).

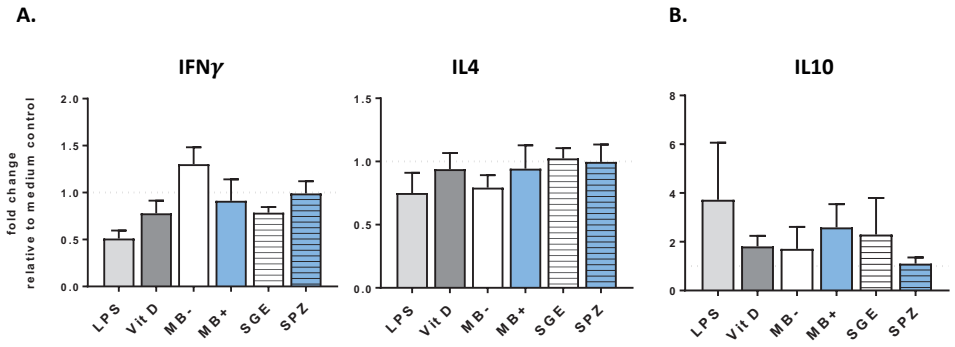

Figure S4. No skewing of naïve CD4^{+} T cells by stimulated DDCs. A. Naïve CD4 T cell polarization. **B.** IL-10 production by naïve CD4 T cells (aCD3/28 stimulation)