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Full Length Research Paper

B cell immunophenotyping reveals decreased frequency of CD11c$^+$ B cells expressing FcRL5$^+$ subset in naturally malaria-exposed Fulani adults

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Abstract

The Fulani populations are more resistant to malaria than genetically distinct populations of the Mossi ethnic group. The Fulani mount an enhanced and broadly reactive immunoglobulin M (IgM). However, which B-cell subsets that are contributing to this enhanced IgM response is not known. In naturally malaria-exposed Fulani (n=30) and Mossi (n=30) adults in Burkina Faso, B cells immunophenotypes were assessed using monoclonal antibodies and flow cytometry. Frequencies of IgM$^+$ naïve B cells ("40.96 ± 1.72" vs "53.84 ± 1.84"; *p=0.0001*), IgM$^+$ activated memory Bcells (MBC) ("17.79 ± 1.09" vs "21.71 ± 1.64"; *p=0.04*), IgM$^+$ atypical MBC ("15.73 ± 1.81" vs "34.64 ± 2.57"; *p=0.00002*) and IgM$^+$ CD11c$^+$ FCRL5$^+$ subsets ("6.73 ± 1.23" vs "19.22 ± 2.40"; *p=0.00013*) were significantly higher in the Fulani than in the Mossi. The frequency of CD11c$^+$ B-cell subset expressing FcRL5 ("34.08 ± 1.70" vs "23.96 ± 1.15"; *p=0.0001*) was significantly lower in the Fulani than in the Mossi. Levels of IgM$^+$ atypical MBC were positively correlated with those of IgM$^+$ CD11c B cells expressing FCRL5$^+$.CD11c$^+$ B cells expressing FcRL5$^+$ subset markedly highlights differences in the Fulani versus the Mossi adults. B cells carrying the FcRL5 negatively affect the acquisition of protective immunity to malaria.

Keywords: CD11c B cells, atypical memory B cells, Fulani, malaria, *P. falciparum*, susceptibility, immunity

INTRODUCTION

Malaria remains a major public health concern to many human populations, despite efforts to eliminate this debilitating and potentially fatal tropical disease. Although, unprecedented success in malaria control, *Plasmodium falciparum (Pf)* claimed about 409 000 deaths and affected 212 million people mainly in sub-Saharan Africa (1).

A need to identify relevant biomarker of protection to *Pf* malaria is a major challenge today for the evaluation of efficient malaria vaccines which may help in the possible eradication of malaria. Immuno-epidemiological studies have offered new insights in the acquisition of natural immunity through multiple *Pf* infections, which suggests new ideas that a protective vaccine is attainable (2). The acquisition of natural immunity through multiple *Pf* infections varies within individuals and different populations. A particular population in West Africa have been identified as Fulani ethnic groups, mounts more broad protective immunity to *Pf* infection (3). In fact, the Fulani had stronger inflammatory immune responses than their neighbor Dogon in Mali or Mossi in Burkina Faso. Looking at antibody responses, the Fulani exhibited a greater enhancement of IgM than IgG responses to a broad array of *Pf* antigens (4). The breadth of the malaria-specific immune response increases with age**,** but plasma IgG responses do not correlate with number of memory B–cells (MBCs) in endemic areas(5). However, natural exposure to malaria parasites induces protective human anti-malarial antibodies (6). This may indicate that long-lived antibody responses are only acquired after years of repeated *Pf* infections and this protective immunity depends on the breadth and the magnitude of protective antibody responses as shown in a cohort study in Mali (7).

Clinical studies of other diseases than malaria have identified flow cytometrically-purified $CD11c^+$ B cells defined by expression of CD11c, T-bet, and other diagnostic cell-surface markers and transcription factors form a discrete B cell subset in response to intracellular bacterial infection (8). Further, these CD11c+ B cells constituted nearly all of the antigen-specific Antibody-Secreting Cells (ASCs) which were detected early during infection in the spleen of mice infected with *Ehrlichia muris* and was shown to be predominant IgM secreting B cells(9). Therefore, a deeper understanding of the molecular and functional characteristics of human $CD11c^+$ B cell expressing $FcRL5^+$ subset in naturally malaria-exposed populations can provide important insights into the development of protective immunity. Interestingly, a study carried out on primary malaria infected naïve individuals identified CD11c as a marker of B cells responding to malaria and further highlighted differences in primary and secondary B-cell responses during infection (10). The formation of this subset of Bcells is triggered by T-Bet expression that involves common initiating features of TLR-driven activation within cytokine milieu such as IL4, IL21 and IFN-γ interaction (11).

Here, we used the B-cell immunophenotyping approach by flow cytometry to characterize the frequency and quality of human anti-malarial B cell subsets that are contributing to the enhanced IgM response to natural *Pf* exposure in Fulani and Mossi adults (20 to 45 years) in Burkina Faso. We found that CD11c+ B cells expressing the FcRL5+ subset markedly highlights differences in Fulani versus Mossi adults suggesting the FcRL5 expression may negatively affect the acquisition of protective immunity to malaria.

MATERIALS AND METHOD

Study Site

The study was carried out in the villages of Barkoundba Peulh and Barkoundba Mossi, located in the Oubritenga Province at 35 km North / East of Ouagadougou Burkina Faso. The study site has been well described and several epidemiological studies have been carried out in this area since 1994 (12).

The study site is located in a savannah area on the central Mossi plateau. The climate is typically sub-Saharan savannah with two seasons: a rainy season from June to October and a dry season from November to May when malaria transmission is low or virtually absent. Malaria is endemic and transmission is high during the rainy season. The annual rainfall varies from 600 to 900 mm. The main vectors of malaria are *Anopheles gambiae*, *A. Arabiensis* and *A. Funestus*. Two chromosome forms of *A. Gambiae* and *A. Funestus* are sympatric in the study area. The annual entomological inoculation rate varies from 10 to 500 infectivity bites per person. *Pf* accounts for more than 90% of malaria infections.

Study population

The populations included in this study live in the villages of Barkoundba Peulh (Fulani ethnic group) and Barkoundba Mossi (Mossi ethnic group) about 5 km apart. All volunteers meeting the inclusion criteria were enrolled to participate in the study. In total 30 Fulani and 30 Mossi adult aged 20–45 years were included in the study.

Study design

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A pilot study enrolling adult patients diagnosed with *Pf* malaria was performed at Barkoundba, Burkina Faso, from 15th to 31st of March 2019. Sixty adult volunteers were included. Study participants were grouped according to ethnicity and malaria infection status. This enabled comparison of immune responses between the two ethnic groups.

Sample collection

Venous whole blood (approximately 18 ml) was collected from study participants, with two additional whole blood spots for diagnosis of *Plasmodium* infection. Thin and thick blood smears were also performed for diagnosis and quantification of malaria parasites by light microscopy. Plasma was collected following centrifugation of venous whole blood, and stored at −80°C. The plasma was subsequently used to measure cytokines responses.

Peripheral blood mononuclear cells (PBMCs) were purified by Ficoll-Paque density centrifugation according to manufacturer's instruction (GE Healthcare, Uppsala, Sweden). One aliquot of PBMC for each participant was cryopreserved in liquid nitrogen for further immunophenotyping assay.

Flow cytometry and B cell immunophenotyping

Frozen PBMCs were thawed in a 37ºC water bath and mixed with an equal volume cold RPMI 1640 Medium supplemented with L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 µg/ml), and 10% heat-inactivated fetal bovine serum (all from Thermo Fischer Scientific). Cells were then rested 15 minutes on ice before being washed twice in RPMI 1640 Medium. After washing, the cells were counted and then stained with Aqua Live/Dead stain (Thermo Fisher Scientific) followed by further washing in RPMI 1640 Medium with 2% FBS. The cells were then incubated for 20 minutes on ice, in 2 steps with 2 washes in between, using an antibody mix targeting primarily B cell surface antigens (Supplemental Table 3). After staining, the cells were washed twice in DPBS with 2% FBS before acquisition on a 3-laser BD LSRII flow cytometer. FlowJo software version 10.4.2 was used to analyze and to define the gate with the gating strategy shown in figure 1A.

T-bet was stained using the intracellular staining technique after staining of the cell surface makers. The cells were then fixed and permeabilized using the FoxP3 buffer staining kit for 30 minutes at room temperature (Thermo Fisher Scientific). After washing, the cells were stained with the antibodies for 30 minutes at room temperature followed by washing and acquisition. To define B–cells subsets, cells were first gated for lymphocytes by forward and side scatter followed by singlets and live cells. B cells were thereafter gated either as $CD19^{+}CD20^{+}$ cells followed by CD10 to distinguish mature ($CD10^{-}$) and immature ($CD10^{+}$) B cells or $CD19^{+}CD20^{lo}$ cells. $CD19^{+}CD20^{lo}$ cells were further gated for $CD27^{+}$ cells, corresponding to plasmablasts/plasma cells

Determination of cytokines in plasma samples using cytometric bead array

Levels of human IL-17A, IFN-γ, TNF, IL-10, IL-6, IL-4 and IL-2 were measured in plasma using cytometric bead array (CBA; BD Biosciences, San Diego, CA, USA) according to the manufacturer's recommendation. Briefly, 24 µL of bead populations with discrete fluorescent intensities coated with cytokine capture antibodies, were added to 24 µL of plasma sample and the mixtures were incubated for 90 min in room temperature. At the same time, standards for each cytokine (range 0-5000 pg/mL) were also mixed with cytokine specific capture beads. After incubation, the samples and standards were washed to remove unbound material and 24 µL of phycoerythrin (PE)-conjugated anti-human inflammatory cytokine antibodies were added where after the mixtures were incubated again for 90 min followed by washings. Then, the samples were acquired on a fluorescent activated cell sorter (FACS) LSR II (BD Biosciences). Samples and standards were analyzed using FCAP Array software v3.0 (BD/Soft Flow Hungary Ltd). Calibration was performed on the flow cytometer before acquisition using BD FACS Comp™ and BD Cali BRITE™.

Statistical analysis

Data were analyzed using GraphPad Prism (version 5.01) and R version 3.5.1 packages. Statistical differences were calculated using one-way analysis of variance (ANOVA) and the Student's *t* test. Nonparametric correlation (Spearman) was used for correlation studies. Values are presented as the mean ± standard deviation (SD). A value of $p < 0.05$ was considered to be statistically significant.

Ethical considerations

The study protocol was reviewed and approved by the Review Board of the University of Sciences, Techniques, Technologies of Bamako (Nº2018/20/CE/FMPOS) Faculty of Medicine, Pharmacy and Dentistry and the Research Ethics Committee of the Ministry of health of Burkina Faso (Nº2018-12-154). Written informed consent was obtained from all study participants. The study was carried out in accordance with the rules of General Data Protection Regulation (GDPR) for personal data use.

RESULTS

Baseline characteristics of the study population

Table I. Antibodies for B-cell Immunophenotyping.

Markers	Mature Cells	BNaive cells	BClassical MBC	Activated MBC	Atypical MBC	Plasmablast/PC	CD11c B cells
CD 10	-	-	-	-	-	-	-
CD 11C	+/-	-	-	-	+	-	+
CD 19	+	+	+	+	+	+	+
CD 20	+	+	+	+	+	-	+
CD 21	+	++	++	-	-	-	-
CD 27	+	-	+	+	-	+	-
FcRL5	+	+/-	+/-	+/-	+	-	+
T-Bet	-	-	-	-	+	-	+
IgM	+	+	+/-	+/-	+	-/+	+/-

Table II. Baseline characteristics of the study population

	Fulani n1 = 30	Mossi n2 = 30	*p*
Female: n (%)	18 (40)	27 (60)	
Male: n (%)	12 (80)	3 (20)	0.01707
Not educated: n (%)	16 (45.71)	19 (54.29)	
Educated: n (%)	14 (56.00)	11 (44.00)	0.6005
Use of bed net	18 (39.13)	28 (60.87)	0.0052
Age (year): median (interquartile range)	27 (21.00, 37.00)	32 (26.25, 36.75)	0.9141
Temperature (°C): median (interquartile range)	36.25 (36.00, 36.88)	36.65 (36.25, 36.90)	0.1964
Haemoglobin (g/dl): median (interquartile range)	12.75 (11.65, 14.4)	12.60 (12.03, 13.30)	0.6055
Parasite density: mean ±SD	No parasite detected	65400 ± 6997.28	-

The mean age of the Mossi adults was 32 years (range: 26–37 years) and seven adults were infected with a mean parasite density of 13624 asexual parasites/µL (Table I). In the Fulani adults, the mean age was 27 years (range: 21–37 years) and we did not find any positive thick smear. In Fulani group, 18 volunteers were female; while in the Mossi group 27 individuals were female. There was no difference between the two groups in terms of education levels. However, the use of bed net was significantly higher in the Mossi than the Fulani group (60.87% vs 39.13%, *p=0.0052*). The mean temperature was similar between the Fulani (36.3°C) and the Mossi (36.7°C, *p=0.19*). The mean hemoglobin was 12.75 g/dL (11.65, 14.40) for Fulani and 12.60 g/dL (12.03, 13.30) for the Mossi.

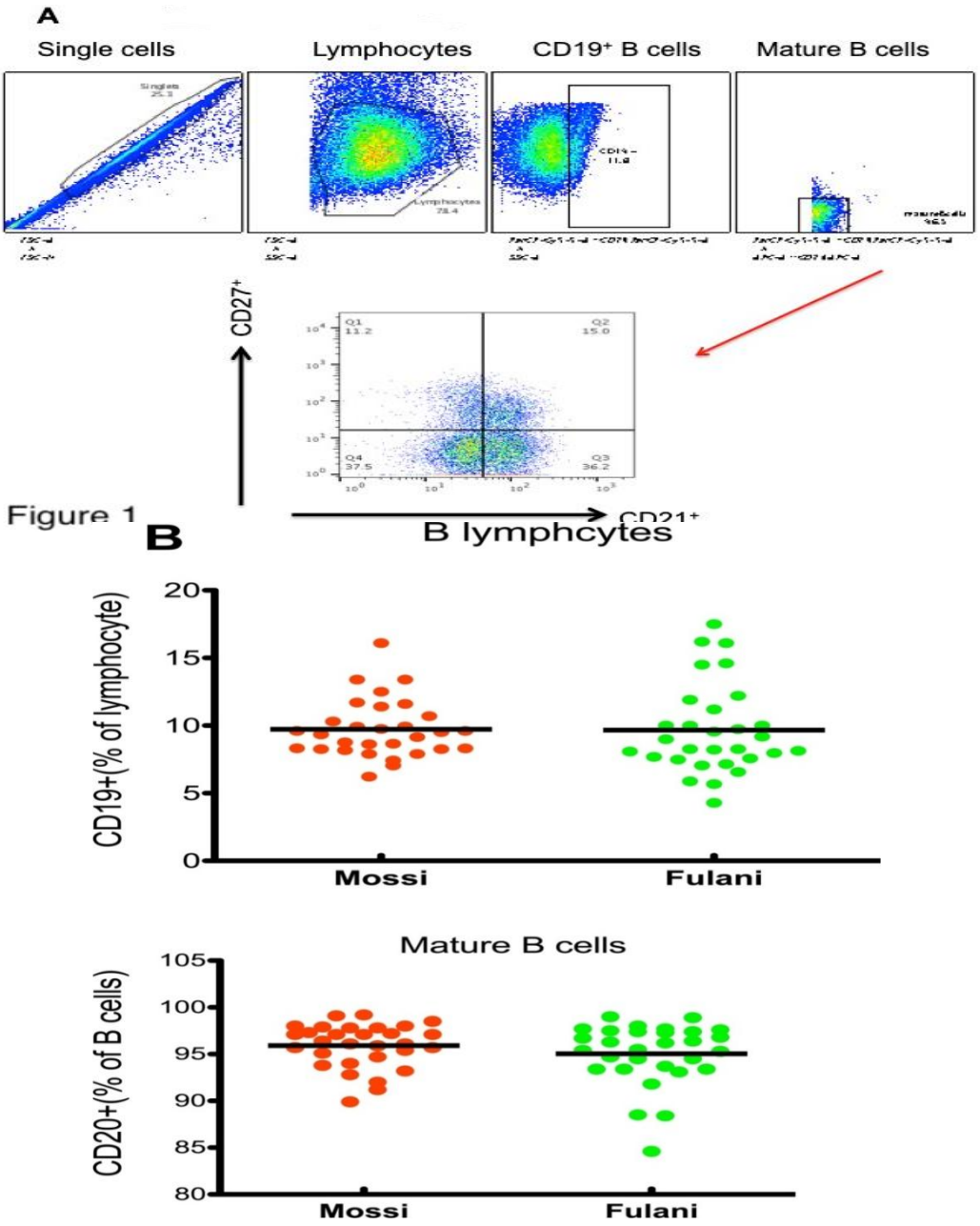
Interethnic differences in the frequency of blood B lymphocyte subsets

Circulating B-cell subpopulations in peripheral blood are identified as a lymphocyte lineage that revealed a broad range of cell surface markers. These cell subpopulations of 60 Fulani and Mossi adults who are living in an intense *Pf* transmission were stained for CD19, CD20, and in combination with CD10, CD11c, CD21, CD27, FcRL5, IgM, and the transcription factor T-bet (Table II, and gating strategies depicted in Figure 1A). The frequency of circulating B cells defined by CD19+ cells was “9.72 ± 0.39 N=30” in the Fulani and “9.66 ± 0.60 N=30” in the Mossi (Figure 1B). Then, we analyzed the different B–cell populations including, naive B cells, classical memory B–cells (MBCs), activated MBCs atypical MBCs and plasma blast/plasma cells. The frequency of circulating naive B cells was “27.98 ± 1.61” in the Fulani and “27.98 ± 1.39” in the Mossi. For classical and activated MBCs, the proportions were “24.02 ± 1.42”; “11.67 ± 0.76” in the Fulani and “22.95 ± 1.22”; “10.89 ± 0.42” in the Mossi respectively. Finally, the proportions of atypical MBCs and plasma blast/plasma cells were “36.32 ± 2.23”; “1.11 ± 0.12” in the Fulani and “38.18 ± 1.78”; “1.10 ± 0.09” in the Mossi respectively.

$CD11c^+$ B-cell subset expressing FcRL5 frequency was significantly lower in the Fulani than in the Mossi

When B cells are chronically stimulated, a phenotypically unique subset expands with an atypical phenotype. This subset, defined as $CD11c^+$ T–bet^+ B cells, is increased in settings of chronic stimulation (13). Further, it has been shown that the $CD11c^+$ T–bet^+ B cells share characteristics

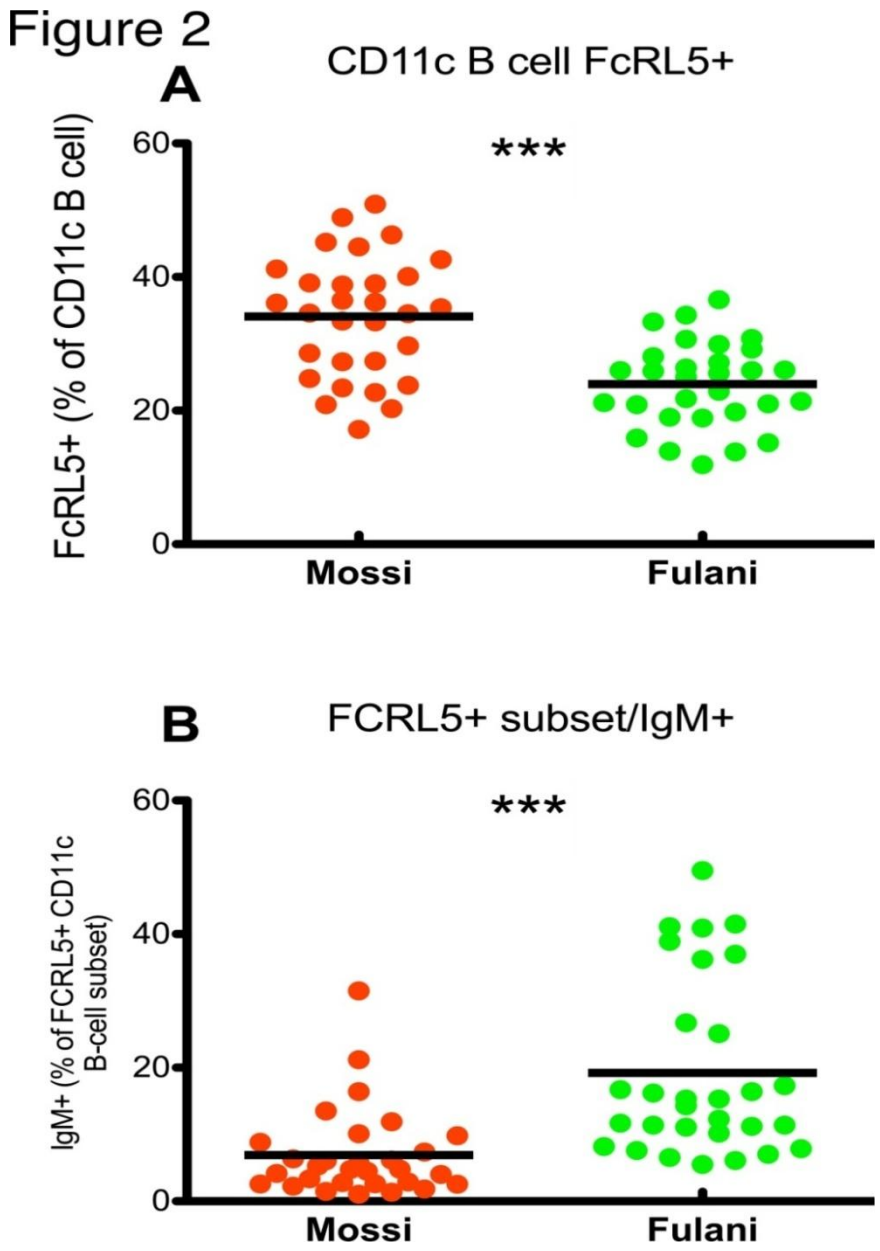
Figure 1. Interethnic differences in the frequency of blood B lymphocyte subsets



PBMCs from the study participants were stained with monoclonal antibodies for subsequent flow cytometric analysis using a FACS LSRII. The samples were categorized as Fulani (n=30) and Mossi (n=30). For the gating strategy of B-cell subsets, cells were pregated for size, singlets, and live cells. The total lymphocyte population was identified on the basis of forward (FSC) and side (SSC) scatter characteristics (Figure 1A). B cells were defined as CD19–expressing cells from the lymphocyte population (Figure 1B). We analyzed the expression of IgM and CD27 on $CD19^+$ B-cells. Naive B cells were defined as $CD10^-$, $CD27^-$ cells, classical memory B–cells (MBC) were defined as $CD21^+$, $CD27^+$ cells, Activated MBCs as $CD21^-$, $CD27^+$ cells and atypical were defined as $CD21^-$, $CD27^-$, $FCRL5^+$ T-Bet^+cells. Additionally, the CD11c B cells were identified as $CD19^+$, $CD20^+$B cells expressing $CD11C^+$, $CD21^-$, $CD27^-$ and $FcRL5^+$ cells. Plasma blast/plasma cells were defined as $CD19^+$, $CD20^-$ $CD27^+$ cells. Red dots in the scatter plot represent Mossi adults; Green dots represent Fulani adults and Black lines are the mean for each group.

of memory B–cells that are maintained under conditions of inflammation and/or low-level chronic antigen stimulation (9). Therefore, we assessed these CD11c+ T–bet+ B cells in the Fulaniand the Mossi.

Figure 2. CD11c^{+} B–cell subset expressing FcRL5 frequency was significantly lower in the Fulani than in the Mossi

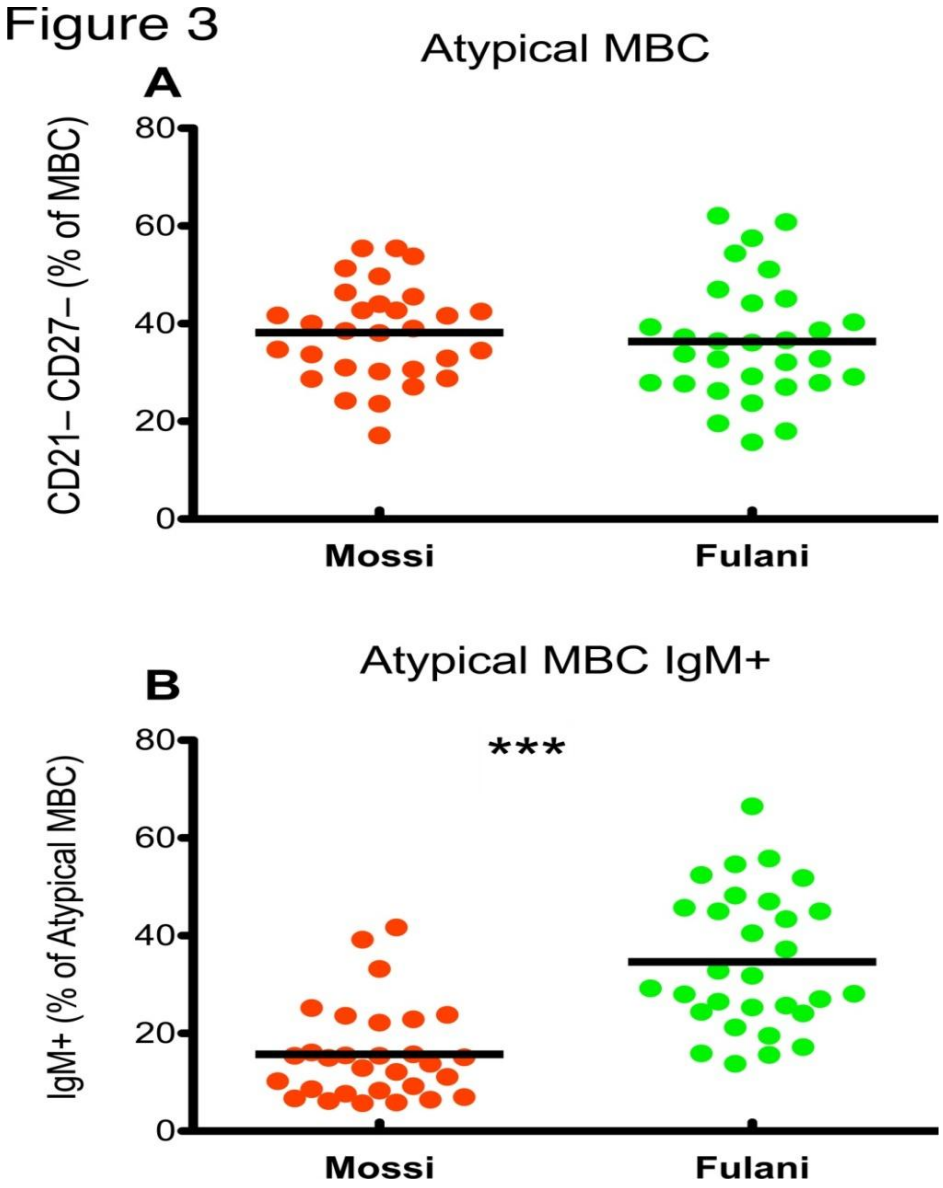


PBMCs were stained with monoclonal antibodies and subsequently analyzed using the BD FACS LSRII. The samples were categorized as Fulani (n=30) and Mossi (n=30). Percentages of CD11C^{+} B-cell subset expressing FcRL5^{+} (A) and FCRL5^{+} IgM^{+} subset (B) in circulating PBMCs were shown. Red dots in the scatter plot represent Mossi adults; Green dots represent Fulani adults and Black lines are the mean for each group. Data were analyzed by Mann-Whitney rank sum test (*** = p<0.0001).

Here it was found that the CD11c+ B-cell subset expressing FcRL5 frequency was significantly lower in the Fulani as compared to the Mossi (“23.96 ± 1.15” vs “34.08 ± 1.70”; *p=0.0001*; Figure 2A). Among these subsets, the proportion of cells expressing IgM was analyzed; the CD11c+ T–bet+ B cells expressing IgM+ frequency was significantly higher in the Fulani than in the Mossi (“19.22 ±2.40” vs “6.91 ± 1.21”; *p=0.0001*) (Figure 2B).

When looking at the infected Mossi, the proportions of CD11c+ B cell subset expressing FcRL5 were higher but not statistically significant compared to the healthy ones. All together, these results suggest that the Fulani have less circulating inhibitory B-cell subset and that they are more prone to produce IgM compared to the Mossi. This may result in a greater predisposition of the Fulani to

Figure 3. IgM$^+$ Atypical Memory B–Cell (MBC) frequency was significantly higher in the Fulani than in the Mossi



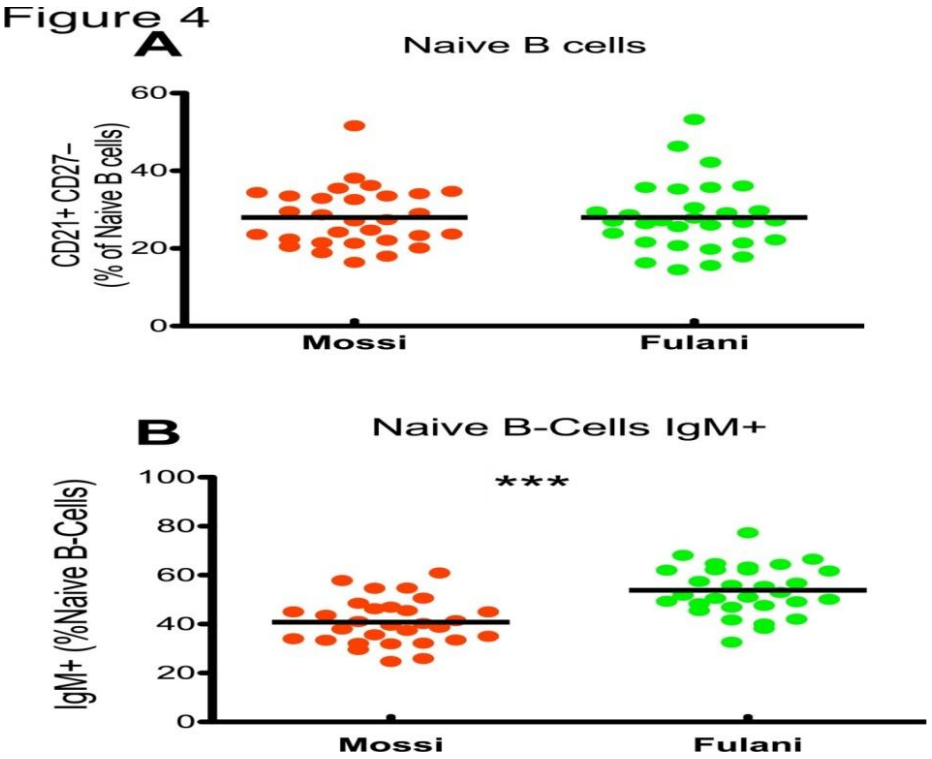
PBMCs were stained with monoclonal antibodies and subsequently analyzed using the BD FACS LSRII. The samples were categorized as Fulani (n=30) and Mossi (n=30). Atypical MBCs were defined by gating on CD19$^+$CD20$^+$CD10$^-$ mature B cells, which were further separated as CD21$^-$ and CD27$^-$. Percentages of Atypical MBC (A) and IgM$^+$ atypical MBC subset (B) in circulating PBMCs were plotted. Red dots in the scatter plot represent Mossi adults; Green dots represent Fulani adults and Black lines are the mean for each group. Data were analyzed by Mann-Whitney rank sum test (*** = *p<0.0001*).

clear the circulating parasites in the blood.

Atypical Memory B–Cell (MBC) IgM$^+$ frequency was significantly higher in the Fulani than in the Mossi

Previous studies have shown that atypical MBCs are greatly expanded in individuals living in a malaria-endemic area (14). These atypical MBCs exhibit markedly reduced signaling and effector function, which may contribute to the inefficient acquisition of humoral immunity to malaria (15) and that the degree of atypical MBC expansion increased with increasing *Pf* transmission intensity (16). We assessed atypical MBCs (CD10$^-$CD19$^+$CD20$^+$CD21$^-$CD27$^-$) from the peripheral blood of 60 Fulani and Mossi adults with lifelong exposure to intense *Pf* transmission (Figure 3). Although the frequency of atypical MBCs was similar in the Fulani and the Mossi (“36.34 ± 2.22” vs “38.18 ± 1.79”; *p=0.52*),

Figure 4. Naïve B–Cell IgM+ frequency was significantly higher in the Fulani than in the Mossi



PBMCs were stained with monoclonal antibodies and subsequently analyzed using the BD FACS LSRII. The samples were grouped as Fulani (n=30) and Mossi (n=30). Naïve B cells were defined by gating on $CD19^{+}CD20^{+}CD10^{-}$ mature B cells, which were further separated as $CD21^{-}$ and $CD27^{+}$. Percentages of naïve B-cell (A) and IgM^{+} naïve B–cell subset (B) in circulating PBMCs were plotted. Red dots in the scatter plot represent Mossi adults; Green dots represent Fulani adults and Black lines are the mean for each group. Data were analyzed by Mann Whitney rank sum test (*** = *p<0.0001*).

we found that the IgM^{+} atypical MBC frequency was significantly higher in the Fulani than in the Mossi ("15.73 ± 1.81" vs "34.64 ± 2.57; *p=0.0002*).Together, these findings indicate that the greater IgM response to malaria was associated with increased proportions of IgM^{+} atypical MBCs in the peripheral blood of naturally malaria–exposed Fulani compared to the Mossi ethnic groups.

IgM^{+} naïve B–Cell frequency was significantly higher in the Fulani than in the Mossi

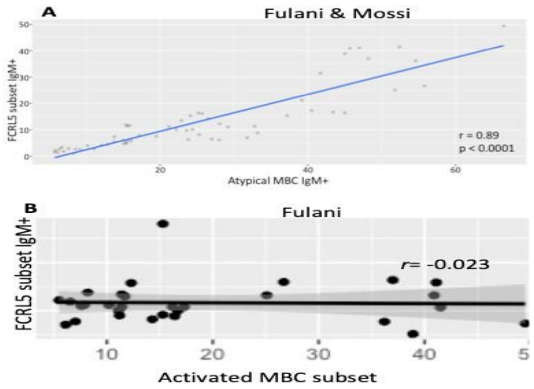
IgM^{+} B cells are generally assumed to represent antigen-inexperienced, naive B cells expressing variable (V) region genes without somatic mutations. However, it has been reported that human IgM^{+} peripheral blood (PB) B cells expressing the $CD27^{+}$ carry mutated V genes, in contrast to $CD27^{-}$ IgM^{+} B cells (17). In addition, IgM^{+} $CD27^{+}$ B cells resemble class-switched and IgM^{-} memory cells in terms of cell phenotype that can reach ~15% of PB B lymphocytes in healthy adults. Therefore, we measured the frequency of this B cell subset in the PBMCs of the Fulani and Mossi adults (figure 4). Although the frequency of naive B cells was similar in the Fulani and Mossi ("27.98 ± 1.62" vs "27.98 ± 1.39;*p=0.52*), we found that the frequency of IgM^{+} naïve B–cell ("40.96 ± 1.72" vs "53.84 ± 1.84"; *p<0.0001*) was significantly higher in the Fulani than in the Mossi. These results suggest that the IgM^{+} naïve B–cell contribute significantly to the higher anti-malarial IgM responses that have been observed in the Fulani as compared to the Mossi ethnic groups.

Levels of IgM^{+} atypical MBC positively correlate with those of IgM^{+} CD11c B cells expressing $FCRL5^{+}$

To better characterize the IgM^{+} CD11c B cells expressing $FCRL5^{+}$ subset, we performed a Pearson r correlation test (Figure 5). Regardless the ethnicity, we found that the levels of IgM^{+} atypical MBCs were strongly and positively correlated with those of IgM^{+} CD11c B cells expressing $FCRL5^{+}$ (r = 0.89, *p=0.0001*; Figure 5A). In addition, when looking at the Fulani groups, we found that the levels of activated MBC were negatively correlated with those of IgM^{+} CD11c B cells expressing $FCRL5^{+}$ (r = –0.023; Figure 5B). Together, these results indicate that high levels of IgM^{+} CD11c B cells expressing $FCRL5^{+}$ in the peripheral blood could be used as biomarker of

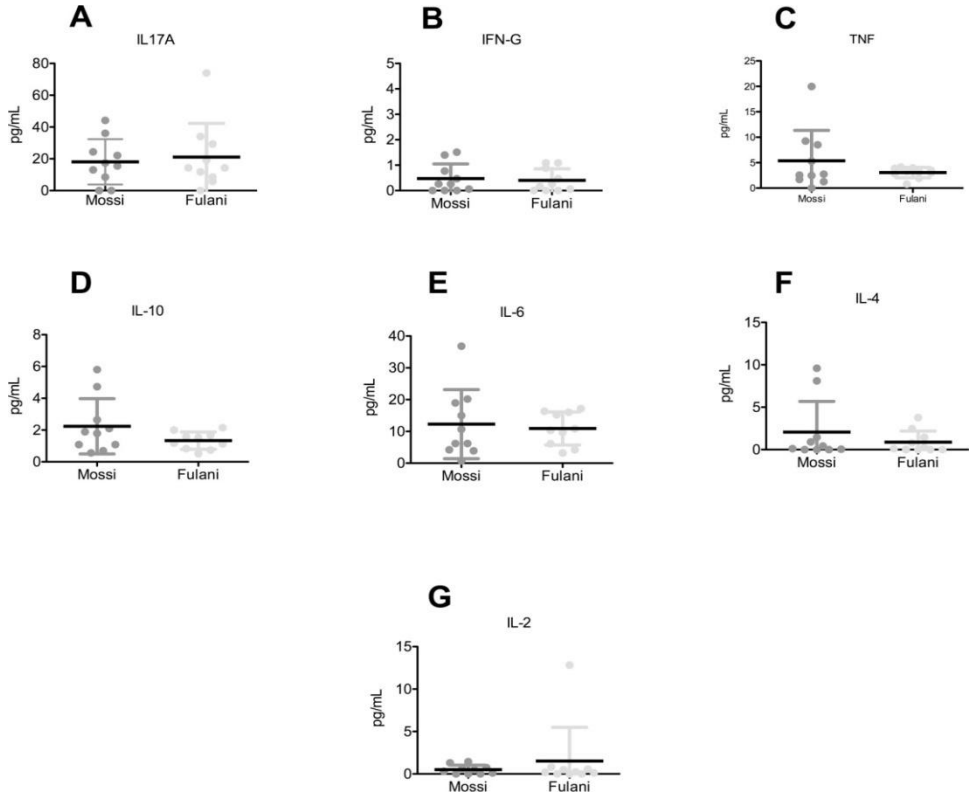
Figure 5. Levels of IgM^+ atypical MBC positively correlate with those of IgM^+ CD11c B cells expressing $FCRL5^+$

Figure 5



Individual cell numbers obtained from the FACS data were plotted using the R Studio package for the Spearman rank linear correlation test, which measures the linear interdependence between two variables X and Y.The scattergrams show a range of correlations. They were generated between Levels of IgM^+ atypical MBC and IgM^+ CD11c B cells expressing FCRL5+ for all the 60 individuals (Fig. 5A); and between levels of CD11c B cells expressing FCRL5+ and Activated MBCs for the 30 Fulani individuals (Figure. 5B).

Figure 6. Cytokine responses in Fulani and Mossi



Blood plasma samples from 60 volunteers were analyzed for circulating cytokines. Levels of IL-17A (Figure 6A), IFN-γ (Figure 6B), TNF (Figure 6C), IL-10 (Figure 6D), IL-6 (Figure 6E), IL-4 (Figure 6F) and IL-2 (Figure 6G) were measured with cytometric bead array using the BD Human Th1/Th2/Th17 CBA Kit. The green box plots showed the Fulani group and the red one for the Mossi. The boxes represent the values between 25% and 75% quartile and the dot indicates the median. Statistical analysis was done by Mann-Whitney U test.

susceptibility in naturally malaria–exposed populations.

Cytokine responses in Fulani and Mossi

Blood plasma samples from the Fulani and Mossi adults were analyzed for levels of IL-17A, IFN-γ, TNF, IL-10, IL-6, IL-4 and IL-2 (Figure 6). Detectable levels of each cytokine were found in 11 Fulani and 15 Mossi were divided according to ethnicity. When comparing the Fulani to the Mossi regardless of infectious status, similar levels of IL-17A (*p=0.93*; Figure 6A), IFN-γ (*p=0.87*; Figure 6B), TNF (*p=0.91*; Figure 6C), IL-10 (*p=0.34*; Figure 6D), IL-6 (*p=0.99*; Figure 6E), IL-4 (*p=0.72*; Figure 6F) and IL-2 (*p=0.75*; Figure 6G) were found between the two groups. Although the breadth levels of IL2 and IL17A seemed to be higher in the Fulani adults, the overall results suggest that the levels of Th1/Th2 cytokines did not vary between these two groups in a healthy condition.

DISCUSSION

Previous studies trying to understand the reason for the Fulani's relative protection against malaria have so far focused on antibody responses and not much is known about the molecular and functional characteristics of human B–cell populations that are contributing to the enhanced antibody responses in the Fulani. Therefore, we investigated B-cell responses in those populations that were genetically isolated and naturally exposed to *Pf* infection over time. Using an explorative approach with targeted flow cytometry, we clearly show that $CD11c^+$ B cell expressing $FcRL5^+$ subset was significantly decreased in the Fulani's peripheral blood. This subset constitutes a selective marker for B cells that respond to *Pf* infection. It may further highlight differences in Fulani versus Mossi adults. Further, we have shown in this study that many B-cell subsets are contributing to the enhanced IgM response observed in the Fulani.

The kinetic of B–cell subsets varies substantially upon infection. For instance, there are dynamic changes in multiple B–cell subsets following acute, uncomplicated malaria, and these subsets are associated with developing immunity to malaria (18). Our result shows variations between the two ethnic groups even within individuals of each ethnic group. This confirms our previous findings that the malaria-resistant Fulani have a higher percentage of activated MBCs compared to the Dogon, and that *Pf* infection was associated with a higher percentage of plasma cells in the Fulani compared to the Dogon (19).

Atypical B cells have been described to be involved in self-reactive or polyreactive B cell responses and constitute a large portion of the CD11c+ B cells (20). Moreover, atypical MBCs are significantly expanded in *Pf*-exposed Malian adults and children compared with healthy U.S. adult controls and was correlated with intense *Pf* transmission (14, 16). Likewise, it seems that chronic exposure to *Pf* is associated with phenotypic evidence of B and T cell exhaustion (21).

In the Mossi groups the levels of IgM^+ atypical MBC were strongly and positively correlated with those of IgM^+ CD11c B cells expressing $FCRL5^+$ suggesting that repeated exposition to *Pf* may drive towards an alteration or an impairment of the immune response, an exacerbated phenomenon in this population as compared to the Fulani who are less susceptible.

$CD11c^+$ T-bet-expressing B cells are now recognized as a novel and distinct B-cell population that is involved in long-term immunity to infections, and in autoimmunity (22). Moreover, $CD11c^+$ $T\text{-}bet^+$ antibody secreting cells (ASCs) are generated independently of T cell help during acute infection. They also contribute to protective immunity during both acute and chronic infection (9). It is likely, that $CD11c^+$T-bet-expressing B–cell subset expressing FcRL5 is a hallmark of altered phenotype that expand with chronic infection, especially in *Pf* infection. The frequency of $CD11c^+$T-bet-expressing B–cell subset expressing FcRL5 was significantly lower in Fulani than in Mossi. The FCRL5 is an inhibitory protein involved in regulation of different B–cell subsets. FCRL5 expression on circulating B cells from healthy donors have shown that $FCRL5^+$ cells are mostly enriched among atypical $CD21^-$ $CD27^-$ tissue–like memory (TLM) B cells, which are abnormally expanded in several autoimmune and infectious diseases (23). Interestingly, the FCRL5 defines functionally impaired MBCs, which were associated with *Pf* exposure (24). CD11c constitutes a selective marker for B cells that respond to *Pf* infection and was upregulated by several B–cell subsets upon infection (10). The fact that this $CD11c^+$ T-bet-expressing B–cell subset expressing FcRL5 is diminished among the Fulani supports our hypothesis that this subset could serve as biological markers of susceptibility. It could also be a marker of cumulative disease, and then because Mossi get sick more often they accumulate more of this B–cell subset expressing FcRL5. This may drive to an alteration or impairment of the immune response, which is often observed in individuals living in malaria-endemic areas. Therefore, it will be important to further study the role of these subsets during infection. For now, the dynamic of acquisition of this subset is not known. A longitudinal study considering such aspects could elucidate the mechanisms of acquisition of the $CD11c^+$B–cell subset expressing FcRL5. This will help to understand the protective mechanisms against malaria in individuals naturally exposed to this disease.

Cytokines are essential mediators during *Pf* infection and the balance between pro- and anti-inflammatory cytokines may be important for the clinical outcome of malaria (25). Previous studies identified that higher levels of all detectable cytokines were found in the Fulani group when comparing Fulani to Dogon children, irrespective of the infectious status (26, 27). In this study we also

measured the cytokines of the Th1/Th2 and Th17 pathways. Although the mean levels of pro-inflammatory cytokines were higher in the Fulani than the Mossi, we did not observe any significant difference between the two groups. One possible explanation for the differing results may have to do with the fact that most of our volunteers were healthy during the time of blood collections, and thus reflecting the reason why the cytokine levels were low or undetectable in the corresponding plasma samples. To rule out if this is the case it would be important to collect plasma early after acute infections.

This study was not able to evaluate a causal relationship between CD11c B cells expressing FCRL5$^+$ and immunity to malaria, but it clearly demonstrates that the accumulation of CD11c B cells expressing FCRL5$^+$ in the peripheral blood in individuals naturally exposed to this disease. Further, it clearly shows a decreased proportion of CD11c B cells expressing FCRL5$^+$ in the Fulani adults, that are more protected from clinical episodes and therefore generally mount a robust anti-*Pf* immunity. As we gain insight into the functional significance of the decreased levels of CD11c B cells expressing FCRL5$^+$ and other immune factors that have been implicated in malaria disease, there will be a hope to harness these processes, as tools and strategies to develop vaccines and target for therapeutic discoveries.

Abbreviations

APC: (allophycocyanin); ASC: (antibody secreting-cell); CBA: (cytometric-bead array); FACS: (fluorescent activated cell sorter); FcRL5: (Fc Receptor–like 5); FITC: (Fluorescein isothiocyanate); IFN: (interferon); Ig: (immunolglobulin); IL: (interleukin); MBC: (memory B-cells); PBMC: (peripheral blood mononuclear cells); PE: (phycoeryhtrin); *Pf:* (*Plasmodium falciparum*); PerCP:(Peridinin-chlorophyll proteins); T-bet: (T-box transcription factor); TLR: (toll like receptor); TNF: (tumor necrosis factor).

Competing interests

CA is a DELGEME post-doctoral fellow funded by DELTAS Africa Initiative [grant 107740/Z/15/Z]. The authors declare that they have no competing interests.

Authors' contributions

CA conceived, designed and coordinated the study and wrote the first draft of the manuscript. CA, MD, SP, analyzed the data. CA, HD, SA, BK, SY, AZ, KB, AD and MS participated to data collection in the field and performed bench work experiments. SBS, IN, MTB, AKÖF and BK revised the manuscript. All authors read and approved the final manuscript.

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