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Rhesus Blood Group C (RH2) is Associated with Protection against SARS-Cov-2 Infections

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Research Article

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Abstract

Background: Previous reports showed a 40% reduction in the odds of being HIV-positive in RH2-heterozygotes. In addition, those lacking the gene were 24 times and 33 times more likely to be HIV-infected than heterozygotes or homozygotes, respectively. A four-year longitudinal study observed lower HIV viral load and slower disease progression in RH2-positive individuals. Thus, the current study sought to establish if the same phenomenon applied to SARS-CoV-2 infections. We also compared the plasma levels of SARS-CoV-2 anti-spike and anti-nucleocapsid protein antibodies following full vaccination against COVID-19.

Materials and Methods: Erythrocytes were phenotyped using anti-C and anti-c. Antibodies against SARS-CoV-2 were also measured in plasma of fully vaccinated persons (Oxford Astra-Zeneca, Johnson & Johnson, Pfizer/BioNTech, Sinovac, and Moderna vaccines) and compared across RH2 phenotypes.

Results: RH2 expression on erythrocytes (n=319) was associated with lower odds for SARS-CoV-2 infection (OR= 0.42, 95%CI: 0.22-0.80, p=0.008). The strength of the serological reaction between anti-C and erythrocytes was predictive of a COVID-19 diagnosis (Mantel-Haenszel Chi Square = 9.44, p=0.002). However, there was no difference in the levels of anti-SARS-CoV-2 antibodies across categories of RH2, regardless of the vaccine taken.

Discussion: RH2-positivity and stronger anti-C reaction were associated with protection against SARS-CoV-2, practically duplicating previous observations regarding HIV infection. Since both pathogens are ssRNA viruses, the primary mechanism likely involves Toll-like receptors that favor a TH1 response. The TH1 polarization is also suggested by protection against infection devoid of a superior humoral response. Further investigations could potentially provide a new approach to treat or prevent both viruses and other single-stranded RNA viruses.

Keywords: SARS-CoV-2; HIV-1; RH2; Blood Group C; Rhesus Blood group; TH polarization

Introduction

Blood groups are antigens carried mostly by erythrocytes but may also be expressed on other tissues as well. The physiological function of many blood group antigens is not known. Some erythrocyte membrane proteins, such as complement receptors, allow them to capture complement-opsonized bacteria and viruses [1,2], thus partially fulfilling an immunological function. In fact, anaemia has been reported to correlate with poor prognosis amongst COVID-19 patients [3,4] as has also been shown in HIV infection [5]. Erythrocytes have been reported to bind HIV-1 [1,6,7], norovirus [8], parvo viruses [9], type 5 adenovirus [10], SARS-CoV-2 [11] and many other microorganisms, including bacteria [12,13] and malaria [14]. This ability to bind a wide variety of microorganisms could arguably be a critical component of innate immunity that has not been adequately explored.

It is plausible that binding pathogens to erythrocyte antigens could lead to destruction of such pathogens in splenic circulation. Alternatively, erythrocytes could provide competitive ligands to act as decoys that distract the pathogen, sometimes getting sacrificed as in the case of malaria infections. Other studies have also reported some level of protection against HIV-1 and SARS-CoV-2 infection in blood group "O" individuals [15,16]. This has been attributed to the presence of soluble blood group antigens adsorbed from the plasma of group A and B individuals [17]. When these viral particles are introduced into group "O" individuals, the virus is neutralized by the anti-A and anti-B present in the plasma of group "O" individuals. Obviously, these antibodies are unable to protect from the virions from other group "O" individuals or individuals of the same blood groups.

We have reported the protective role of blood group C, also denoted as RH2 antigen, against HIV-1 infection [18], demonstrating that the blood group is associated with enhanced CD4 and CD8 cell counts [19], lower viral load (VL) and slower CD4 cell decline over a four-year follow-up period [20]. In addition, we reported the extreme rarity of HIV-1 infection in blood group C homozygotes, being up to 33 times less likely to be HIV-positive [20]. Based on UNAIDS data and published reports on RH2, we estimated that nearly 60% of global HIV-1 prevalence may be explained by RH2 relative frequency alone, despite there being other important risk factors for infection. Thus, data favouring increased cytotoxic T cells suggests a TH1-polarization of the immune response associated with blood group C.

Inheritance of a single RH2 gene has been associated with a significant increase in the antibody response to swine influenza virus A (H1N1) pdm09 [21]. Unexpectedly, homozygotes for RH2 in that study exhibited an inferior antibody response against the virus compared to heterozygotes. Although this observation may demonstrate the typical reciprocal inhibition of the TH1 and TH2 immune responses [22,23], the study did not include such an assessment.

Based on these observations, we studied both the antibody response to COVID-19 vaccination, our hypothesis being that RH2 positivity would incline the immune response towards a TH1 response, and therefore lower antibody levels than RH2-negatives.

Materials and Methods

Study Population

We used whole blood samples from a cross-sectional study whose aim was to determine the antibody responses to various COVID-19 vaccines used in Botswana. Participants aged 18 years and above, who had received full vaccination with any of the vaccines approved for such use by the Botswana Medicines Regulatory Authority (Oxford Astra-Zeneca, Johnson & Johnson, Pfizer/BioNTech, Sinovac, and Moderna) were recruited in Gaborone, Botswana. This data was used to specifically probe the effect of erythrocyte RH2 antigen density by comparing the strength of serological reactions among individuals of different RH2 phenotypes.

Specimen Collection and Testing

Demographic and vaccination information was obtained through interviews and examination of vaccination records supplied by the participants. Participants were also asked if they had been diagnosed with COVID-19, which was also corroborated by a positive antibody test against SARS-CoV-2 nucleocapsid protein (anti-N). Timing of SARS-CoV-2 infection relative to vaccination, vaccine side effects and any underlying conditions, including HIV-status, were also collected.

Blood samples were drawn into EDTA-anticoagulated tubes and an aliquot was submitted for routine phenotyping using anti-C and anti-c (Lorne Laboratories, Berkshire, UK). Serological reactions were graded on a scale on 0-4 as follows:

0: No reaction
1+: Small agglutinates with free cells in the background
2+: Small agglutinates with a clear background
3+: Large fragmented agglutinates with a clear background
4+: A single large agglutinate (all cells agglutinated), clear

background

Total antibody levels against SARS-CoV-2 spike (anti-S) and nucleocapsid proteins (anti-N) were measured using the Roche Elecsys® Anti-SARS-CoV-2 Spike and Anti-SARS-CoV-2 Nucleocapsid immunoassay (Roche Diagnostics, Mannheim, Germany) as per manufacturer's instructions.

Ethical Considerations

Ethical clearance was obtained from the Health Research and Development Committee of the Botswana Ministry of Health. Written Informed consent was obtained from participants.

Statistical Analysis

The Statistical Package for Social Sciences version 27 (IBM Corp, Armonk, NY) was used to compare proportions of RH2 individuals positive or negative for HIV or COVID-19. Trend analysis was achieved by use of the Mantel-Haenszel analysis to study the relationship between reaction strength and COVID-19 diagnosis. The Independent samples t-test and the independent median tests were used to compare continuous variables (anti-S, anti-N) across categories of RH2. Results were considered statistically significant if P-values were less than 0.05.

Results

Three hundred and nineteen participants were enrolled in November 2021, being 50 males and 269 females. The age ranged from 20-67 years with a mean of 40.8. 127/319 (40%) reported having been diagnosed with COVID-19. Of these 127, 71 (56%) were diagnosed before taking any vaccines, while the remaining 44% were diagnosed after taking at least the first dose.

RH2 expression on erythrocytes (n=319) was associated with a negative SARS-CoV-2 diagnosis (OR= 0.42, 95%CI: 0.22-0.80, p=0.008). The strength of the serological reaction between anti-C and erythrocytes was predictive of a COVID-19 diagnosis (Mantel-Haenszel Chi Square = 9.44, p=0.002). Ninety five percent of participants who reported having been diagnosed with COVID-19 also tested positive for anti-N (p<0.001). However, there was no difference in the mean or median levels of anti-S or anti-N antibodies across categories of RH2, regardless of the vaccine taken. Interestingly, in samples collected between December 2021 and June 2022, the significant relationship between COVID-19 diagnosis and RH2 antigen expression was not observed. This coincided with the time when Omicron was first detected in Botswana and South Africa as well as accelerated vaccine coverage.

Discussion

The physiological function of the RH2 and other Rhesus antigens, on the other hand, are not known. Our previous reports provided strong evidence on RH2 protection against HIV [18-20]. However, the mechanism remains unclear. Specifically, our previous studies documented higher CD8+ cell counts in RH2-positive, HIV-positive individuals [19], compared to RH2-negatives. In this study, we report that the RH2-associated protection extends to SARS-CoV-2 infection as well.

We investigated the relationship between infection with COVID-19 and carriage of the RH2 antigen. In addition, we quantified the strength of the serological reaction as a surrogate for antigen density on the red blood cells [24]. Evidence obtained in this study suggests that the level of RH2 expression on erythrocytes is more important than the mere presence of the gene. Our results also indicate that carriage of this antigen is associated with a significant 60% reduction in the odds of being infected with COVID-19.

The level of protection against COVID-19 and HIV diagnoses were statistically comparable [18-20]. Similarly, the trend between serological reactions and a positive diagnosis was comparable between these two ssRNA viruses, leading us to conclude that a common mechanism probably underpinned the protection.

In this study, we used SARS-CoV-2 anti-nucleocapsid antibodies (anti-N) to detect individuals who had a previous COVID-19 infection. Only a small proportion (5%) of individuals with a COVID-19 diagnosis tested negative for anti-N antibodies. Although RH2 was associated with protection against COVID-19, mean and median anti-S and anti-N antibody levels did not differ across categories of RH2 phenotypes.

Based on our observation of a clear protection devoid of antibody dominance, we hypothesize that the blood group likely promotes a Th1 response, consistent with previous studies that showed enhanced CD8+ T-cell counts in HIV-positive individuals [19]. Interestingly, one study reported that underlying conditions that exacerbate COVID-19 such as asthma, diabetes, hypertension and old age, were associated with Th2 (humoral) rather than a Th1 (cell-mediated immunity) response [23]. A study of cytokine profiles of acute phase COVID-19 patients reported that a Th2 response was associated with a fatal outcome [25]. Conversely, a strong cytotoxic response was associated with survival in patients admitted to ICU [26,27]. That notwithstanding, in the current study, SARS CoV-2-infected RH2-positive and Rh2-negative participants did not differ in the severity of symptoms reported. This could be a reflection of the

inadequate quantification of "severity" of symptoms in this study.

Rh2 antigens are expressed only on erythrocytes and are highly hydrophobic [28] with the bulk of the antigen buried in the lipid bilayer. It remains to be determined how an antigen expressed only on erythrocytes impacts cytotoxic T-cells. However, we cannot exclude the possibility that erythrocyte-bound viruses, as documented by others, [6,7,29] are ultimately phagocytosed by antigen-presenting cells leading to the activation of toll-like receptors that favour a TH1 response, enhancing both innate and adaptive immune responses.

Our data shows loss of the relationship between Rh2 and COVID-19 diagnosis in samples obtained after November 2021. We hypothesize that this may be reflective of the effect of widespread vaccination which obliterated the infections in RH2-negative individuals. Moreover, the Omicron variant was detected in Botswana and South Africa on 24th November 2021, and soon became the dominant strain. We cannot also exclude the possibility that the more infectious variant may have further blurred susceptibility between RH2-positive and RH2-negative individuals.

Limitations of the Study

Samples for this study were obtained from individuals who had already received COVID-19 vaccines. Ideal samples would have been those from natural infections without vaccination so that the vulnerability of different phenotypes could be studied in the absence of vaccine protection. However, under the circumstances, these were the only samples available, and the results are, in our view, significant.

Conclusion

There is strong evidence of association between RH2-expression and protection against HIV and COVID-19 diagnosis. This study provides additional evidence of an immune function attributable to an antigen with hitherto unknown physiological function. Although a TH1 response is suggested, more studies are necessary to establish the mechanism and explore how it may be applied to treat or prevent SARS_CoV-2 and HIV infections.

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Authorship Contributions

Modisa S Motswaledi: Conceptualization, formal analysis writing original draft. Sikhulile Moyo: Validation, formal analysis, review and editing. Mogomotsi Matshaba: Writing review, fund acquisition. Mosepele Mosepele and Kereng Masupu: Fund acquisition, Project administration. Simani Gaseitsiwe: Resources, project administration. Basetsana Nthoiwa Charity Ralegoreng, Sethunya Gotulweng: Experimental work. Matshediso Zachariah, Irene Gobe, Kaelo K. Seatla, Margaret Mokomane: Discussion and review of draft Nabila Youssouf, Ponego Lloyd Ponatshego: Data curation.

Disclosure of Conflict of Interest

The authors declare that they have no conflict of interest in this study.

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