Figures and figure supplements

Atypical memory B-cells are associated with *Plasmodium falciparum* anemia through anti-phosphatidylserine antibodies

*Juan Rivera-Correa et al*
Figure 1. Specific autoantibodies correlate with malarial anemia in P. falciparum-infected returned travelers. Non-parametric Spearman correlation analysis comparing hemoglobin with (A) parasitemia, (B) anti-PS IgG antibodies, (C) anti-PfEBA IgG antibodies, (D) anti-erythrocyte IgG antibodies and (E) anti-DNA IgG antibodies.

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Figure 2. Plasma from *P. falciparum* patients mediates erythrocyte lysis, which can be partially inhibited by Annexin V. (A,B) Correlation of plasma anti-PS IgG antibodies with the LDH levels (A) or with the erythrocyte lysis capacity (B) of the plasma of *P. falciparum* patients. (C) Complement-mediated lysis of erythrocytes exposing PS by *P. falciparum* patient’s plasma compared to plasma from uninfected controls, expressed as percentage of maximal lysis. (D) Complement-mediated lysis of erythrocytes exposing PS, pre-incubated or not with Annexin V, before incubation with the plasma of *P. falciparum* patients (*n* = 6). Results show the means and standard deviations of triplicated determinations. Significance was assessed by nonparametric Spearman correlation analysis (A,B) or unpaired Student’s t-test (C,D). *p*≤0.05, **p**≤0.01.

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Figure 3. Atypical MBCs expand in \textit{P. falciparum}-infected patients and decline after treatment. (A) Gating strategy for the characterization of FcRL5$^+$ T-bet$^+$ B-cells (CD19$^+$) with representative plots of one uninfected control and one \textit{P. falciparum} patient. (B) Percentage of CD19$^+$ FcRL5$^+$ T-bet$^+$ B-cells in samples from uninfected controls and \textit{P. falciparum} patients. Significance assessed by unpaired Student’s t test. ****p\leq0.0001.

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Atypical MBCs do not correlate significantly with patient background.

Comparison of the percentage of atypical MBCs in the circulation of *P. falciparum* patients by background (visiting friends or relatives (VFR) and tourists). Significant assessed by unpaired Student’s t test.

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Atypical MBCs do not correlate significantly with patient gender. Comparison of the percentage of atypical MBCs in the circulation of *P. falciparum* patients by gender. Significant assessed by unpaired Student’s t test.

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Figure 3—figure supplement 3. The time after treatment at which samples were collected correlates significantly with atypical MBCs but not with hemoglobin levels. Non-parametric Spearman Correlation analysis comparing the days after treatment when samples were collected and levels of (A) atypical MBCs and (B) hemoglobin.

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Figure 4. The atypical MBC subset correlates with the development of anemia in *P. falciparum* patients. Correlation analysis of atypical (A) and classical (B) MBC subsets from the PBMC of *P. falciparum* patients compared with hemoglobin levels. Significance was assessed by non-parametric Spearman correlation analysis.

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Figure 4—figure supplement 1. Gating strategy for relevant B-cell sub-populations. B-cell subpopulations of human PBMC (Weiss et al., 2009). DOI: https://doi.org/10.7554/eLife.48309.016
Figure 4—figure supplement 2. Expression of T-bet in FcRL5+ cells compared to classical MBCs. Significance assessed by unpaired Student’s t test. **p<0.01.
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Figure 4—figure supplement 3. Atypical MBCs do not correlate significantly with parasitemia. Non-parametric Spearman correlation analysis comparing the percentage of atypical MBCs and parasite levels.

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Figure 4—figure supplement 4. Atypical MBCs correlate significantly with patient’s age. Non-parametric Spearman correlation analysis comparing the percentage of atypical MBCs and patient’s age.
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Figure 4—figure supplement 5. Atypical MBCs do not correlate significantly with thrombocyte levels. Non-parametric Spearman correlation analysis comparing the percentage of atypical MBCs and thrombocyte levels in the circulation.

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Correlations of other B-cell subsets with hemoglobin levels in *P. falciparum* patients. Non-parametric Spearman correlation analysis of relevant B-cells subsets from the PBMC of *P. falciparum* patients: (A) naive B-cells (CD27^-CD21^+CD10^-), (B) immature B-cells (CD10^+), and (C) plasma cells (CD27^+CD21^-CD20^-) compared with hemoglobin levels.

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Figure 5. Anti-PS IgG antibodies show distinct correlations with classical and atypical MBC subsets in *P. falciparum* patients. Correlation analysis of levels of (A) atypical and classical (B) MBCs with anti-PS IgG antibody levels from the plasma of *P. falciparum* patients. (C) Correlation analysis of atypical and classical MBC levels. Significance was assessed by non-parametric Spearman correlation analysis.

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Anti-PS IgG antibodies do not correlate with other B-cell subsets in *P. falciparum* patients. Non-parametric Spearman Correlation analysis of (A) naïve B-cells (CD27−CD21+CD10−), (B) immature B-cells (CD10+), and (C) plasma cells (CD27+CD21−CD20−) with anti-PS IgG antibody levels from the plasma of *P. falciparum* patients.

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Correlations of anti-RBC IgG antibodies with B-cell subsets in *P. falciparum* patients. Non-parametric Spearman Correlation analysis of (A) atypical MBCs (CD27^−^CD21^−^FcrL5^+^), (B) classical MBCs (CD27^+^CD21^+^), (C) naive B-cells (CD27^−^CD21^+^CD10^−^), (D) immature B-cells (CD10^+^), and (E) plasma cells or plasmablasts (CD27^+^CD21^−^CD20^−^) with anti-erythrocyte lysate IgG antibody levels from the plasma of *P. falciparum* patients.

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Anti-DNA IgG antibodies do not correlate with the B-cell subsets analyzed in *P. falciparum* patients. Non-parametric Spearman correlation of (A) atypical MBCs (CD27−CD21−FcrL5+), (B) classical MBCs (CD27+CD21+), (C) naive B-cells (CD27−CD21−CD10−), (D) immature B-cells (CD10+), and (E) plasma cells or plasmablasts (CD27−CD21−CD20−) with anti-DNA lysate IgG antibody levels from the plasma of *P. falciparum* patients.

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Figure 6. There is no significant correlation of anti-parasite PfEBA antibodies with relevant B-cell subsets from *P. falciparum* patients. Correlation analysis of (A) atypical MBCs, (B) classical MBCs, (C) plasma cells, (D) naive B-cells, and (E) immature B-cells with anti-*P. falciparum* (PfEBA) IgG antibody levels from the plasma of *P. falciparum* patients. Significance was assessed by non-parametric Spearman correlation analysis.

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Figure 7. *P. falciparum* drives the expansion of human FcRL5⁺ T-bet⁺ B-cells that secrete anti-PS antibodies in vitro. (A) Percentage of T-bet⁺ FcRL5⁺ B-cells that expanded from the PBMCs of a healthy naive donor after in-vitro exposure to either uninfected erythrocyte lysate (uLysate) or *P. falciparum*-infected erythrocyte lysate (iLysate). (B) ELISPOT of enriched populations for either FcRL5 (gray bars) or CD27 (black bars) from PBMCs of healthy naive US donors after in-vitro exposure to *P. falciparum*-infected erythrocyte lysate (iLysate) (N = 3). ASC, antibody-secreting cells. Significance assessed by unpaired Student’s t test. **p < 0.01, ***p < 0.001.

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Figure 7—figure supplement 1. Total antibody-secreting cells among the CD27⁺ and FcLR5⁺-enriched PBMC.
Total number of anti-IgM spots of antibody-secreting cells (ASCs) from either CD27⁺ or FcRL5⁺-enriched PBMC that were stimulated with *P.-falciparum*-infected erythrocyte lysate. Significant assessed by unpaired Student’s t test, ****p<0.0001.
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Figure 7—figure supplement 2. Stimulation with *P. falciparum* Histidine Rich Protein II (HRPII) does not stimulate the expansion of atypical MBCs in vitro. Stimulation in vitro of naïve PBMC from healthy US donors (n = 3) with medium, *P. falciparum* HRPII, uninfected erythrocyte lysate (uLysate) or *P. falciparum*-infected erythrocyte lysate (iLysate). Significance was assessed by one-way Anova. *p<0.05, **p<0.01.

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