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The Effect of Rh2 Phenotype on Cytotoxic T- Cell Counts

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Abstract

Sub-Saharan Africa and the Caribbean countries bear a disproportionate burden of the HIV pandemic. This suggests a genetic predisposition arising from a common ancestry. A 40 % HIV risk-reduction associated with Rh2 blood group was reported in a previous study by the same authors. The current study seeks to elucidate potential mechanisms for this risk reduction. Lymphocyte sub-populations and viral load measurements were achieved by routine diagnostic laboratory methods in 102 untreated HIV-1 infected patients. The results were compared across the categories of RH2 blood group. Carriage of the Rh2 antigen was associated with a higher proportion of T-cells (82±8% versus 75±10%, *P*=0.001), especially single-positive ($CD8^+$) cytotoxic T-cells (64±14 versus 54±13%, *P*=0.004). High absolute CD8 counts were more prevalent among the Rh2-positive than the Rh2-negative subjects (90 % versus 65 %, p=0.023). Among the Rh2-positive subjects, the increase in CD8 count paralleled the viral load in comparison to the Rh2-negatives (r^2=0.630, *P*<0.0001 versus r^2=0.148, *P*<0.001, respectively). The results suggest that Rh2 enhances the CD8 counts in an HIV infection, and its cells are known to play a vital role in immunity against HIV. This probably explains the protective role observed against HIV-1 infection.

Keywords: Rh2; HIV-prevalence, CD8 count, CD4 count, Viral load; African.

1. Introduction

HIV prevalence in Botswana has been ranked among the highest in the world (Weiser *et al.*, 2006). Many studies have revealed risk factors that have contributed to the spread of the virus, some of which include poverty, multiple sexual partners, alcohol and drug abuse, as well as the improper use of protective devices (Keetile, 2014). In some studies, the risk of infection in women was partly attributed to cultural norms that subject women to sexual abuse or assign them to a lower economic status (Shannon *et al*., 2012).

The high prevalence of HIV in Africa as well as those communities comprised of people of African descent, such as the Caribbean, strongly suggests a common genetic link. However, few such links have been alluded to. In at least two studies, the Duffy antigen receptor for chemokines was reported to promote HIV infection (He *et al*., 2008; Lachgar *et al*., 1998) among individuals of African extraction. However, these reports were strongly refuted by other investigators (Winkler *et al.*, 2009). In yet another study, the over-expression of the P^k blood group was reported to protect against HIV infection (Lund *et al*., 2009). However, this antigen is rare among Africans(Cooling, 2014). While it is generally accepted that host genetic factors play a role in immunity against the virus (Chatterjee, 2010), no specific hereditary factors have been advanced to explain the pandemic in Africa.

Although the function of many blood groups remains unknown, some reports have demonstrated that erythrocytes (red-blood cells) bind HIV, and such HIV becomes more efficiently transferred to CD4+(Beck *et al*., 2009; Beck *et al*., 2013; Garcia *et al*., 2012) cells. Erythrocyte antigens are therefore logical targets for erythrocyte-virus interactions. In a previous work by the authors of this study, it was observed that a 40 % risk reduction for HIV-1 infection in individuals of the blood group C (Rh2), while blood groups P_1 and Lu^b were associated with double and triple risks, respectively (Motswaledi *et al.*, 2016). Of an epidemiological importance was the observation that this protective blood group was very rare among Africans, while the risk-associated blood groups were much more common, which raises the question of whether this antigenic profile could have contributed to the peculiar susceptibility of this population to infection with HIV-1.

Blood group C is a component of the Rh blood group system. Antigens in this system are inherited as a block of genes in close proximity to each other on chromosome 1. This ensures that the genes are always inherited together. The RHD gene codes for the D antigen, while the RHCE gene carries a polymorphism that leads to the production of a range of RH phenotypes that include CE, Ce, cE or ce (Ripoche *et al*., 2004). The Rh gene products are organized on the erythrocyte membrane as a complex of proteins that include the Rh-associated glycoproteins (RhAG), LW, CD77, Duffy and CD47, which serve as ammonia transporters (Anstee and Tanner, 1993; Pourazar, 2007; Ripoche *et al*., 2004).

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The current study performed a viral load testing, CD4 and CD8 counts and compared the results among patients expressing or negative for Rh2. From these results and the review of some available literature, the current study sought to find out if the observed risk reduction for HIV infection could be corroborated by clinical laboratory data.

2. Materials and Methods

One hundred and two (102) HIV-1-infected (39 males and 63 females) and treatment-naïve individuals have been enrolled in this study. Since, no drug tests were done to verify their treatment-naïve status, all subjects with undetectable viral loads were excluded on the presumption that they could have been exposed to treatment.

Anonymized EDTA-anticoagulated samples from a central HIV testing laboratory in Gaborone have been used in this study. The ethical clearance was obtained from the University of Botswana's Office of Research and Development, Human Research and Development Committee of the Ministry of Health and Wellness, Gaborone District Health Management Team and the Research Ethics Committee of the Faculty of Health and Wellness Sciences at Cape Peninsula University of Technology. Individual consent was not needed since the study used anonymized residual samples (ISO, 2007).

The viral load was measured using the Cobas® Taqman 48 analyser (Pleasanton, CA., USA). CD3, CD4, CD8 and CD45 counts were obtained using the FacsCalibur ® flow cytometer (Becton-Dickinson, San Jose, CA, USA) and the CD3/CD8/CD45/CD4 Trucount panel. The samples were further phenotyped for the C antigen using specific anti-C and anti-c antibodies (Fortress Diagnostics, Antrim, UK). The reactivity of the antisera was confirmed with C/c-positive and negative cells selected from an antibody identification panel, Bio-Rad DiaPanel®, Lot 45241.88.1, (Cressier FR, Switzerland).

The results of the laboratory tests were interpreted in line with the reference ranges previously determined for the Botswana population (Mine *et al*.,2011). CD4 and CD8 counts from that study of normal individuals were also used to compare counts in the current study in a one-sample t-test.

The results were analyzed using the IBM SPSS version 24 statistical software. The independent t-test was used to compare means. Correlations were used to study the effect of blood RH2 on the relationship between CD8%, log viral load, CD8% and CD4%. Results were considered significant only if P<0.05.

3. Results

3.1. Results from Experimental Data

The mean viral load did not differ among individuals positive (n=21) or negative (n=81) for C (*P*=0.398). Representative dot plots for lymphocyte populations are shown in Figure 1.

In the untreated HIV-1-infected subjects, the CD4 count was significantly lower than that in the general uninfected population. On the contrary, the absolute CD8 count was significantly higher in the infected than in the uninfected subjects. The T-test results are shown in Table 1.

Individuals expressing the Rh2 antigen, 19/21 (90 %)) had a high absolute CD8 count compared to 52/80 (65 %) individuals who were Rh2-negative. A high CD8 count was therefore more associated with C-positive individuals than with the C-negative (*P*=0.023) population. The mean CD8 count between the two categories of Rh2, though higher in the C-positive group, did not reach statistical significance. However, the proportion of T-lymphocytes (CD3+) and cytotoxic (CD8+) T-cells was higher in the C-positive population as shown in Table 2.

Among C-positive subjects, the increase in CD8 counts paralleled the viral load more strongly than in the C-negatives. These results are shown in Figure 2.

Table 1. Comparison of CD4 and CD8 in untreated, HIV-infected individuals and normal controls.

Lymphocyte population	Mean in HIV-infected Subjects (n=101)	Mean in normal population (n=261)	*P*-value
CD4	307±208	859*	<0.0001
CD8	927±492	540*	<0.0001

*One sample (T-test) mean used from a previous study (Mine *et al.*, 2011).

Table 2. Comparison of mean Lymphocyte sub-populations in untreated HIV-infected patients

Lymphocyte Sub-population	C-Positive (Mean±sd) n = 21	C-Negative (Mean±sd) n = 80	*P*-value
CD3+ (%)	82±8	75.0±10	0.001
CD8+ (%)	64±14	54±13	0.004
CD8 Absolute count (/µL)	1058±444	893±500	0.171

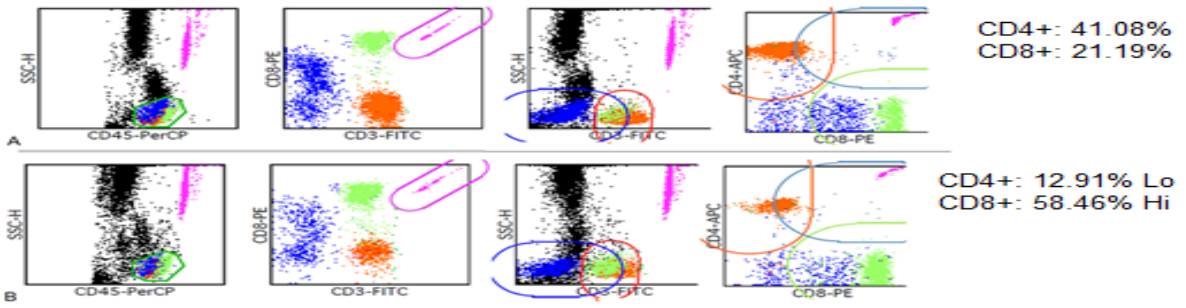

Figure 1. Lymphocytes were gated on the SSC-H vs CD45 and SSC-H vs CD3. A=normal control showing adequate CD4+ cells relative to CD8. The reverse is observed in the patient (B).

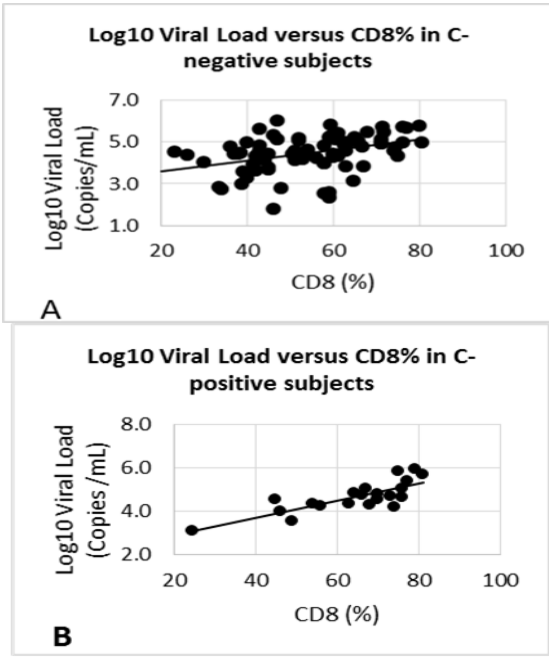

Figure 2. Relationship between viral load and CD8% in C-positive subjects shows strong positive correlation (r2=0.630, p<0.0001 versus r2=0.148, *P*<0.001).

3.2. Results from Country HIV Prevalence Data

To test this observation in real life, a literature search was undertaken to investigate HIV prevalence in countries where the frequencies of the Rh2 are known. Data was obtained for twenty countries or communities as shown in Table 3. Countries or communities with an Rh2 frequency less than 30 % consistently presented a high HIV prevalence (Pearson χ^2=20.0, *P*<0.0001).

Table 3. Comparison of Rh2 frequencies with HIV prevalence across geographical regions

Country/Community	Rh2 Frequency	HIV Prevalence
Botswana	29.70	21.90
South Africa (Blacks)	18.80	18.90
Nigeria	28.2	2.9*
Cote d'Ivoire	21.97	2.70
Caribbean	24.50	1.60**
Uganda	3.62	6.5***
Thailand	51.50	1.10
Ethiopia (Blacks)	41.18	1.10
South Africa (Asians)	99.53	1.00
Mali	58.8	1.00
Brazil	63	0.6
Mauritania	42.69	0.5
Argentina	62	0.4
Laos	60.30	0.30
Southern India	88.00	0.30
India	87.00	0.30
Northern India	84.76	0.26
Sudan	58.40	0.20
Netherlands	68.00	0.20
Iran	75.90	0.10
China	78.22	0.04

*5.8% in 2001. Some states at some point exceeded 8%. Nigeria carries the world's heaviest HIV burden(Gwaram, 2013).
**Prevalence rates range from 1-5% for different islands, depending on the reporting dates(Coggins, 2006; UNAIDS, 2016a). RH2 information for Curacao used here.
***Prevalence is down from 24.8% in 1994 (Kilian *et al*., 1999).

4. Discussion

4.1. Rh2, CD8 Parameters and Protection against HIV Infection

This study seeks to establish the mechanism of protection provided by carriage of Rh2 observed in a previous study. In this study, the expression of this antigen was associated with a higher proportion of cytotoxic (CD8+) T-cells (CTL), which corroborates protection against HIV. In C-positive subjects, the CD8 absolute count varied in a direct proportion to the viral load, suggesting a coordinated response to viremia. This correlation was much weaker in the C-negative group. Moreover, the proportion of individuals with high CD8 counts was greater in the C-positive group. All these observations suggest a role for this antigen in cytotoxic T-cell kinetics and function.

CTLs are important in the control of viremia in acute HIV-1 infections (Goonetilleke *et al*., 2009). They also correlate negatively with infection rates (Oxenius *et al*., 2004) and with good prognosis (Rinaldo *et al*., 1995). Moreover, they have been linked to the slower progression of disease in non-progressors by producing HIV-1-specific CTL responses (Alimonti *et al*., 2006; Betts *et al*., 2001) and increasing perforin production in the HIV-infected non-progressors (Migueles *et al*., 2002).

The enhancement of CTLs points to a mechanism involving major histocompatibility class I (MHC-I) response, in which a cell-mediated rather than humoral response is invoked, and this kind of response is critical for viral infections such as HIV. This was an unexpected finding for an erythrocyte antigen to be involved in an immune reaction. However, it is noted that erythrocytes and erythrocyte-bound platelets selectively bind infectious HIV virions (Beck *et al*., 2013). Furthermore, platelets have been shown to engage in direct MHC-I antigen presentation to CTLs (Chapman *et al*., 2012), a phenomenon that has also been documented in murine megakaryocytes (Zufferey *et al*., 2015). This study maintains that the Rh antigens co-localize on the membrane with CD47 (Ripoche *et al*., 2004), an activator of CTLs (Seiffert *et al*., 2001). Furthermore, both CD47 and Rh2 have been implicated as prognostic indicators in adenocarcinoma of the lungs (Schulze *et al*., 2018), noting that cell-mediated immunity is also critical for immunity against cancer cells.

CD47 also interacts with its ligands on monocytes and T-lymphocytes in signal-transducing events. In this regard, it binds to signal regulatory protein-α (SIRPα) in a high-affinity interaction that results in T-cell activation (Seiffert *et al*., 2001). The binding of CD47 to SIRPβ2 enhances adhesion of T-cells to antigen-presenting cells, and therefore enhances cell-mediated immunity (Piccio *et al*., 2005). In activated T cells, such as those occurring following the HIV infection, CD47 promotes Fas-mediated apoptosis (Manna *et al*., 2005). All these events work to eliminate HIV-infected CD4+cells, and therefore minimize the chances for an infection to be established.

4.2. Evidence from Empirical HIV Prevalence Data

To further investigate its potential role in the epidemiology of HIV worldwide, this study compared the frequency of the Rh2 antigen across populations with varying degrees of HIV prevalence. A thorough literature

search yielded Rh2 data from twenty countries. Invariably, countries with low frequency of Rh2 were consistently associated with the highest prevalence rates in their regions. In Southern Africa, antigen frequency data were available only for Botswana (Motswaledi et al., 2016) and South Africa (Tax *et al*., 2002). Cote d'Ivoire and Nigeria (Gwaram, 2013) likewise had the lowest antigen frequencies (Bogui *et al*., 2014) and the highest HIV prevalence in West Africa (UNAIDS, 2016a). In contrast, other African countries with higher antigen frequencies had low HIV-prevalence rates comparable to or slightly above those in other non-African countries, such as Argentina (Cotorruelo *et al*., 2008), Brazil (Guelsin *et al*., 2011), Thailand (Nathalang *et al*., 2001), Laos (Keokhamphoui *et al*., 2012), India (Makroo *et al*, 2013), Netherlands (Tax *et al*., 2002), Iran (Shokouhi Shoormasti *et al*., 2011), and China (Ma *et al*., 2018), where the Rh2 frequency is higher than 40 %. These African countries include Mali (Ba, *et al*., 2015), Mauritania (Hamed *et al*., 2013), Ethiopia (Tax *et al*., 2002), Sudan (Elfadni *et al*., 2014), and among South Africans of Asian origin (Tax *et al*., 2002).

Rh2 data for Caribbean Islands were not readily available, except for Curacao. The Caribbean Islands represent the second highest HIV-prevalence outside Africa (UNAIDS, 2016b). Apparently, a significant proportion of this population originates from Africa, and the Rh2 antigenic profile performed in Curacao (Tax *et al*., 2002) is similar to that of the African countries where high HIV rates are found. This study proposes that the Rh2 antigen may be important for HIV immunology and probably explains the genetic basis for the geographical distribution of the pandemic.

5. Conclusion

The low frequency of the Rh2 antigen in Botswana and other countries with similar frequencies may have contributed to the rapid spread of HIV-1 among the populations of these countries. Results also suggest an immunological role of an Rh-system antigen, unreported hitherto. However, the current study has been limited in that it has not measured specific CD8 immune responses such as the interferon gamma response to specific HIV-1 peptides in C-positive and negative individuals. Furthermore, other co-variables that are known to affect the risk of infection were not considered in this cross-sectional study.

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