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SARS-CoV-2 and *Plasmodium falciparum* common immunodominant regions may explain low COVID-19 incidence in the malaria-endemic belt

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Abstract

Coronavirus disease 2019 (COVID-19) has caused significant morbidity and mortality and new cases are on the rise globally, yet malaria-endemic areas report statistically significant lower incidences. We identified potential shared targets for an immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by immune determinants' shared identities with *P. falciparum* using the Immune Epitope Database and Analysis Resource Immune 9.0 browser tool. Probable cross-reactivity is suggested through HLA-A*02:01 and subsequent $CD8^+$ T-cell activation. The apparent immunodominant epitope conservation between SARS-CoV-2 (N and open reading frame (ORF) 1ab) and *P. falciparum* thrombospondin-related anonymous protein (TRAP) may underlie the low COVID-19 incidence in the malaria-endemic zone by providing immunity against virus infection to those previously infected with *Plasmodium*. Additionally, we hypothesize that the shared epitopes which lie within antigens that aid in the establishment of the *P. falciparum* erythrocyte invasion may be an alternative route for SARS-CoV-2 via the erythrocyte CD147 receptor, although this remains to be proven.

Keywords: CD147 receptor, epitope, homology, *Plasmodium falciparum*, SARS-CoV-2
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Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, formerly HCoV-19), has led to significant morbidity and mortality in addition to adversely affecting healthcare systems and the global economy [1,2]. This acute respiratory illness ranges from a self-limiting acute upper respiratory tract infection to severe pneumonia, multiorgan failure and death [3]. There is currently no approved treatment or vaccine.

Most of the confirmed cases are confined to subtropical and temperate zones; countries in the equatorial and tropical zones seem to have the lowest incidences of COVID-19 (Supplementary Fig. S1). Interestingly, those countries have a high burden–high incidence (HBHI) of malaria infection [4]. According to World Health Organization (WHO) reports, the African region is characterized by a high prevalence of malaria (~150.9 million in 2018), with dominant infection caused by *Plasmodium falciparum* (99% compared to ~0.7% of Plasmodium

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vivax in 2018), and have the lowest number of cases of confirmed COVID-19 (~187625 as of 17 June 2020) compared to other regions [4–6].

Malaria infection and its severity have shown an association with the ABO blood group system. Individuals with the O blood group are less susceptible to the infection compared to individuals with the A blood group [7]. The mechanism for this preferential protection is attributed to the rosette phenomenon, which is initially induced by the parasite proteins thrombospondin-related adhesive protein (MTRAP) and RH5. MTRAP and RH5 interact with the CD147 receptor on the erythrocyte surface, which results in making infected red blood cells (RBCs) stickier and therefore facilitate their binding to healthy erythrocytes, forming RBC clusters termed rosettes [7,8]. These rosettes assist parasites to escape recognition by phagocytosis. Clump formation is weakly seen in the case of individuals with O blood group.

The relationship between the ABO blood groups and susceptibility to COVID-19 infection has already been documented and is shown to follow a pattern similar to that of malaria [9]. Blood group A is associated with a high risk for acquiring the disease, whereas O blood group individuals have the lowest risk [10]. Several recent reports have highlighted the notably lower incidence of COVID-19 in malaria-endemic belts [11,12]. Though no mechanistic laboratory-based clarifications of this lower number of COVID-19 cases in the African malaria zone have been explained, several hypotheses have been put forward to answer the open query. The following factors have been widely suggested to be relevant: (a) warmer climate [13], (b) limited amount of international air traffic to and from Africa compared to the other continents, (c) public socioeconomic conditions, (d) early lockdown measures, (e) demographic factors, (f) protection provided by repeated use of antimalarial drugs and finally (g) factors related to host susceptibility, which might include immunologic and genetic factors [14–17].

The genome of coronaviruses encodes 16 nonstructural proteins and four major structural proteins. A number of them are being targeted therapeutically and are being considered for candidates for vaccine development for COVID-19 [18,19]. In this study, we computationally and statistically analysed cases of COVID-19 in malaria-endemic regions and investigated possible shared immunogenic regions between dominant proteins of *P. falciparum* and SARS-CoV-2.

Methods

Statistical analysis of malaria-endemic regions and COVID-19 outbreak

We collected and analysed data from WHO reports and countries' status profiles for malaria HBHI in 2018 and the daily reports covering the COVID-19 outbreak [5,20]. Two-tailed tests of logistic regression coupled with chi-square, odds ratio and confidence interval analyses were performed by GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA, USA). Correlation confidence was used, and one-way ANOVA was performed with Bonferroni multiple comparison tests.

Defining immunodominant epitopes from SARS-CoV-2

SARS-CoV-2 B- and T-cell major histocompatibility complex (MHC)-restricted immunodominant regions and their corresponding epitopes, which are potential targets for immune responses, were identified by Grifoni et al. [21]. These potential epitopes were determined on the basis of sequence-shared identities with the closely related SARS-CoV and by parallel bioinformatic prediction approaches. Epitope sequences from SARS-CoV-2 were then retrieved and used to identify common immunodominant epitopes for *P. falciparum*.

Immunodominant regions of *P. falciparum* and homology with SARS-CoV-2

The Immune Epitope Database and Analysis Resource (IEDB, https://www.iedb.org) is a comprehensive repository of epitope data reported from the scientific literature. It includes antibody and T-cell epitopes for infectious disease, allergy, autoimmunity and transplantation. IEDB was used to search the immunome of *Plasmodium falciparum* (ID 5833) to identify the most immunogenic proteins (i.e. 23 proteins were determined) and retrieve their immunodominant epitopes. These proteins are apical membrane proteins 1 (AMP-1), circumsporozoite protein (CSP), merozoite surface protein 1 (MSP-1), thrombospondin-related anonymous protein (TRAP), liver stage antigen 3 (LSA3), circumsporozoite-related antigen (EXP1), sexual stage–specific protein 16 (Pfs16), sporozoite threonine and asparagine-rich protein (STARP), uncharacterized protein (UniProtQ8I5P1), DNAJ protein, putative (UniProt: Q8I0U6), serine-repeat antigen protein, RING finger protein (PFF0165c), *Plasmodium* exported protein (PHISTc), erythrocyte binding antigen 175, reticulocyte-binding protein homolog 5 (RPH5), rhoptry-associated protein 1 (RAP1), merozoite surface protein 3 (MSP-3), heat-shock 70 kDa protein, S antigen protein, early transcribed membrane protein 13 (ETRAMP13), *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP-1), erythrocyte binding antigen 140 (EBL-140) and erythrocyte binding antigen-181 (EBL-181).

These proteins were mapped back to the *P. falciparum* representative reference sequence using the IEDB's Immune 9.0 browser tool to display graphs and a data table that depict and list all the epitopes by their position and response frequency (RF, or how frequently a residue was found in a positive epitope) [22,23]. All B- and T-cell epitopes for the 23 proteins were selected on the basis of their sequence RF > 0.0. For extra refinement of the results, we went further to identify T-cell epitopes with recognition restricted by human leukocyte antigen (HLA) MHC (class I (HLA-A, -B) and class II (HLA-DR, -DP, -DQ)); only positive assays were selected. Next, we aligned the *P. falciparum* B-cell, T-cell and T-cell MHC restriction epitopes to the SARS-CoV-2 immunodominant epitopes in order to calculate the percentage identity between each of the *P. falciparum*–dominant epitopes and SARS-CoV-2. Four or five amino acid shared residues were considered significant [24].

Prediction of T-cell MHC restricted epitopes

To identify and predict potential T-cell MHC restricted epitopes by alternative methods for selected *P. falciparum* antigenic targets (AMP-1, MSP-1, CSP, TRAP, SSP-2) and SARS-CoV-2 open reading frame (ORF) proteins (ORF3a, ORF1ab, ORF7a, ORF8, ORF10), we used the IEDB MHCI and MHCII online prediction tools (respectively http://tools.iedb.org/mhcI and http://tools.iedb.org/mhcII). These tools use different methods to determine the ability of the submitted sequence to bind to specific MHCI and –II molecules. The artificial neural network method was used to calculate the half-maximal inhibitory concentration (IC_{50}) values of the peptide binding. For both frequent and nonfrequent alleles, the peptide length was set to 9 amino acids before the prediction. For MHCI epitopes, the alleles with affinity binding at IC_{50} of $\leq$500 nM were considered [25], while for MHCII, all epitopes that bind to many alleles with $\leq$1000 nM at IC_{50} were selected for further homology analysis [26].

Results

COVID-19 and malaria-endemic regions

We obtained results of malaria (year 2018) versus COVID-19 (up through 12 April 2020) cases worldwide in different regions including Africa (respectively 150887242 vs. 4943), Eastern Mediterranean (5202933 vs. 79695), West Pacific (1080872 vs. 19868), South-East Asia (742114 vs. 15735), the Americas (764980 vs. 46417) and Europe (0 vs. 880106) (Fig. 1). To examine the relative risk of exposure to COVID-19 and malaria coinfection, HBHI regions of malaria were assigned as cases and compared to Europe (which was considered the control), with zero cases of local malaria infection in 2018. The odds ratio indicated that all regions of malaria-endemic areas had a statistically significant lower relative risk of COVID-19 infection ($p < 0.0001$). Our results also demonstrated a significant reverse correlation between *P. falciparum* and COVID-19 death rates ($r^2 = -0.218$, $p < 0.001$). Bonferroni multiple comparison tests were used to compare the mean of death rates and showed a significant variation between the death rate caused by *P. falciparum* and *P. vivax* against COVID-19 ($p < 0.005$) (Tables 1 and 2).

Plasmodium falciparum immunodominant regions

We screened the *P. falciparum* immunome to identify immunodominant regions. Twenty-three proteins were selected as promising immunogenic proteins. A total of 763 B-cell–immunodominant epitopes and 1084 T-cell–immunodominant epitopes were identified with RF > 0.0. Many of these epitopes clustered in four proteins (AMP-1, MSP-1, CSP, TRAP) with a total of 190 and 918 B- and T-cell epitopes respectively. The analysis showed that AMP-1 has ten immune-conserved regions (residues 14–35, 51, 200, 317–334, 350–365, 374, 397, 387–399, 446–463, 571–588). MSP-1 has six regions located at both the N and C terminals of the protein; CSP has only two regions with potential interest. Residues 113–318, 335; and 43–53, 101, 120, 122–130, 221–240, 302–320, 371, 390, 421–440, 509–523 were identified for TRAP (Fig. 2). For SARS-CoV-2, ten immunodominant regions which were identified in reference to SARS-CoV by Grifoni *et al.* [21] via experimental data and confirmed with prediction approaches were used to define conserved shared regions with Plasmodium's tested immunogenic proteins.

Homology of *Plasmodium* B- and T-cell–immunodominant epitopes with SARS-CoV-2

All tested B-cell epitopes shared no significant homology with SARS-CoV-2. As such, no antibodies to Plasmodium could be proposed as eliciting an immune response against infection with SARS-CoV-2 through cross-reactivity. On the other hand, $\geq$40% of shared identities were noted between SARS-CoV-2 N protein (215–227 aa) and Plasmodium TRAP epitopes located at (509–523 aa) and also between ORF1ab (3661–3669 aa) and TRAP (101–130 aa). Although the phylogenetic distance between the two organisms would be expected, four or five shared amino acids in a single immunodominant epitope would be considered significant (Table 3).

Experimental and predicted T-cell MHC restriction homology with SARS-CoV-2

T-cell epitopes with recognition restricted by HLAs were also analysed. Typically, polymorphisms associated with MHC molecules result in the recognition of different epitopes.

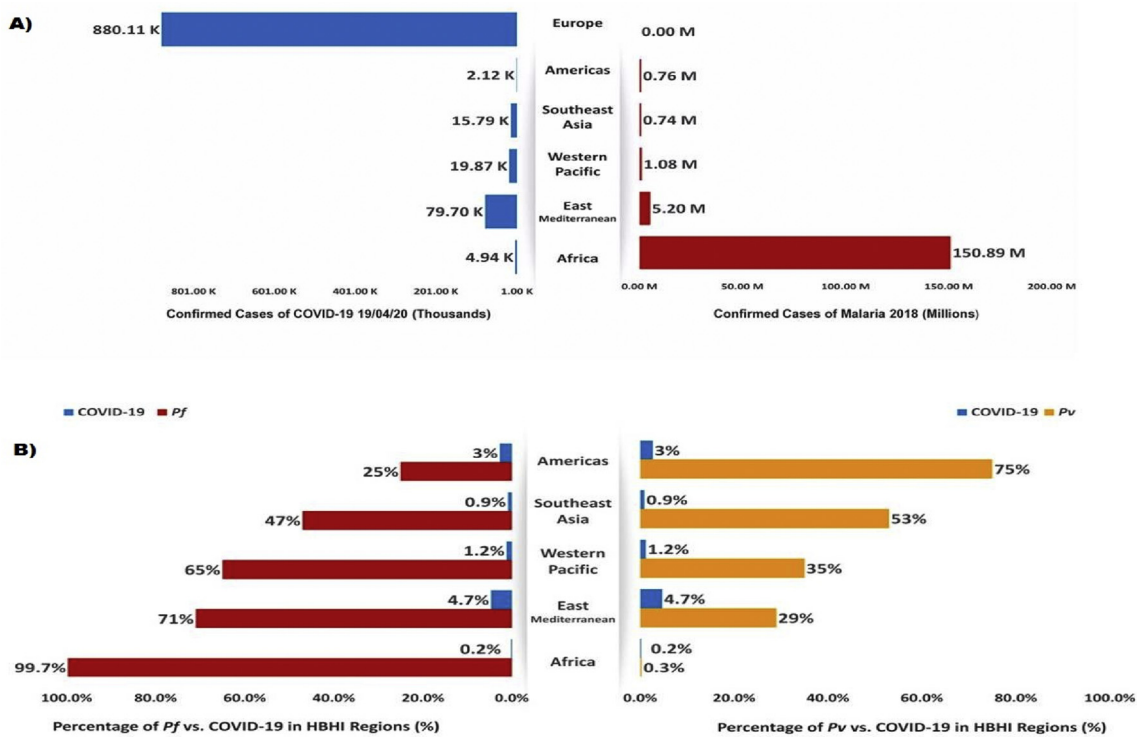

FIG. 1. Coronavirus disease 2019 (COVID-19) vs. malaria. (A) Comparison between COVID-19 and malaria case numbers within different high burden–high incidence (HBHI) malaria regions. (B) Comparison between *Plasmodium falciparum* (Pf), *P. vivax* (Pv) and COVID-19 case percentages within HBHI malaria regions.

P. falciparum whole immunome was screened for T-cell MHC-immunodominant epitopes. The pool of MHCI- and MHCII-positive assays contained about 1610 immunodominant epitopes, most of them recognized in humans (n = 1575) and the rest reported in mice (n = 35) and rhesus macaques (n = 11). The same T-cell epitopes reported above have MHC restriction and equal identities with the same shared SARS-CoV-2 N and ORF1ab quarto-immunodominant epitopes. Interestingly, the N protein epitope located at 219–227 aa is extremely conserved in SARS-CoV (100% identity, RF = 0.29) and recognized by HLA-A*02:01. This sequence is also partially shared by TRAP (504–513 aa) (44.4% identity, RF = 0.37), which is recognized by the same HLA-A*02:01. Similarly, the ORF1ab epitope (3661–366aa) (89% shared SARS-CoV identity, RF = 0.42, recognized by HLA-A*02:01 identified in HLA-transgenic mice) is 44.4%, homologous to TRAP precursor (114–122 aa, RF = 1) and also recognized by the same HLA molecule. Experimentally, HLA-A*02:01 was assayed using interferon gamma enzyme-linked immunospot assay and reported to be restricted to $CD8^+$ T-lymphocyte response to malaria in endemic areas (Table 4).

We then analysed the epitopes generated from the prediction approach, which resulted in the same sequences shared between TRAP epitopes and SARS-CoV-2 N and ORF1ab proteins with other MHCI and MHCII restrictions. Additionally, there was a novel immunodominant epitope identified in

TABLE 1. Logistic regression analysis associated with reduction factor of COVID-19 outbreak

WHO region	No. of malaria cases in 2018	No. of COVID-19 cases[a]	OR	95% CI	p
African	150887242	4943	179.1	174.1–184.1	<0.0001
Eastern Mediterranean	5202933	79695	12.04	11.96–12.12	<0.0001
Western Pacific	1080872	19868	45.30	44.68–45.93	<0.0001
South-East Asia	742114	15735	56.93	56.06–57.82	<0.0001
Americas	764980	46417	19.18	19.01–19.34	<0.0001
Europe	0	880106	—	—	

CI, confidence interval; COVID-19, coronavirus disease 2019; OR, odds ratio; WHO, World Health Organization.
[a]As of 12 April 2020.

TABLE 2. Average risk factor analysis by one-way ANOVA and Bonferroni multiple comparison test

	Means (all regions)						
Death rate	**Malaria cases in 2018**	**COVID-19 cases[a]**	**95% CI**	**Mean difference**	**r^2**	**t**	**p**
COVID-19 vs. malaria	30370	65890	−117300 to 188300	35520	−0.21	0.7331	0.6780224
COVID-19 vs. Pf	30370	25860000	−693000 to 17650000	−25830000	−0.218	1.946	0.6781664
COVID-19 vs. Pv	30370	590500	−440300 to 42910000	−560100	−0.75413	0.04221	0.0832416

CI, confidence interval; COVID-19, coronavirus disease 2019; Pf, *Plasmodium falciparum*; Pv, *Plasmodium vivax*.
[a]As of 12 April 2020.

sporozoite surface protein 2 (SSP-2) located at 80–90 which shared a pentapeptide (55.6%) with SARS-CoV-2 S protein (1192–1200 aa) (100% identity with SARS-CoV, RF = 0.29). Both sequences also bind the HLA-A*02:01 molecule and can be another potential candidate for the cellular immune response. Upon testing the sequence homology between SARS-CoV-2 predicted T-cell MHC epitopes for the accessory ORF proteins against the experimentally identified T-cell MHC epitopes from the *P. falciparum* immunome, we found that ORF3a epitopes (IVDEPEEHV, 236–244 aa; ALLAVFQSA, 51–59) share 44.4% homology with TRAP epitopes (DLDEPEQFRL, 543–552 aa; GLALLACAGL, 504–513 aa) respectively. These epitopes are again restricted by the HLA-A*02:01 molecule (Table 5).

Discussion

The outbreak of COVID-19 caused by SARS-CoV-2 has caused significant devastation on multiple fronts, with 8061550 total confirmed cases and 440290 deaths globally as of 17 June 2020 [27]. This pandemic has adversely affected social practices, healthcare systems and economies. Despite the incremental increase in incidence in various parts of the world, Africa has remained the continent with the lowest number of confirmed cases and deaths. The poor socioeconomic status of Africa makes it one of the most vulnerable regions in the world as a result of malnutrition, endemic tropical infections, fragile healthcare systems, poverty and social practices that encourage close gatherings [4]. However, the consensus is that the actual situation of the pandemic in Africa remains unclear. According to the Director of the Africa Centres for Disease Control and Prevention (Africa CDC, UN), only 1.3 million COVID-19 tests were conducted by mid-May across the continent (one test for every 1000 individuals), compared to 3.6 million tests performed in Italy by the 27 May 2020 [28]. The social distancing applied by many governments in Africa to mitigate the pandemic is now creating extra financial hardship and food insecurity among African women and may not be effective in the future [29]. Despite the higher relative youth percentage in the African population (43% under 15 years old) compared to the European population (14% under 15 years old), the majority of the cases now are reported among individuals younger than 65 [30]. Nevertheless, regardless of the abovementioned challenges, as of 17 June 2020, it is apparent that African countries located within the HBHI of malaria (42 countries) reported fewer numbers of confirmed cases (n = 111852) and mortality (0.95%) due to SARS-CoV-2 compared to the 12 African countries outside the malaria zone (n = 149089 and 1.72% respectively). These findings are supported by our statistical analysis, which indicated a significantly lower risk of COVID-19 in malaria-endemic areas ($p < 0.0001$) with emphasis on *P. falciparum*–endemic areas ($p < 0.001$) and a reverse correlation between *P. falciparum* and COVID-19 death rates ($r^2 = -0.218$, $p < 0.001$).

In malaria-endemic areas, the net immune response of one disease can be influenced by the predominant pathogen. The immune response to SARS-CoV-2 infection in malaria-endemic areas could be affected by prior exposure to the Plasmodium parasite [31]. However, considering the immune response through cross-reactivity could also be of value. The concept of 'original antigenic sin' is well known and had been used to explain many immunologic phenomena. Essentially, an adaptive immune response against one antigen can be used to combat another exposure by an unrelated antigen [32], with the second antigen relying on the memory established by the first antigen to initiate response [33,34]. In T-cell cross-reactivity, closely related sequences recognition is common and typically occurs between genetically related organisms. This is well documented for the same epitope isolated from different hepatitis C virus strains. Each epitope had a minor variation constricted to a single amino acid change. In this case, cross-reactivity occurs, but with substantial differences in the priming and magnitude of response [35]. Nonetheless, cross-reactivity can also be seen among random peptides or peptides with no shared sequences, or between those bearing relativity low homology [36,37]. The mechanism of recognition between T cell Receptor (TCR) and the epitope–MHC complex, where binding is not governed by the chemical principles of the epitope sequence, is well documented [38]. Searching the T-cell–immunodominant epitopes as

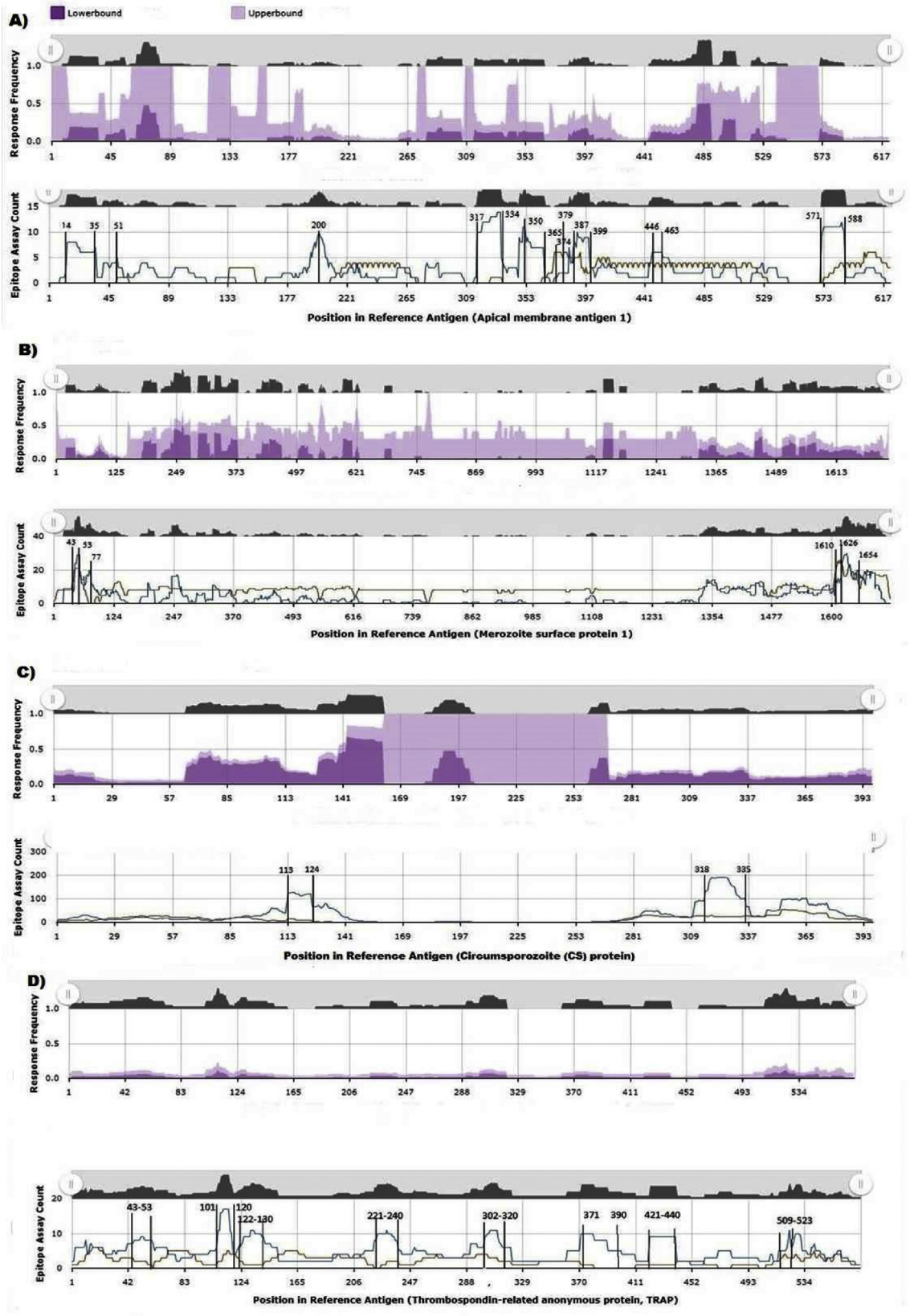

TABLE 3. Experimental T-cell–immunodominant epitopes from *Plasmodium falciparum* sharing homology with SARS-CoV-2 T-cell epitopes

P. falciparum					SARS-CoV-2			
ID	Sequence	Mapped start–end	Epitope name	RF	Sequence	Protein name	Mapped start–end	Identity (%)
55033	RNNENRSYNRKHNNTPKHPE	471–490	TRAP	0.03	ALNTPKDHI	N	138–146	44.4
31137	KHNNTPKHPEREEHEKPDNN	481–500		0.02				44.4
34480	KYKIAGGIAGGLALL	509–520	TRAP	0.1	GDAALALLLL	N	215–224	40
20213	GIAGGLALL	515–523		0.37				44.4
34480	KYKIAGGIAGGLALL	509–520	TRAP	0.1	LALLLLDRL	N	219–227	44.4
20213	GIAGGLALL	515–523		0.37				44.4
28326	IRLHSDASKNKEKALIIIKS	101–120	TRAP	0.05	SMWALIISV	ORF1ab	3661–3669	44.4
7640	DASKNKEKALIIIKS	106–120		1				44.4
32526	KNKEKALII	109–117		0.37				44.4
30453	KEKALIIIKSLLSTNLPYGK	111–130		0.02				44.4
549167	KEKALIIIRSLLSTNLPYGR	111–130		1				44.4

ORF, open reading frame; RF, response frequency; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TRAP, thrombospondin-related anonymous protein.

well as T-cell MHC-restricted epitopes for shared sequences between *P. falciparum* and SARS-CoV-2 revealed several conserved tetrapeptides and pentapeptides between the two organisms. Because both organisms are evolutionarily distant, the four or five amino acids shared were considered to be of significance. These similarities were observed among immunodominant sequences N protein from SARS-CoV-2/SARS-CoV and TRAP from *P. falciparum*; and S protein–SARS-CoV-2/SARS-CoV and the predicted epitope in SSP-2 from *P. falciparum*. Separately, both epitopes can stimulate $CD8^+$ T-lymphocyte response through HLA-A*02:01 recognition.

In this argument, we have carefully applied the doctrine of original antigenic sin to explain possible cross-reactivity between SARS-CoV-2 and *P. falciparum*. TRAP is a type I membrane protein essential for sporozoite motility and cellular invasion. Twenty-one percent of MHCI-restricted $CD8^+$ T-cell epitopes are found in this protein, and 28.6% to 100% of subjects from malaria-endemic areas responded to 504-GLALLACAGL-513–immunodominant epitope with restriction to HLA-A*02:01 [39]. Four amino acid determinants found in this epitope were shared by SARS-CoV-2 nucleocapsid protein 219-LALLLLDRL-227, the recognition of which is also restricted by HLA-A*02:01 [21]. Our assumption here is that the memory of the cellular adaptive immunity mounted against the abovementioned TRAP-immunodominant epitope could recognize the 219-LALLLLDRL-227–HLA-A*02:01 complexes originating from SARS-CoV-2 infection in malaria-endemic regions and trigger an immune response. Of course, such an assumption needs further empirical testing to prove its validity and ascertain the strength of the primed response.

Although we could not find any shared immunodominant epitopes between *P. falciparum* and SARS-CoV-2 B-cell epitopes, many recent letters to the editors have suggested that antibodies against glycol immunodeterminants on *P. falciparum* infection could recognize SARS-CoV-2 envelope glycoproteins and induce activation of the complement system and proinflammatory cytokines [40,41].

Death due to malaria is attributed in part to Plasmodium invasion of erythrocytes, a process mediated by RH5-CD147 interactions. Recent findings have indicated infection of the host cell by SARS-CoV-2 through spike protein–CD147 interaction [42]. Though the CD147 receptor is also expressed on the erythrocyte surface, these authors focused only on the lung's receptor. Ulrich and Pillat [43] proposed the CD147 receptor as a target for COVID-19 treatment. In a recent study, homology modelling and molecular docking revealed that SARS-CoV-2 could attack haemoglobin and inhibit haeme metabolism. The authors also showed that the viral nonstructural proteins ORF3a and ORF1ab are assisted with the viral haemoglobin attack [44]. Of COVID-19 patients admitted to the intensive care unit with acute pneumonic complications, ~49% presented with coagulopathy and thrombotic events [45]. High levels of D-dimer, or fibrinolysis-degraded fragments of fibrin, are often associated with COVID-19 infection and are used to detect in-hospital mortality [46,47], a situation that is also common with *P. falciparum* and P. vivax malaria [48]. Although not yet conclusively demonstrated, it is alleged that mature and immature erythrocytes could be implicated in SARS-CoV-2 entry into the body [49]. Both cells have CD147 and sialic acid receptors but lack angiotensin-converting

FIG. 2. T-cell–immunodominant regions based on *Plasmodium falciparum*–targeted proteins. (A) Apical membrane protein 1 (AMP-1). (B) Merozoite surface protein 1 (MSP-1). (C) Circumsporozoite protein (CSP). (D) Thrombospondin-related anonymous protein (TRAP). Specific epitope mapping response frequency (RF) score for each amino acid position was calculated and plotted over severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) consensus sequences. Cross-immune reactivity between P. falciparum and SARS-CoV-2 was identified.

TABLE 4. Experimental T-cell MHC restriction immunodominant epitopes from *Plasmodium falciparum* that share homology with SARS-CoV-2 T-cell MHC epitopes

P. falciparum						SARS-CoV-2			
ID	Sequence	Mapped start–end	Epitope antigen name	Epitope parent name	MHC allele	Sequence	Protein name	Mapped start–end	Identity
34480	KYKIAGGIAGGLALL	509–523	Sporozoite surface protein 2	TRAP	HLA-DRB1*01:01, HLA-DRB1*04:01, HLA-DRB1*07:01, HLA-DRB1*08:02, HLA-DRB1*09:01, HLA-DRB1*11:01, HLA-DRB1*12:01, HLA-DRB1*13:02, HLA-DRB5*01:01	GDAALALLLL	N	215–224	40
34480	KYKIAGGIAGGLALL	509–523	Sporozoite surface protein 2	TRAP	HLA-DRB1*01:01, HLA-DRB1*04:01, HLA-DRB1*07:01, HLA-DRB1*08:02, HLA-DRB1*09:01, HLA-DRB1*11:01, HLA-DRB1*12:01, HLA-DRB1*13:02, HLA-DRB5*01:01	LALLLLDRL	N	219–227	44.4
20762	GLALLACAGL	504–513	TRAP precursor	TRAP	HLA-A*02:01	GDAALALLLL	N	215–224	40
20762	GLALLACAGL	504–513	TRAP precursor	TRAP	HLA-A*02:01	LALLLLDRL	N	219–227	44.4
35388	LEDIINLSKKKKKSINDTSF	2557–2576	Putative erythrocyte binding protein EBL-1	EBL-140	HLA-DRB1*11:01	WLMWLIINL	ORF1ab	2292–2300	44.4
32526	KNKEKALII	109–117	Sporozoite surface protein 2	TRAP	HLA-B8	SMWALIISV	ORF1ab	3661–3669	44.4
2632	ALIIIRSLL	114–122	TRAP precursor	TRAP	HLA-A*02:01				

HLA, human leukocyte antigen; MHC, major histocompatibility complex; ORF, open reading frame; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TRAP, thrombospondin-related anonymous protein.

TABLE 5. Experimental T-MHC restriction epitopes from *Plasmodium falciparum* that share homology with predicted SARS-CoV-2 ORF T-cell MHC epitopes

P. falciparum						SARS-CoV-2			
ID	Sequence	Mapped start–end	Epitope antigen name	Epitope parent name	MHC allele	Sequence	Protein name	Mapped start–end	Identity (%)
9041	DLDEPEQFRL	543–552	TRAP precursor	TRAP	HLA-A*02:01	IVDEPEEHV	ORF3a	236–244	44.4
20762	GLALLACAGL	504–513	TRAP precursor	TRAP	HLA-A*02:01	ALLAVFQSA	ORF3a	51–59	44.4
68914	VICSFLVFL	9–17	Hypothetical protein PFL0800c	Uncharacterized protein	HLA-A*02:03, HLA-A*02:06	LVFLGIITTV	ORF8	4–13	44.4
68915	VICSFLVFLV	9–18			HLA-A*02:03, HLA-A*02:06				40
16939	FLVFLVFSNV	13–22			HLA-A*02:06				40
16939	FLVFLVFSNV	13–22	Hypothetical protein PFL0800c	Uncharacterized protein	HLA-A*02:01, HLA-A*02:03	FLVFLGIITT	ORF8	3–12	50
68914	VICSFLVFL	9–17			HLA-A*02:01, HLA-A*02:03				55.6
68915	VICSFLVFLV	9–18			HLA-A*02:01, HLA-A*02:03				50
16939	FLVFLVFSNV	13–22	Hypothetical protein PFL0800c	Uncharacterized protein	HLA-A*02:06	LVFLGIITT	ORF8	4–12	44.4
68914	VICSFLVFL	9–17			HLA-A*02:06				
68915	VICSFLVFLV	9–18			HLA-A*02:06				

HLA, human leukocyte antigen; MHC, major histocompatibility complex; ORF, open reading frame; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TRAP, thrombospondin-related anonymous protein.

enzyme 2 (ACE2) receptors. Unlike nucleated erythrocytes, mature RBCs lack the required machinery to support virus replication and provoke an immune response through MHC molecules. Therefore, erythrocyte-mediated infections are often fatal, as they go undiscovered by the immune clearance system. Nevertheless, viruses attacking erythrocytes would attract circulating antibodies, leading to antibody clumping. This would initiate a cascade of inflammatory responses that eventually leads to blood clotting. Given that *P. falciparum* antigens (TRAP and SSP-2) share immunodominant epitopes with SARS-CoV-2 antigens N, S, ORF1ab and ORF3a (Tables 3–5), in addition to the involvement of the CD147 receptor in the invasion process of the virus to the erythrocyte, a receptor that is commonly used by *P. falciparum* in the blood stage, and the subsequent finding that the nanolipid Metadichol could Moderately inhibit both SARS-CoV-2 by blocking the ACE2 receptor and the malaria parasite [50], could indicate a plausible SARS-CoV-2 infection route related to the blood system.

Conclusion

The shared immunodominant epitopes with cross-immunogenic reactivity between SARS-CoV-2 antigens S, N, ORF1ab and ORF3a to that of the *P. falciparum* antigens TRAP and SSP-2 which are reported in this investigation could suggest an answer for the ambiguous reason why the lowest number of COVID-19 infections and mortality rates exist in malaria-endemic regions compared to the rest of the world. These results support other recently published data that relate to erythrocyte CD147 receptor and surrogate entry for the virus. This, in addition to the several shared epitopes between SARS-CoV-2 with those antigens from *P. falciparum* which are related to the later RBC invasion, are collectively suggested as a probable alternative route for SARS-CoV-2 via RBCs, although this remains to be practically demonstrated and warrants future investigations to confirm their validity.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nmni.2020.100817.

Conflicts of interest

None declared.

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