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MAJOR ARTICLE

Comparison of CD4 T-Cell Response in *Plasmodium falciparum* and *vivax* Malaria

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Background. *Plasmodium falciparum* and *vivax* are parasites responsible for most malaria cases globally. In areas where these species coexist, individuals gain protection from *P vivax* more rapidly, and important biological differences between species may affect immune responses. CD4 T cells are key drivers of immunity to malaria as effector and helper cells, with T follicular helper cells having key roles in antibody development. Comparative studies on CD4 T cell responses between these species are limited.

Methods. We assessed CD4 T cells in adults with either *P falciparum* or *P vivax* malaria. Activation and proliferation of CD4 T cells were measured ex vivo, and functional capacity was determined by intracellular cytokine staining via flow cytometry.

Results. The phenotype, activation, and proliferation of CD4 T cell subsets were largely comparable between species. However, within the peripheral T follicular helper (pTfh) cell compartment, there was some evidence for species-dependent activation, with relatively increased pTfh1 cells in *P falciparum* infection. Additionally, in *P falciparum*, increased IL-10 production was detected, including within IL-21–producing CD4 T cells.

Conclusions. While activation and function of CD4 T cells in malaria are largely comparable, some species-dependent responses are detected within the pTfh-cell compartment that may affect antibody development.

This study directly compares CD4 and T follicular helper cell responses in acute *Plasmodium falciparum* and *vivax* infections, identifying largely shared activation profiles but distinct responses associated with peripheral T follicular helper 1 and IL-10 in *P falciparum* malaria.

Keywords. CD4; *falciparum*; malaria; T follicular helper cells; *vivax*.

Malaria is a disease of global importance, with *Plasmodium falciparum* and *vivax* infection causing significant disease morbidity and mortality [1]. In areas where both species exist, individuals develop immunity faster to *P vivax* irrespective of transmission intensity as compared with *P falciparum* [2, 3]. Rapid acquisition of immunity to *P vivax* is linked to higher genetic diversity when compared with *P falciparum* [4]. Nonetheless, there are important biological differences between the species, such as *P vivax* hypnozoite–mediated relapses [5] and splenic life cycle [6], which could contribute to immune acquisition. Direct comparative studies in controlled human malaria infection (CHMI) have suggested that parasite species mediate differences in cellular responses [7]. Whether these differences are also observed in natural infection is unknown.

CD4 T cells play important roles in naturally acquired and vaccine-induced malaria immunity [8]. IFNγ+ from CD4 T cells and other cell subsets can drive parasite clearance through activation of macrophages [9], and Th1 CD4 T cells, which produce IFNγ, are associated with protection to *P falciparum* infection [10–15]. While data are limited in *P vivax* malaria, the frequency of Th1 CD4 T cells negatively correlates with parasitemia in endemic settings [16], suggesting that Th1 cells play important roles in immunity to both species. T follicular helper (Tfh) CD4 T cells are essential in malaria immunity by providing help to B cells to produce antibodies [17–19]. Activated peripheral Tfh (pTfh; CXCR5+PD1+) expands during *P falciparum* [19–21] and *P vivax* infection [16, 22]. pTfh can

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be categorized into subsets based on the expression of CXCR3 and CCR6 (pTfh1, CXCR3+CCR6−; pTfh17, CXCR3−CCR6+; pTfh2, CXCR3−CCR6−), with different subsets associating with antibody development in a pathogen-dependent manner [23]. In *P vivax* and *P falciparum* malaria, pTfh responses are dominated by pTfh1 [16, 19, 21, 22]. Aside from Th1 and pTfh, other CD4 T cells with roles in malaria immunity include type 1 regulatory (Tr1) T cells, which coproduce IL-10 with IFNγ and dominate the immune response in children [14, 24–27]. These cells are associated with protection from clinical malaria, possibly at the expense of parasite clearance [28]. FoxP3+ T regulatory (Tregs) cells also expand during malaria, although these cells are reduced with continuous exposure [29, 30].

To date, few studies have investigated species-associated differences in CD4 T cells during *P falciparum* and *P vivax* malaria. A recent study using *P falciparum* and *P vivax* blood-stage CHMI in naive individuals highlighted potentially important differences in the CD4 T cell response [7]. *P vivax* infection induced a comparatively higher systemic type I inflammatory response, increased Th1 transcriptional profile, and elevated IFNγ and CXCL9, whereas *P falciparum* induced greater T-cell activation and terminal differentiation during acute infection, including within the Treg compartment. pTfh was not investigated in this study, and to the best of our knowledge, no comparative studies of pTfh in the 2 species have been reported nor have differences between the infections been comprehensively investigated in naturally acquired malaria.

MATERIALS AND METHODS

Ethics Statement

Written informed consent was obtained from all study participants. Studies were approved by the Northern Territory Department of Health and Menzies School of Health Research (Darwin, Australia; HREC 05-16, HREC 03-64, HREC 10-1397, QIMR Berghofer HREC P3444 and HREC P1479), Alfred Hospital Ethics Committee (188/23), the Indonesian National Institute of Health Research and Development (Jakarta, Indonesia; NIHRD KS.02.01.2.1.4042), and the Oxford Tropical Research Committee (Oxford, United Kingdom; OXTREC 013-04).

Study Participants

Peripheral blood mononuclear cells (PBMCs) were obtained from studies conducted in Timika between 2004 and 2007 to assess antimalarial drug efficacy in patients with blood smear–confirmed *P falciparum* or *P vivax* infection and fever within the last 48 hours [31–33]. Timika is coendemic for *P falciparum* and *P vivax*, with perennial transmission and minimal seasonal variation. Between April 2004 and March 2006, the incidence rate of malaria was 234 cases per 1000 person-years for *P falciparum* and 152 for *P vivax* [34]. For the current study, PBMC samples were from adults with *P falciparum* or *P vivax* monoinfection, as selected by sample availability. All adults with malaria were considered clinically nonimmune on the basis of clinical presentation. Healthy endemic adults from the same study site were malaria negative by blood smear, with no history of malaria in the previous month. For individuals who were malaria naive, PBMCs were collected from healthy Australian adults with no history of malaria exposure.

Enzyme-Linked Immunosorbent Assay

Total plasma IgG specific to recombinant *P falciparum* and *P vivax* apical membrane antigen 1 (AMA1) was quantified by a 2-step enzyme-linked immunosorbent assay. MaxiSorp 96-well plates (Nunc) were coated with AMA1 (0.5 μg/mL) and incubated overnight at 4 °C. Plates were washed with phosphate-buffered saline (PBS)–Tween (0.05% v/v) and blocked with PBS-casein sodium salt (1% w/v) for 2 hours at 37 °C. Plasma was incubated (1:100) for 1 hour at room temperature. Plates were washed and IgG quantified by mouse anti-human IgG (H + L, MII0303, 1:8000; Thermo Fisher) and goat anti-mouse horseradish peroxidase (AP308P, 1:8000; Merck), detected with TMB, and stopped with 1M HCl. Optical density was measured at 450 nm. PfAMA1 and PvAMA1 were produced in EXPI293F-BirA cells.

Flow Cytometry

PBMCs were thawed in RPMI 1640 media (Gibco), L-glutamine, 25nM HEPES, 10% fetal calf serum, and 0.02% benzonase nuclease. Cells were resuspended at 1×10^6 cells/100 μL in 96-well plates and rested for 2 hours at 37 °C, 5% CO_2. Cells were stained with anti-CCR7 for 45 minutes at 37 °C, followed by surface antibody staining for 15 minutes at room temperature (Supplementary Tables 4 and 5). Intracellular staining was performed with FoxP3 Fix/Perm staining (eBioscience; Supplementary Tables 3 and 4).

For cytokines, 1×10^6 cells/100 μL were rested overnight at 37 °C, 5% CO_2, and stimulated with PMA (25 ng/mL) and ionomycin (1 μg/mL) for 6 hours. Brefeldin A and monensin were added after 2 hours. Antibodies to CXCR3, CCR6, CCR7, and CD107a and human Fc block (BD Biosciences) were added during stimulation. After stimulation, cells were stained for 15 minutes at room temperature with Live/Dead Blue (Invitrogen) and then with surface antibodies. Intracellular staining was performed with the BD Cytofix/Cytoperm kit (BD Biosciences). Antibody details are in Supplementary Table 5. For all panels, cells were resuspended in 2% fetal calf serum/PBS and acquired with Gallios (Beckman Coulter) for subcohort 1 and Aurora (Cytek) for subcohort 2. For Gallios, conventional compensation was applied with single-stained compensation beads (BD Biosciences). For Aurora, spectral unmixing with SpectroFlo (Cytek) was used on single-stained or unstained controls.

For manual gating, populations were gated with FlowJo (version 10.7.2) as indicated (Supplementary Figures 2A, 3, 5A, 9A). A minimum of 5000 live CD4 T cells per sample was required for the analysis. In subcohort 1, samples in which CXCR3 and CCR6 staining failed were excluded for Th-cell subset analyses (*P falciparum*, n = 6; *P vivax*, n = 4).

Unsupervised clustering was performed in FlowSOM [35] and projected on dimensional reduction in UMAP [36] via R Spectre (version 1.1.0) [37]. Nonnaive CD4 T cells (Supplementary Figure 5A) were exported from FlowJo. For FlowSOM, the random seed was set as default, while the metaclusters *k* value was set at 40 to enable overclustering. Cells were clustered by expression of FOXP3, CD45RA, CD25, CCR4, CCR6, CXCR5, CXCR3, and CD127. Clusters were individually grouped and annotated by marker expression. Annotated cell clusters were visualized on UMAP at 10 000 total cells. For CD38, ICOS, Ki67, CCR7, and CD161, expression was identified by positive expression (Supplementary Figure 5F) and for granzyme B with median fluorescent intensity.

Statistics

Group comparisons (naive, endemic control, *P falciparum*, and *P vivax*) were made by an unpaired Wilcoxon test. Correlations were performed per Spearman rank (version 0.7.2; rstatix). Principal component analysis (PCA) was calculated via singular value decomposition and plotted with factoextra (version 1.0.7). All analyses were performed in R (version 4.3.3).

RESULTS

Study Cohort

To investigate species-dependent impacts of malaria on CD4 T cell activation, we analyzed cells in adults with clinical *P falciparum* and *P vivax* malaria and compared them with uninfected endemic controls or malaria-naive individuals [31–33] (subcohorts 1 and 2; Supplementary Tables 1 and 2). As a surrogate of prior malaria exposure, we measured total IgG to *P falciparum* and *P vivax* AMA1 in endemic controls and infected individuals (Supplementary Figure 1). Among controls, there was a range of IgG to PfAMA1 and PvAMA1, consistent with previous exposure. For PfAMA1 IgG, persons with *P falciparum* had higher responses than those with *P vivax* but similar levels to controls. PvAMA1 IgG was similar across all groups. Despite not reaching statistical significance, IgG to PfAMA1 in *P falciparum* cases and IgG to PvAMA1 in *P vivax* cases were higher than other groups, suggesting boosting from current infection (Supplementary Figure 1). Data suggest that all individuals had prior exposure to both species, consistent with previous analysis of PfMSP5 and PvMSP5 IgG in the same parent study [38].

Activated CD4 T Cells Are Comparable Between *P falciparum* and *P vivax* Malaria

We analyzed CD4 T cells to identify Tregs (FOXP3+CD25+), pTfh (CXCR5+PD1+), and T-helper subsets (Th1, CXCR3+CCR6–; Th17, CXCR3–CCR6+; Th1–17, CXCR3+CCR6+), along with a chemokine-negative subset that contained naive CD4 T and Th2 cells (CXCR3–CCR6–). Activation and proliferation were determined by ICOS and Ki67 (Supplementary Figure 2A). ICOS+ and Ki67+ CD4 T cells were significantly higher in *P falciparum* and *P vivax* malaria as compared with endemic controls but comparable between the species (Figure 1*A*). In CD4 T cell subsets, proportions of pTfh and naive/Th2 were higher in *P falciparum* than controls, with naive/Th2 also increased in *P vivax* malaria (Figure 1*B*). All subsets of CD4 T cells were activated (ICOS+) and proliferating (Ki67+) during malaria, except for Tregs (Figure 1*C*, Supplementary Figure 2B). Between species, no differences were observed across subset frequencies or by cell activation and proliferation, expect for pTfh, where Ki67 expression was higher in *P falciparum* malaria. Furthermore, in proliferating cells, the proportion of pTfh was increased in *P falciparum* (Supplementary Figure 2C). Levels of activation or proliferation on either total CD4 T cells or pTfh were not associated with parasitemia (Supplementary Figure 2D).

Activation of pTfh Cells Is Influenced by the Infecting Species

pTfh cells were categorized into pTfh1 (CXCR3+CCR6–), pTfh2 (CXCR3–CCR6–), pTfh17 (CXCR3–CCR6+), and pTfh1–17 (CXCR3+CCR6+), and activation and proliferation were quantified (Supplementary Figure 3A). The phenotypes of pTfh subsets were significantly different between the species, with a higher proportion of pTfh1 in *P falciparum* (Figure 2*A*). ICOS+ pTfh1 and Ki67+ pTfh1 were also increased during malaria as compared with controls and was higher in *P falciparum* (Figure 2*B* and 2*C*). Correlations were explored among all subsets along with parasitemia and IgG to PfAMA1 and PvAMA1. Across all individuals, including controls, there was a strong correlation among many ICOS+ and Ki67+ subsets, and these were associated with parasitemia (Supplementary Figure 4A). When each species was considered separately, Th1 and pTfh1 responses were strongly correlated in *P falciparum*, but these were not associated with parasitemia (Supplementary Figure 4B). For *P vivax,* there was evidence for a coordinated response, with strong correlations among Th1 and Th17 responses, along with ICOS+ and Ki67+ Th2 and Th17 but not parasitemia (Supplementary Figure 4C). To further explore these data, we performed PCA. The PCA revealed clear separations among controls, *P falciparum*, and *P vivax* (Figure 2*D*). pTfh1-associated responses were the main contributors to *P falciparum* infection, whereas pTfh2-associated responses predominated in *P vivax*.

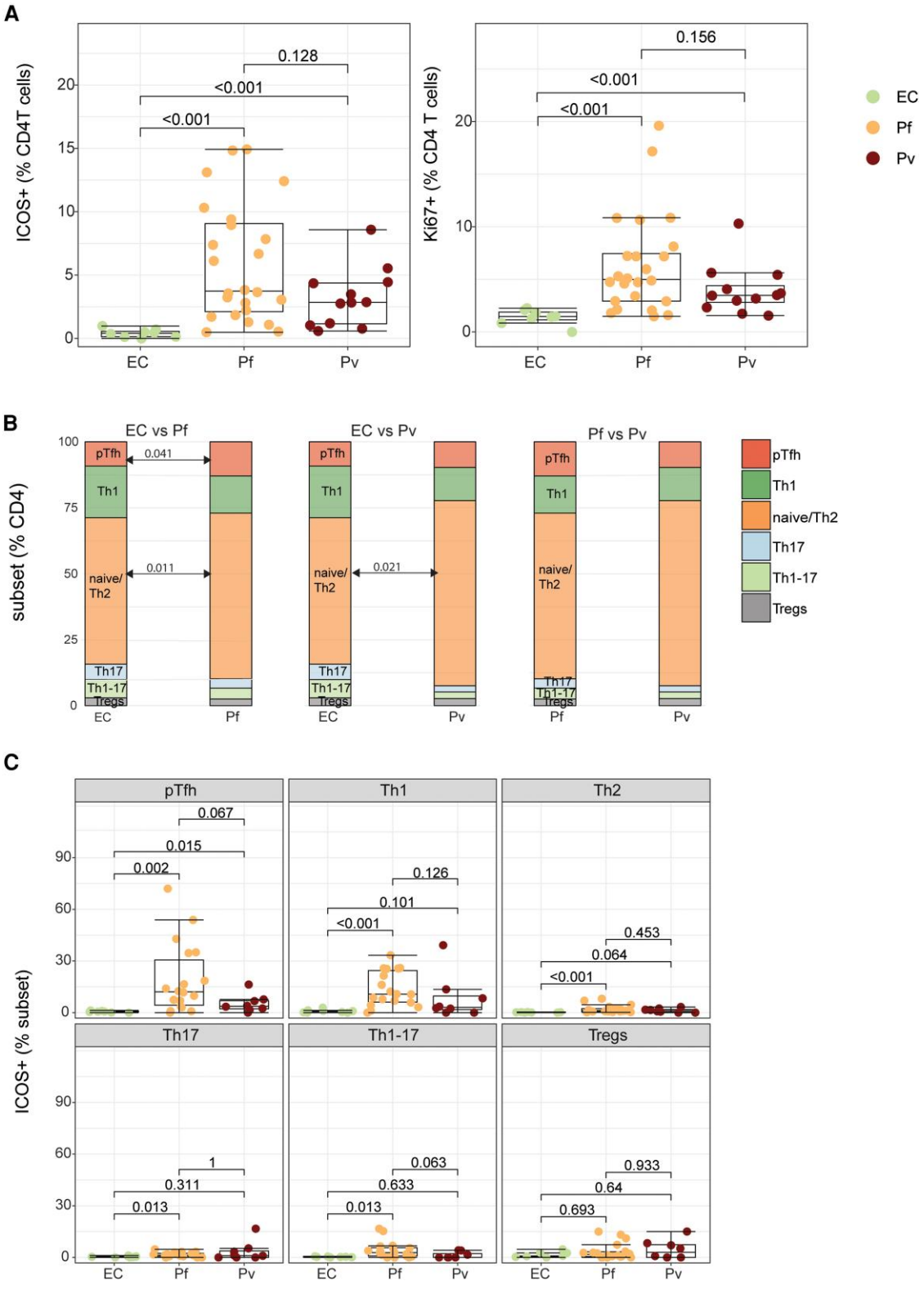

Figure 1. CD4 T cells are activated in *Plasmodium falciparum* and *Plasmodium vivax* malaria. CD4 T cells were assessed in *P falciparum* (Pf; n = 24) or *P vivax* (Pv; n = 12) malaria and in endemic controls (EC; n = 8). *A*, ICOS+ and Ki67+ cells as a proportion of CD4 T cells. *B*, Frequency of CD4 T-cell subsets. *C*, ICOS+ as a proportion of each subset. *A* and *C*: Data are presented as box plots with median (line), first and third quartiles (box), and 1.5 IQR from quartiles (error bars). *B* and *C*: EC, n = 8; Pf, n = 18; Pv, n = 8.

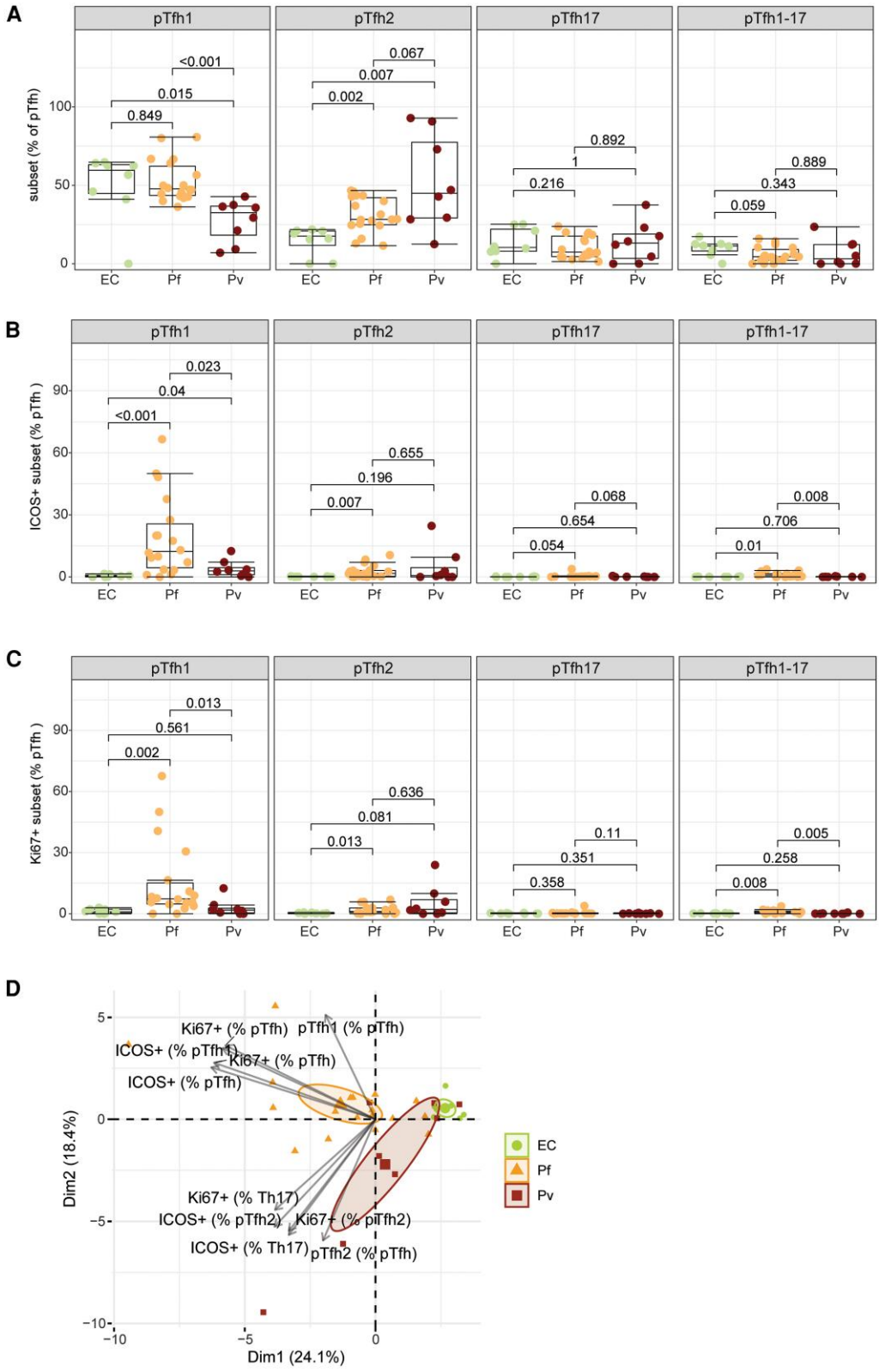

Figure 2. pTfh cells during *Plasmodium falciparum* and *Plasmodium vivax* malaria. pTfh-cell subsets were categorized into pTfh1 (CXCR3+CCR6–), pTfh2 (CXCR3—CCR6–), pTfh17 (CXCR3—CCR6+), and pTfh1–17 (CXCR3+CCR6+). Responses were compared among uninfected endemic controls (EC; n = 8), *P falciparum* (Pf; n = 18), and *P vivax* (Pv; n = 8). *A*, pTfh subsets as a proportion of total pTfh cells. *B* and *C*, Frequency of ICOS+ and Ki67+ pTfh cell subsets as a proportion of total pTfh cells. *A–C*: Data are presented as box plots with median (line), first and third quartiles (box), and 1.5 IQR from quartiles (error bars). *D*, Principal component analysis from all measured variables (parasitemia; cellular and antibody responses). Arrows indicate variable loadings with the top 10 contributors shown. Ellipses show 95% confidence regions for each group centroid. Percentage variance explained is shown on each axis. pTfh, peripheral T follicular helper.

Unsupervised Analysis of CD4 T Cells in *P falciparum* and *P vivax* Malaria

To expand these findings, we designed a comprehensive CD4 T cell panel and analyzed cells with clustering analysis (subcohort 2). Lymphopenia, quantified by the proportion of T cells within all live cells, was evident in *P falciparum* and *P vivax* malaria but was not different between species (Supplementary Figure 5A and 5B). We identified pTfh (CXCR5+), Tregs (CD25+FOXP3+), Temra (CD45RA+), Th1 (CXCR3+CCR6–CCR4–), Th2 (CXCR3–CCR6–CCR4+), Th17 (CXCR3–CCR6+CCR4+), and Th1–17 (CXCR3+CCR6+) subsets (Figure 3A, Supplementary Figure 5C and 5D). The proportions of subsets were largely comparable across groups (Supplementary Figure 5E). The frequencies of ICOS+, CD38+, and Ki67+ pTfh and Th1 were higher in acute *P falciparum* and *P vivax* when compared with controls, but activation was less pronounced in other subsets (Figure 3B, Supplementary Figure 6A–C). The expression of CCR7, CD161, and granzyme B on cell subsets was similar across groups (Supplementary Figure 6D–F). Within pTfh cells, subsets were identified by CXCR3 and CCR6 (Supplementary Figure 7A). While the proportions of pTfh subsets did not vary, higher frequencies of ICOS+, CD38+, and Ki67+ pTfh1 were observed in malaria, regardless of species, as compared with uninfected individuals (Figure 3C, Supplementary Figure 7B–D). ICOS+, CD38+, and Ki67+ frequencies were positively correlated among pTfh subsets, along with parasitemia and AMA1 IgG levels (Supplementary Figure 8A). No clear correlations were observed within *P falciparum* or *P vivax* malaria, possibly due to the limited sample size (Supplementary Figure 8B and 8C). However, PCA identified distinct responses to *P falciparum* and *P vivax* as compared with malaria uninfected controls, driven by cell activation and proliferation (Figure 3D).

Cytokine Production From CD4 T Cells in *P falciparum* and *P vivax* Malaria

To elucidate the functional potential of CD4 T cells, cells were stimulated with PMA/ionomycin and degranulation marker CD107a, as well as IFNγ, TNFα, IL-4, IL-17, IL-21, and IL-10, and quantified (subcohort 2; Supplementary Figure 9A and 9B). Frequencies of IFNγ+, TNFα+, IL-4+, IL-17+, IL-21+, and CD107a+ CD4 T cells were comparable between the endemic control and malaria groups (Figure 4A). In contrast, IL-10+ cells were higher in *P falciparum* than in endemic controls and *P vivax* malaria. IL-21, the hallmark cytokine of Tfh [23], was produced by Tfh (CXCR5+) and non-Tfh cells with similar proportions across groups (Supplementary Figure 10A and 10B). Coproduction of other cytokines with IL-21 can modulate antibody development, and IL-10 frequency within IL-21+ CD4 T cells was higher in *P falciparum* malaria when compared with endemic controls (Figure 4B). IL-21+ and IL-10+ cells also expressed IFNγ, and these cells were increased in *P falciparum* malaria vs endemic controls (Figure 4C). Production of other cytokines with IL-21 was similar across groups, and there were no differences in cytokines from Tfh (CXCR5+) cells (Supplementary Figure 10C and 10D).

Discussion

We analyzed CD4 T cells between naturally acquired *P falciparum* and *P vivax* malaria. Utilizing supervised and unbiased analyses, we show that CD4 T-cell activation and phenotypes are largely similar between *P falciparum* and *P vivax* malaria. Focusing on pTfh, we show some evidence for increased activation of pTfh1 in *P falciparum*. We also observe increased IL-10 production within CD4 T cells and increased IL-10+ coexpression with IL-21+ cells during *P falciparum* malaria as compared with uninfected endemic controls. To the best of our knowledge, this is the first study to directly assess the species-dependent response of pTfh in acute malaria, and taken together, the results inform our understanding of the immune response to acute *P falciparum* and *P vivax* malaria.

CD4 T cells have multiple roles in immunity to malaria, with production of IFNγ by Th1 cells contributing to parasite killing [39] and Tfh having essential roles in antibody development [18]. A recent CHMI study comparing *P falciparum* and *P vivax* malaria responses showed higher CD4 T cell activation and Th1-associated gene signatures in *P falciparum* [7]. In comparison, our study examines naturally acquired malaria, where we observed that the magnitude of CD4 T cell activation and subset distribution was largely species independent. Although history of exposure is unknown in our cohort, individuals are presenting with symptomatic malaria, suggesting that any acquired immunity was not sufficient to control the current infection. Nevertheless, study design and analytic differences, including sample collection time point relative to peak parasitemia, may explain disparity in findings between our study and CHMI studies [7]. Indeed, within the CHMI studies, peak CD4 T cell activation is observed at 6 days after treatment as compared with the acute infection time point analyzed here [7], and lymphopenia at acute infection should be considered when interpreting our findings.

While global CD4 T cell responses were comparable between *P falciparum* and *P vivax* infection, we observed some evidence of a species-dependent response in pTfh. Specific subsets of pTfh have varying capacity to provide B-cell help [23]. Previous studies in *P falciparum* [19, 20] and *P vivax* [16] malaria showed that pTfh1 is the dominant subset. Our data suggest that pTfh in *P falciparum* malaria have a greater skew toward pTfh1 than *P vivax*. We have previously shown that pTfh2 is associated with antibody development in CHMI [19]. While pTfh1 is not associated with antibodies in Malian children [21], pTfh1 and pTfh2 are increased in Ugandan children with the highest levels of protective malaria antibodies [40], and we have recently identified additional diversity with

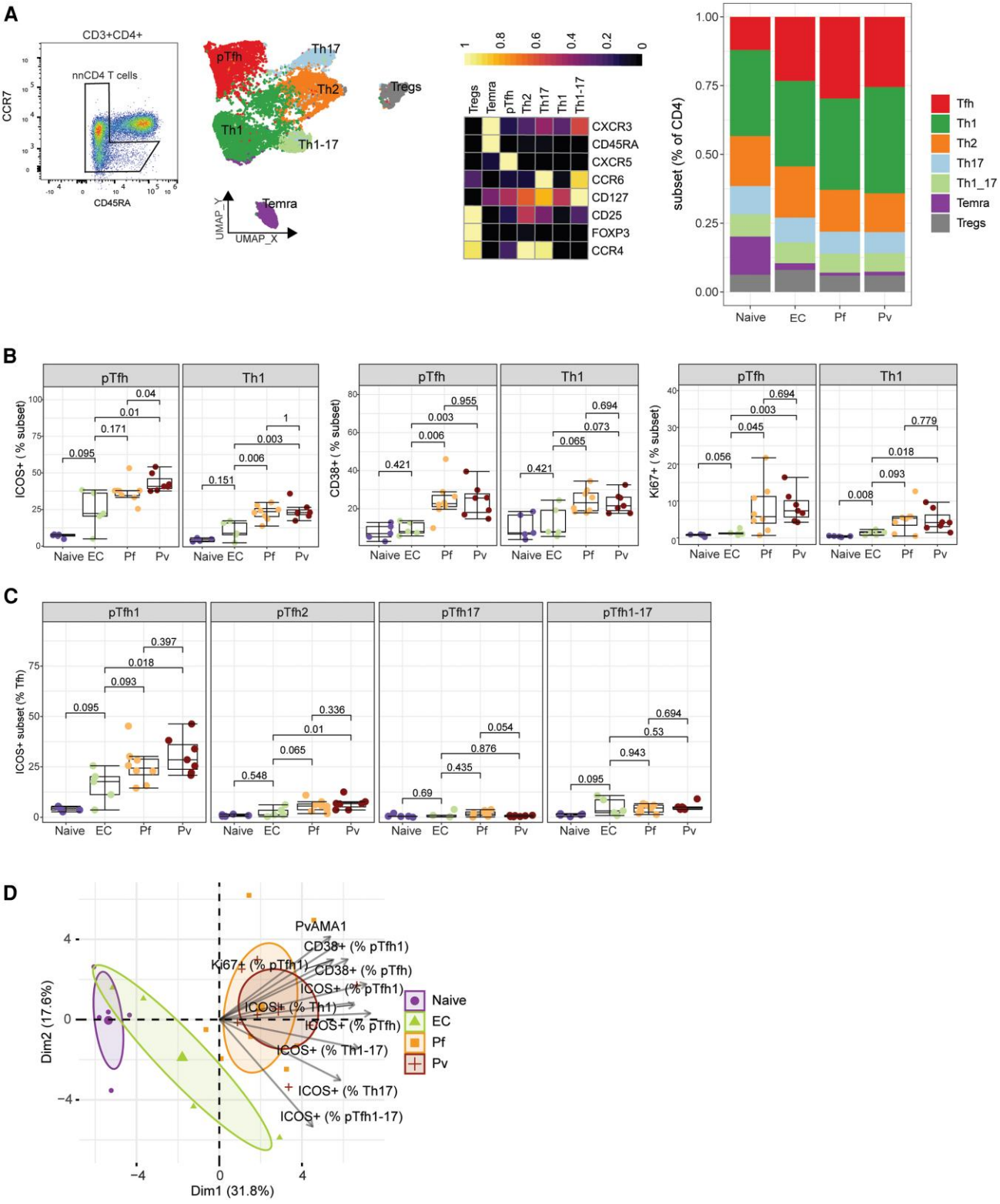

Figure 3. Unsupervised analysis of CD4 T-cell and pTfh subsets. CD4 T cells were analyzed with a comprehensive panel in 4 groups: malaria naive (n = 5), endemic controls (EC; n = 5), *Plasmodium falciparum* (Pf; n = 8), and *Plasmodium vivax* (Pv; n = 7). *A*, Nonnaive CD4+ T cells were identified by CCR7 and CD45RA, analyzed with unbiased clustering, and visualized with UMAP. Heat map of normalized median expression of markers (middle panel) and subsets as percentages of nonnaive CD4 T cells (right panel). *B*, Frequency of ICOS+, CD38+, and Ki67+ pTfh and Th1 cells. *C*, Frequency of ICOS+ pTfh subsets as proportions of total pTfh. *B* and *C*: Data are presented as box plots with median (line), first and third quartiles (box), and 1.5 IQR from quartiles (error bars). *D*, Principal component analysis from all measured variables (ex vivo phenotyping, parasitemia, and antibody response). Arrows indicate variable loadings with the top 10 contributors shown. Ellipses show 95% confidence regions for each group centroid. Percentage variance explained is shown on each axis. pTfh, peripheral T follicular helper.

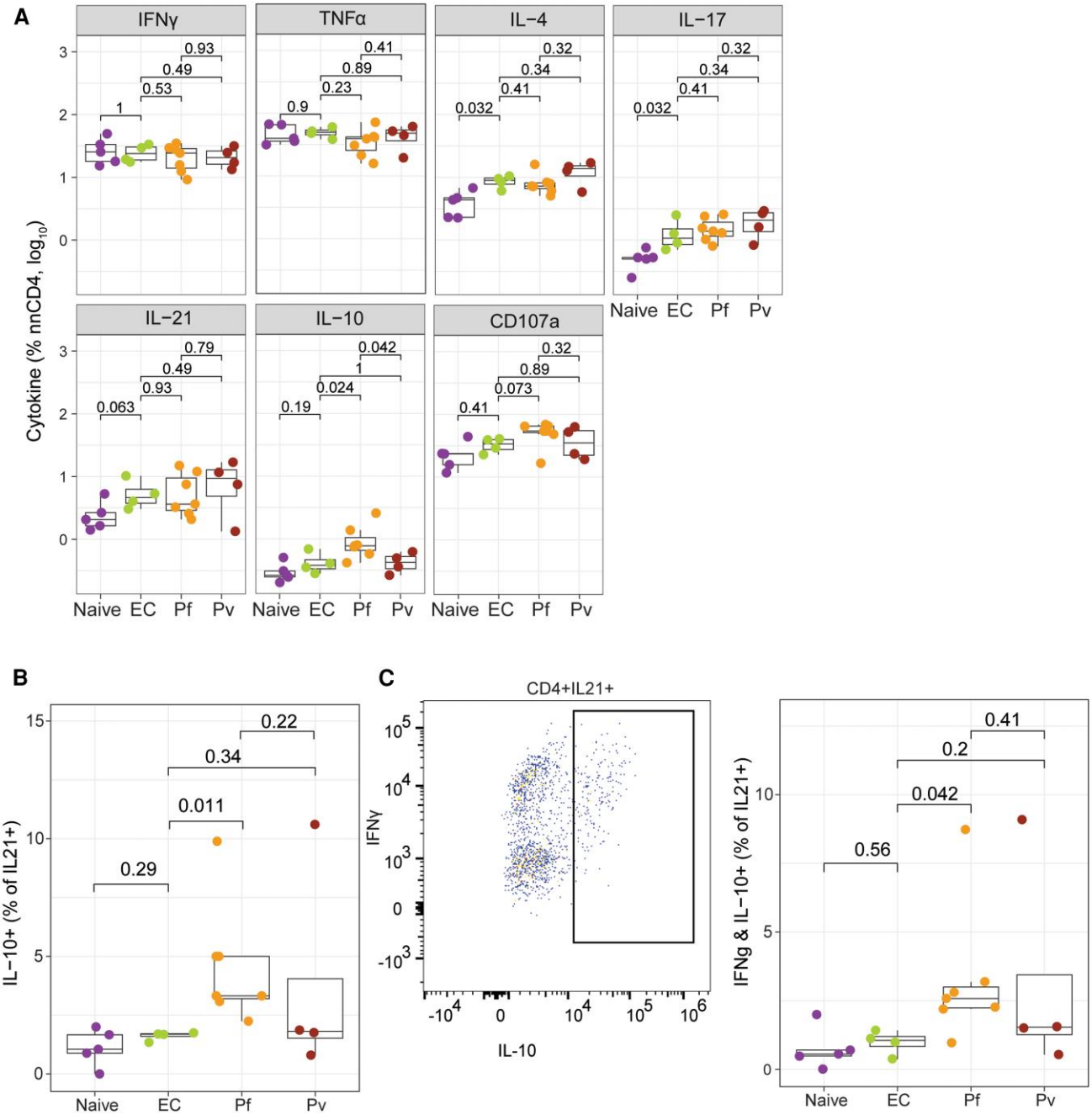

Figure 4. Functionality of CD4 T cells *Plasmodium falciparum* and *Plasmodium vivax*. Peripheral blood mononuclear cells were stimulated with PMA/ionomycin among 4 groups: healthy naive (n = 5), endemic controls (EC; n = 5), *P falciparum* (Pf; n = 7), and *P vivax* (Pv; n = 4). *A*, Cytokine-producing cells in nonnaive CD4 T cells (nnCD4 T cells). *B*, IL-10+ cells within IL-21+ nnCD4 T cells. *C*, Representative plot shows IL-10+ gating strategy and IL-10+/IFNγ+ cells in IL-21+ nnCD4 T cells. Data are presented as box plots with median (line), first and third quartiles (box), and 1.5 IQR from quartiles (error bars).

functional relevance within pTfh during CHMI [41]. Further studies are required to dissect the capacity of antigen-specific Tfh in providing help to B cells and inducing antibodies during malaria and whether this is modulated by different *Plasmodium* species.

We observed some evidence for increased production of IL-10, including within IL21+ CD4 T cells in *P falciparum*. Regulatory Tr1 that secretes IL-10 has been identified as a dominant CD4 T response in highly *P falciparum*–exposed populations [25, 42], and these cells emerge rapidly [43]. Consistent with our findings, IL-10 is coproduced with IL-21+ in pregnant women infected with *P falciparum* [44], and recent studies report significant overlap between Tr1 and Tfh cells in children with high *P falciparum* exposure [28]. Further studies are

required to identify the underlying phenotypes and functions of CD4 T cells that produce IL-10+ and IL-21+ and whether these cells are indeed a Tr1-like Tfh subset. However, IL-10–producing Tfh has been found within human lymph nodes following BNT162b2 mRNA (for SARS-CoV-2) and influenza vaccination [45, 46]. While the function of Tr1-like Tfh in malaria is unknown, in lymphocytic choriomeningitis virus mouse models, these cells support antibody production in the presence of persistent antigen [47]. The role of IL-10–producing Tfh in human malaria infection is a focus of future research.

Study Limitations

Our current study is limited to relatively small sample sizes in a single study site with cross-sectional analysis. While prior exposure is likely similar to both species across our cohort, we are unable to investigate individual exposure histories. Furthermore, parasitemia in all individuals was assessed only via blood smear. It is possible that the control group had low-density parasite infections, and we are unable to quantify non-circulating biomass. Cohorts used here had samples available only at acute infection, at which we observed significant lymphopenia [7, 48]. While lymphopenia was similar between species, we cannot discount differential sequestration of key CD4 T cell subsets. Further analysis of CD4 T cell and Tfh activation in larger cohorts of different demographic profiles and age with more comprehensive clinical data and sample availability post-treatment is required to confirm and understand the broad relevance of our findings, particularly in children [20, 40]. Additionally, our analysis was on total CD4 T cells without considering antigen specificity. While assessing *P falciparum*–specific CD4 T cell responses is possible with infected red blood cell stimulation, this is highly challenging for *P vivax* due to the lack of a continuous in vitro culture system for this parasite.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Data availability statement. All data required for conclusions are available in the article. Additional data are available from the corresponding authors upon reasonable request and appropriate agreements. Flow cytometry files for all data are publicly available at doi:10.5281/zenodo.17567448 (https://zenodo.org/records/17567448).

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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