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Research Article

Human Leukocyte HLA-B7 and HLA-B27 Antigen Typing among Healthy Adults Primedwith HIV-1 DNA Boosted with Recombinant Modified Vaccinia Ankara in Dar Es Salaam

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ABSTRACT

Background: The human leukocyte antigens are genetically inherited proteins present on thesurface of the cells of the immune system. Cells of the immune system recognize antigens only when presented to them next to an MHC self-molecule. Three main types of HLA Class I namely, HLA-A, HLA-B and HLA-C they are expressed by most human cells. Class I HLAs are involved in discriminating and presenting antigens to T-lymphocytes. T-lymphocytes kill the cell presenting it. T-lymphocytes destroy cells infected together with viruses, including HIV. Developing a vaccine for HIV may be aided by a complete understanding of those rare cases in which some HIV-infected individuals control replication of the virus. Most of these elite controllers express the histocompatibility alleles HLA-B57 or HLA-B27, these alleles remain the most robust associations with low concentrations of plasma virus.

Objectives: To evaluate the magnitude of Human leukocyte antigen production before and uponadministration of multiclade, multigene HIV-1 DNA prime/MVA boost among healthy individuals in Dar es Salaam, Tanzania.

Methods: EDTA plasma samples collected at baseline and post DNA prime and MVA boostimmunizations and archived in HIV vaccine immunogenicity study (HIVIS03) was used for HLA typing whereby HLA-B7 and HLA-B27 were determined using quantitative ELISA. Results were read and interpreted as per manufacturer's instructions Data analysis was done using IBM SPSS software.

Results: A total of 42 samples from Police officers were tested at baseline and after HIV-1DNA MVA boost vaccine the mean age of tested subjects was 24 there were 34 males and 8 females,20 of them the vaccine was administered intradermally and 22 of them the vaccine was administered intramuscularly, educational level of participants was certificate of secondary education The mean, median and range of levels of HLA-B7 in males was Both HLA B7 andHLA B27 levels increased from baseline to vaccines group, but didn't show significant difference between placebo and vaccines. Moreover HLA B7 and HLA B27 didn't show association on mode of delivery of the vaccine, sex and age.

Conclusions: Both HLA-B7 and HLA-B27 were detected No statistically significant differencein levels we recommend further HLA typing using molecular or cellular methods.

Keywords: MVA; HIV-1; DNA; HLA

INTRODUCTION

The human immune system has several components including the HLA, which is controlled by genes located on the short arm of chromosome 6. The HLA loci are part of the genetic region known as the Major Histocompatibility Complex (MHC) [1]. The genes for regulation of the MHC are integral to normal function s of the immune response. The essential role of the HLA antigens bases in the control of self-recognition and thus defence against pathogens [2, 3]. The HLA loci, by virtue of their extreme polymorphisms ensure that few individuals are identical and thus the population at large is well equipped to deal with pathogenic attacks. Because some HLA antigens are recognized on all of the tissues of the body (rather than just blood cells), the identification of HLA antigens is described as Tissue Typing or HLA Typing [4].

Human leukocyte antigens (HLAs) are an inherent system of alloantigen, which are the products of genes of the MHC, these genes span a region of approximately 4 centi-morgans on the short arm of human chromosome 6 and encode the HLA class I and

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class II antigens, which play a central role in cell-to-cell interaction in the immune system. These antigens interact with the antigen-specific cell surface receptors of T lymphocytes (TCR) thus causing activation of the lymphocytes and the resulting immune response [5, 6, 7]. Class I antigens restrict cytotoxic T-cell (CD8+) function thus killing endogenous infected cells such as occur with viral infected targets, while class II antigens are involved in presentation of exogenous antigens to T-helper cells (CD4+) by antigen presenting cells (APC). The APC processes the antigens, and the immunogenic peptide is then presented at the cell surface along with the MHC molecule for recognition by the TCR. Since the MHC molecules play a central role in regulating the immune response, they may have an important role in controlling resistance and susceptibility to diseases [5, 8, 3, 9].

MHC molecules are highly polymorphic glycoprotein's that are expressed at the cell surface after assembly within the cell, together with beta 2 microglobulin and short antigenic peptide fragments [10]. This combination can then be recognized by CD8+CTLs. Specificity for a particular MHC/peptide combination is conferred by the TCR, which is an immunoglobulin -like molecule [11,12]. CTLs play a major role in the adaptive immune response to intracellular infections and tumours, specifically killing appropriate target cells and also releasing pro-inflammatory cytokines such as IL-1 and TNF-.

The principal human leukocyte antigen (HLA) MHC class I alleles are denoted HLA-A, HLA-B and HLA-C [36]. CTLs can be detected according to their function, for example in killing assays or by analysis of cytokine production, according to either their antigenic specificity (using MHC class I multimers) or their phenotype i.e. their TCR or other expressed molecules [1, 6].

Althoug about one-thousandth of the total human genome the HLA system contains several genes that have important functions in biology and medicine [13]. MHC classes also play a very crucial role in donor selection for organ and bone marrow transplantation. Other important areas in which HLA has played a great role include paternity determination, identification of susceptibility or predisposing genes for a wide variety of diseases, particularly those with infectious and autoimmune aetiology, prediction of risk development for disease in families, anthropological characterization of different races and ethnic groups, and for understanding the control and regulation of the immune system [13].

MHC class I and II molecules are necessary in increasing the efficiency of the immune system for handling a broad spectrum of peptides [14]. Although the general rules for peptide–MHC interactions for both classes of MHC molecules are essentially similar, X-ray crystallographic studies have indicated that distinct peptides bind to class I or class II molecules owing to differences in their binding clefts [15, 16]. In accordance with the distribution of hydrogen bonds in the peptide-binding groove of the MHC, the anchor residues are placed at the terminal ends of the class I groove [15]. Class I MHC molecules has closed ends, peptides bound by short, generally 9 – 10 amino acids. The residues occupy deep pockets in the antigen-binding site. On the other hand, the MHC class II cleft is more open ended, accommodating peptides that are more variable in size and much longer than nonamers, generally 14–18 amino acids [7]. The groove anchored stretch of the peptide exactly nine amino acids long, with its flanking parts protruding out of the groove's ends [7]. In relation to the MHC class II motif structure, relative positions of five peptide binding pockets designated as P1, P4, P6, P7 and P9 have been defined, each of which accommodates specific peptide side-chains. B-cells and antigen-presenting cells such as macrophages, dendritic cells, fibroblasts, Langerhans cells and Kupffer cells constitute intracellular surveillance which is sub-served mainly by T cells. The TCR recognizes peptides only if they are presented in association with the self-MHC molecule, while CD81 T-cells are restricted by MHC class I– peptide complexes and CD41 T-cells by class II–peptide complexes. Since CD4 T-cells are placed at the initial phase of the immune response and provide helper signals to both CD81 T-cells as well as B-cells, they are central to both extracellular and intracellular surveillance [7].

HLA-B57 and the closely related HLA-B5801 are over-represented among HIV-1 infected long-term non progressors (LTNPs) [9]. It has been suggested that this association between HLA B57/5801 and asymptomatic survival is a consequence of strong CTL responses against epitopes in the viral Gag protein. Moreover, CTL escape mutations in Gag protein genes would coincide with viral attenuation, resulting in low viral load despite evasion from immune control [9]. A potential loss of viral fitness has been most clearly demonstrated for escape mutations in epitopes that are restricted by HLA-B27 and HLA-B57/5801. This loss of fitness has been used to explain the protective effect of these HLA types and a long-term non progressive clinical course of HIV infection, HLA-B57 is also present in up to 11% of HIV infected patients with progressive disease, which is similar to the frequency in the Caucasian population [17]. As the HLA class I loci have been implicated in the course of many infectious diseases, including human immunodeficiency virus type 1 (HIV-1), the consistent association of certain alleles such as HLA-B57 and HLA-B27 with HIV-1 disease progression and the association of HIV-1–specific CTL responses with reduced plasma viral load in early infection suggest an HIV-1–specific CTL control of HIV-1 replication once infection has been established [4]. Moreover one of the African specific allele HLA-A74:01 is associated with protection from HIV-acquisition and disease progression as reflected in previous studies [4]. According to this community-based cohort study, it was concluded that African-specific allele HLA-A74:01 displayed a protective effect for HIV-1 acquisition and disease progression in the setting of a mixed subtype epidemic [4]. Moreover Experimental evidence shows that CD8+T cells play a central role in controlling HIV-1 viremia during primary infection as well as in the long-term suppression of viral replication [8]. The correlation between expressions of specific MHC class I alleles and HIV-1 control strongly supports the idea that CD8+T cells have a role in controlling HIV-1 viremia. Early studies performed in the late 1980s on small cohorts of AIDS patients reported an increased frequency of HLA-C04 and HLA-B35 in HIV-1-infected patients compared to non-infected controls [8] an elevated HLA-C04 frequency in patients with Kaposi's sarcoma and of HLA-C07 in patients with opportunistic infections [8]. Recent studies have reported an increase in prevalence of HLA-C07 in HIV-1 positive individuals [2]. A larger study published in 1999 established the association of HLA-C04 and HLA-B35 with rapid development of AIDS-defining conditions in Caucasians [18] while showing maximum homozygosity at the HLA-B locus to be more detrimental than homozygosity at either HLA-A or HLA-C. A follow up study done later by the same group showed that peptide presentation properties of particular HLA-B35 subtypes explain much of the HLA-C04 and HLA-B35 association [15]. The dominance of HLA-B in the control of HIV-1 infection is supported by several other studies which associated HLA-B57, HLA-B5801 and HLA-B27 with slower disease progression rates (time to AIDS) and strong virologic (viral load) and immunologic control (CD4 count) [27, 19]. On the other hand, the HLA-B35, HLA-B5802 and HLA-B18 were associated with ineffective control of viral replication and rapid progression to disease [20, 17]. Research by Kiepiela in 2004 have shown that, a significantly greater number of CD8+T cell responses are HLA-B restricted, compared to HLA-A and -C variation in viral set-point, absolute CD4 count, and rate of disease progression are strongly associated with particular HLA-B, but not HLA-A or HLA-C alleles [21]. Recently, a study on blood

donors in China who were contaminated by a narrow source blood born virus showed that HLA-B exerts a greater selection pressure on HIV evolution than other HLA molecules [22]. Expression of HLA-DR at the cell surface induces a redistribution of mature Gag products into late endosomes by enhancing nascent HIV-1 particle internalization from the plasma membrane through a process that relies on the presence of intact HLA-DR-□ and □-chain cytosolic tails. These reports raise the possibility that major histocompatibility complex class-II molecules might influence endocytic events at the plasma membrane and as a result promote endocytosis of progeny HIV-1 particles [1].

Several studies have been done to study the association between HLA subtypes and HIV vaccines. Most HIV vaccine induces T-cell responses in individuals receiving an HIV-1 recombinant adenoviral vaccine [40]. Certain HLA alleles HLA-B27, HLA-B57, HLA-B35, and HLA-B14 are preferentially utilized to mount HIV-specific responses, whereas other alleles (A02 and B07) are rarely utilized [23, 41]. Higher affinity of specific peptides to specific HLA alleles as well as higher avidity of T-cell receptor, HLA and peptide interaction and higher surface expression of certain HLA are said to be important factors for HLA immunodominance that plays a substantial role in vaccine-induced T-cell responses [23, 42]. Moreover, An Ad5-vectored HIV-1 vaccine elicits cell-mediated immunity, in which HIV RNA levels, CD4+T cell counts, time to initiation of ART, and AIDS-free survival were similar in vaccine and placebo recipients, but there were marked increase in levels of HLA types namely, HLA-B27, HLA-B57, and HLA-B58 [23].Study by Kipelainen [24] concluded that Epitopes such as HLA-A*30:01, HLA-A*30:02, HLA-B*58:01 and HLA-C*07:01 contributed to confer both stronger and broader cellular immunogenicity to an HIV-1 nef vaccine. Participants who received a Trivalent Adenovirus Type 5 HIV recombinant vaccine expressed HLA B 27, HLA B57, HLA B58 and exhibited a greater killing than those possessing these alleles [17]. Studies on other disease [25] have shown HLA Class II subtypes HLA-DR3+ and HLA-DR7+ to regulate the human immune responses to hepatitis B surface antigens. However, there is still limited information on the distribution of HLA subtypes and its association with HIV DNA primed MVA boost immunization Tanzania.

It has been documented that HLA types HLA-A74:01 confers a protective role on HIV progression in individuals with chronic HIV infection [4]. It is also reported that HLA-B27, HLA-B57 and HLA-B35 are commonly associated with HIV protection in sub Saharan Africa [3, 9]. However, there are no data on the distribution of HLA types and its association with HIV DNA prime MVA boost immunization in Tanzania. This fact has led us to conduct such a study to gather and mine information on the role and distribution of HLA types following vaccine intervention in healthy individuals.

MATERIAL AND METHODS

Study design

This was a randomized, controlled, double blinded placebo controlled study that involved health adults aged 18 to 40 years, administered with a multiclade, multigene HIV-1 DNA prime/ MVA boost.HIV-1 DNA was given at weeks 0, 4 and 12 as injections. MVA boost injection was given at month 9 and 21 after the last DNA priming injection.

Study subjects and setting

The study was carried at MNH/MUCHS, Dar es Salaam, Tanzania. Participants were recruited from healthy police force volunteers attending pre-screening sessions in Dar es Salaam Tanzania in May 2007. The sample analysis was done in May 2013 to June 2013.

Study population

This study was part of a bigger project that involved 258 Healthy volunteers from police force attending pre-screening sessions and 42 samples were analyzed for HLA subtypes HLA-B7 and HLA-B27. Recruitment and study visits are elaborated by Bakari [26].

Ethical considerations

The ethical clearance was obtained from both Muhimbili University of Health and Allied Sciences (MUHAS) Ethics Review Sub-committee.

Mode of Vaccine Delivery

All immunizations were performed with the Bioject device according to the manufacturer's instruction. An intramuscular injection was given in the deltoid muscle. Ampoules 1 was given in the left arm and ampoules 2 in the right arm. During the procedure the overlying skin was stretched flat prior to applying the Bioject device.

DNA priming

Was effected with DNA plasmids derived from puC8 with akanamycin resistance gene, hCMV promoter, HPV 16 poly A and origin of replication for E. coli. Plasmid carries HIV-1 genes of subtypes A, B and C. These are pKCMVgp160A, KCMVgp160B, pKCMVgp160C, pKCMVrev, pKCMVp37A (ba), pKCMVp37B, and pKCMVpRTB and will code for their respective proteins.

MVA boosting

Boosting was effected by a live recombinant poxvirus ModifedVaccinia Ankara vaccine (MVA-CMDR). This is a vector vaccine that has been genetically engineered to express the following HIV-1 genes:

- gp160 (Subtype E, CM235), and

- gag and pol (integrase-deleted and reverse transcriptase non-functional, Subtype A, CM240)

Laboratory investigations

Blood samples at baseline and post DNA prime and MVA boost immunization were collected in EDTA vacutainer tubes, EDTA blood tubes were and centrifuged at 1500 rev/min for 30 minutes, then plasma was separated from other components of blood and stored at -80°C until the day of HLA typing.

HLA typing

HLA typing was performed using quantitative ELISA in which HLA-B27 and HLA-B7 were determined as previously described (Zachary et.,al 2012). Briefly, the test specimens, control sera and calibrators were added into their respective wells on a 96 wells ELISA plate and incubated at 37 °C for 2 hour, wells coated with a synthetic peptide and antigens specific to HLA antibodies in the sample and calibrators were allowed to bind to antigens on the microwell. Excess sample and other debris were washed subsequently; a conjugate was to binds to any specific antibody already bound to the antigen on the wells. In principle, samples not containing specific antibody do not cause the conjugate to bind to the wells. Unbound Conjugate was then washed away with phosphate buffered saline and a solution containing 3, 3', 5, 5'-TMB and hydrogen peroxide (H2O2) was added to the wells. Wells with bound Conjugate developed a purple colour which was converted to an orange colour when thereaction was stopped with sulphuric acid. After incubation stoppage of the enzymatic reactions sulphuric acid the colour was read spectro-photometrically at 450 nm. The amount of conjugate, and hence colour, in the wells is said to be directly related to the concentration of antibody to HLA in the sample. Calibrators Optical densities or absorbencies obtained were then used to plot a standard curve against their known concentrations

Statistical analysis

Statistical analysis was performed using IBM SPSS software and Student t-test and ANOVA were used to compare the means between age groups, mode of delivery, placebo and vaccines group and sex. Differences at $p \leq 0.05$ between groups were considered as statistically significant.

RESULTS

A total of 42 samples were tested at baseline and post DNA and MVA boost, the mean age of the tested subjects was 24, there were 34 males and 8 females. The tested population was police officers having certificate of secondary education as the highest level of education. HIV-1 MVA boost vaccine was administered to 42 study participants with 20 individuals receiving intradermal administration while 22 of the subjects received the vaccine intramuscularly. EDTA plasma specimens were analysed for HLA-B7 and HLA-B27. Healthy participants were analysed by age, sex, mode of delivery as well as those receiving placebo group versus vaccines group. The levels of HLA-B7 between baseline (visit 3) and after injection of injection of the vaccine HIV-1 DNA MVA boost (visit 21) groups are shown in Figure 3. The placebo consists of saline alone, however there was an increase in HLA when comparing visit 3 and visit 21 due to the fact that visit 21 received MVA boost a vector vaccine that has been genetically engineered to express HIV-1 genes which are gp160 (Subtype E, CM235), and gag and pol (integrase-deleted and reverse transcriptase non-functional, Subtype A, CM240). The distribution of HLA-B7 plasma concentration between males and females at baseline and after MVA boost administration at visit 21 showed no significant difference (p=0.127, p=0.391, respectively). Evaluation of distribution of plasma concentration of HLA-B7 between placebo and vaccine groups both at baseline and after visit 21 revealed some differences but not statistically significant (p=0.88, p=0.47, respectively). The baseline characteristics of the study population is summarised in Tables 1-5 below and ELISA test was done only in subjects whose samples were available for analysis (Figure 3).

Table 1: Immunizations performed at weeks 0, 4 and 12

Volunteers	DNA immunization	MVA boost	
20	DNA intramuscularly by Bioject	MVA 108	i.m
20	DNA id by Bioject	MVA 108	i.m
10	Saline intramuscularly by Bioject	Saline i.m	-
10	Saline id by Bioject	Saline i.m	-

Table 2: Doses of the vaccine.

Vaccine and delivery method	Route	Ampoule1 (env), Left arm	Ampoule2 (gag, Rtmut), Right arm
DNA by Biojector	Intramuscularly	1.0 ml (tot 2.0 mg DNA)	0.9ml (tot1.8mg DNA)
DNA by Biojector	Intradermally	3 injections of 100ul (Total 0.6mg DNA)	2 injections of 100ul (total 0.4mg DNA)

Table 3: Comparison between groups mean, median and range in HLA-B7 levels.

Category	Mean	Median	Range
Gender			
Males baseline	0.21 ng/ml	0.22 ng/ml	0.20 ng/ml - 0.25 ng/ml0.50
Males vaccine	0.52 ng/ml	0.53 ng/ml	0.52 ng/ml - 0.60 ng/ml
Females baseline	0.21 ng/ml	0.22 ng/ml	0.20 ng/ml - 0.24 ng/ml
Females vaccine	0.52 ng/ml	0.50 ng/ml	0.50 ng/ml - 0.58 ng/ml
Mode of delivery			
Intradermally	0.58 ng/ml	0.55 ng/ml	0.50 ng/ml - 0.60 ng/ml
Intramusculary	0.55 ng/ml	0.54 ng/ml	0.50 ng/ml - 0.58 ng/ml
Age <20			
Baseline	0.28 ng/ml	0.28 ng/ml	-
Vaccine	0.50 ng/ml	0.50 ng/ml	-
21-30			
Baseline	0.23 ng/ml	0.24 ng/ml	0.20 ng/ml - 0.29 ng/ml
Vaccines	0.55 ng/ml	0.55 ng/ml	0.50 ng/ml - 0.58 ng/ml
31-40			
Baseline	0.24 ng/ml	0.23 ng/ml	0.20 ng/ml - 0.2 ng/ml
Vaccine	0.60 ng/ml	0.58 ng/ml	0.50 ng/ml - 0.67 ng/ml
Placebo and Vaccines			
Baseline	0.54 ng/ml	0.53 ng/ml	0.48 ng/ml - 0.58 ng/ml
Vaccines	0.56 ng/ml	0.55 ng/ml	0.50 ng/ml - 0.60 ng/ml

Table 4: Comparison of group mean, median and range for HLA-B27 levels.

Category	Mean	Median	Range
Gender			
Males baseline	0.58 ng/ml	0.55 ng/ml	0.52 ng/ml - 0.58 ng/ml
Males vaccine	0.67 ng/ml	0.60 ng/ml	0.52 ng/ml - 0.57 ng/ml
Females baseline	0.582 ng/ml	0.55 ng/ml	0.52 ng/ml - 0.58 ng/ml
Females vaccine	0.66 ng/ml	0.60 ng/ml	0.52 ng/ml - 0.58 ng/ml
Mode of delivery			
Intradermally	0.62 ng/ml	0.57 ng/ml	0.50 ng/ml - 0.67 ng/ml
Intramuscularly	0.60 ng/ml	0.55 ng/ml	0.50 ng/ml - 0.67 ng/ml
Age <20			
Baseline	0.81 ng/ml	0.60 ng/ml	-
Vaccine	0.67 ng/ml	0.62 ng/ml	-
21-30			
Baseline	0.57 ng/ml	0.50 ng/ml	0.48 ng/ml - 0.58 ng/ml
Vaccines	0.69 ng/ml	0.60 ng/ml	0.50 ng/ml - 0.70 ng/ml
31-40			
Baseline	0.56 ng/ml	0.50 ng/ml	0.48 ng/ml - 0.58 ng/ml
Vaccine	0.66 ng/ml	0.58 ng/ml	0.52 ng/ml - 0.68 ng/ml
Placebo and Vaccines			
Placebo	0.71 ng/ml	0.58 mg/ml	0.50 ng/ml - 0.71 ng/ml
Vaccines	0.66 ng/ml	0.53 ng/ml	0.50 ng/ml - 0.60 ng/ml

Table 5: Baseline characteristics of study population and volunteers preliminarily enrolled in the main study

Characteristic		Vaccine recipients		Placebo recipients	
	Overall N=60	Group I (N=20)	Group II (N=20)	Group IIIA (N=10)	Group IIIB (N=10)
Age (years)					
Mean (SD)	29(6)	31.2(6.8)	29.0(5.4)	27.5(6.2)	27.8(5.1)
Median	28	29.5	27.5	25	28
Range	20-47	21-47	22-40	20-40	21-39
Sex					
Male	45(75%)	14(70%)	16(80%)	6(60%)	9(90%)
Female	15(25%)	6(30%)	4(20%)	4(40%)	1(10%)
Education					
Primary	16(27%)	7(35%)	6(30%)	1(10%)	2(20%)
Secondary	36(60%)	12(60%)	11(55%)	7(70%)	6(60%)
Post secondary	8(13%)	1(5%)	3(15%)	2(20%)	2(20%)
Marital status					
Single	37(62%)	11(55%)	13(65%(	7(70%)	6(60%)
Married	23(38%)	9(45%)	7(35%)	3(30%)	4(40%)
Weight in kg		67.7	64.9	65.7	
Mean (SD)	65.2 (12.6)	-13.7	-11.1	-12.9	60.3 (13.1)
Presence of vaccinia scar	32(54%)	10(53%)	13(65%)	4(40%)	5(50%)

Exploration of distribution of HLA-B7 by age showed no statistical difference at baseline (p=0.641) but there were evidence of marginal significant difference (p=0.062) after vaccination as reflected by the HLA-B7 increased plasma levels. Comparisons were also done on the distribution of HLA-B7 with mode of delivery of the vaccine between those participants to whom the test compounds was administered intra-dermally or intramuscularly. The results revealed differences in distribution of HLA-B7 between the modes of administration, with evidence of statistical significance between (p<0.001) signifying that route of administration for a vaccine or vaccine product influence the availability to the systemic circulation and thus other body tissues. Ideally, intramuscular and intra-dermal administration of test compounds (placebo vs MVA vaccine boost) should have differences in terms of availability of the vaccine or placebo to both tissues.

We also determined HLA-B27 by sex (Figure 1 and Panel II), and the distribution revealed no evidence that within the groups, males and females differed significantly in levels of the HLA- B27 (p=0.07) at baseline. After vaccine boostadministration thegroups malesand female showed a significant difference in HLA-B27 levels (p<0.001). Determination of HLA-B27 distribution among age groups revealed significant difference (p=0.02) within the age group 21-30 both at baseline and after vaccine administration (Figure 1 and Panel I). There was no significant difference in HLA-B27 distribution at age group 31-40p=0.08 when compared at baseline and after vaccine administration. The assessment of HLA-B27 distribution by mode of vaccine delivery, between the groups showed no evidence of statistical difference between intra-dermal and intramuscular administration(Figure1 and Panel III).We also determined the plasma concentration of HLA-B27 at baseline before subjecting individuals to either placebo or vaccine groups, There was homogeneity in plasma levels of HLA-27 between the groups (Figure 2).However, after administering either placebo or vaccine to the groups respectively, unexplainable increase in level of HLA-B27 was observed with a significant difference (p=0.04) at visit 21 in the placebo group while no significant difference in HLA B-27 plasma concentration (p=0.1) in the vaccine group. We also determined HLA-B27 by sex (figure 1, Panel V), and the distribution revealed no evidence that within the groups, males and females differed significantly in levels of the HLA-B27 (p=0.07) at baseline. After vaccine boost administration the groups males and female showed a significant difference in HLA-B27 levels (p<0.001). Determination of HLA-B27 distribution among age groups revealed significant difference (p=0.02) within the age group 21- 30 both at baseline and after vaccine administration (Figure1 and Panel VI). There was no significant difference in HLA-B27 distribution at age group 31-40p=0.08 when compared at baseline and after vaccine administration. The assessment of HLA-B27 distribution by mode of vaccine delivery, between the groups showed no evidence of statistical difference between intradermal and intramuscular administration (Figure 1 and Panel VII). We also determined the plasma concentration of HLA-B27 at baseline before subjecting individuals to either placebo or vaccine groups, There was homogeneity in plasma levels of HLA-27 between the groups. However, after administering placebo or vaccine to the groups respectively (Figure 1 and Panel VIII), resulted into unexplainable increase in level of HLA-B27 with significant difference (p=0.04) at visit 21 in the placebo group while no no significant difference in HLA B-27 plasma concentration (p=0.1) in the vaccine group. The combined plasma levels of HLA-B7 and HLA-B27 and changes between visit 3 and visit 21 have also been plotted (Figure 2). There was significant 'negative' change inHLA-B27 plasma levels between the two visits for the age <20 yrs as at visit 3 high levels were recorded as compared to visit 21 (Figure 1 and Figure 2).

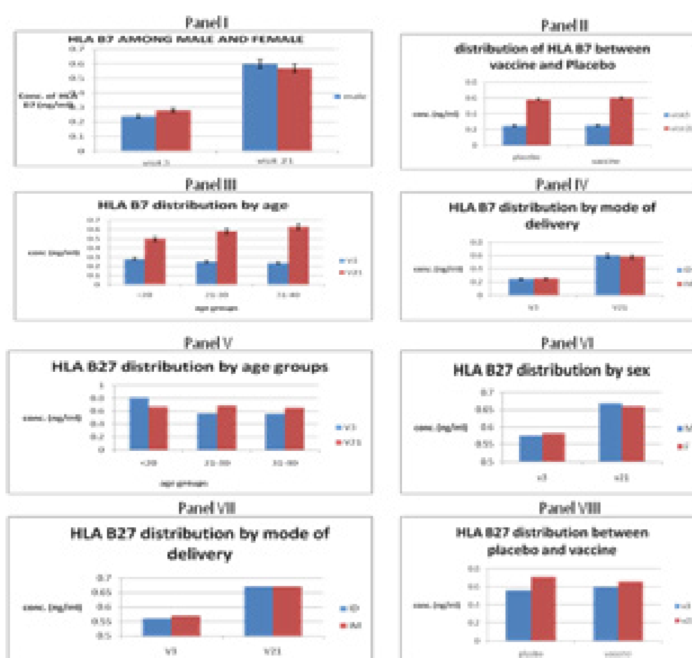

Figure 1: Distribution of HLA B7 between males and females at baseline group and after vaccine administration (Panel I)), distribution of HLA-B7 between placebo and vaccines group (Panel II), HLA distribution by age (Panel III) and HLA distribution by model of delivery (Panel IV). The distribution of HLA-B27 by age, sex, by mode of delivery as well as HLA-B27 distribution between placebo and vaccines groups are shown in Panels V, VI, VII and VIII, respectively.

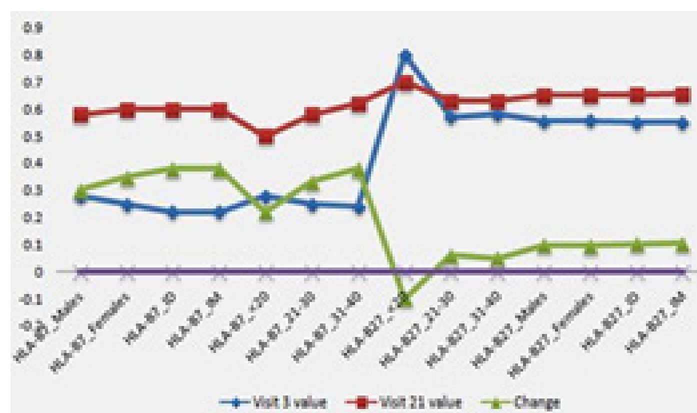

Figure 2: The plot of distribution of HLA-B7, HLA-B27 and their change in plasma level between visit 3 and visit 21. The levels and changes in HLA-B7 and HLA-B27 were also sought in relation to sex, age and route of administration and can easily be comparing.

DISCUSSION

Several studies suggest that innate and adaptive immune responses occurring during primary HIV infections plays a crucial role in determining levels of viral replications and restriction towards disease progression HLA-B7 and HLA-B27 being one of the molecules involved in presenting peptides to specific T cytotoxic lymphocytes [27, 28], [30,29]. Our study aimed at checking the immunogenicity of the vaccine, CD8 (+),CTLs and HLA are some of the markers of immunogenicity, in our study we checked the plasma concentration of the two MHC molecules which are HLA-B7 and HLA-B27. The kit we used in this study has a detection range of 0.525 ng/mL – 4 g/mLl for HLA-B7. In our study, 12

samples had readings below the detection range and none of them had a reading above the detection range. The kit detection range for HLA-B27 is 0.312 ng/mL – 20 ng/mL, for HLA-B27 the lowest concentration we recorded was 0.48 ng/ml none of them had a reading above the detection range meaning that all were within the range,HLA-B7 and HLA-B27 levels in the placebo group from the two visits i.e. visit 3 and visit 21 didn't show a significant difference and the levels of HL-B7 and HLA-B27 were almost the same.

The explanation for the 12 samples with readings below the detection range for HLA-B7 could be that the samples had very low concentration of HLA-B7 expression at the time when the samples were collected or the long storage time of samples that might have led to degradation of HLA-B7 molecules despites the fact that there is a standby generator to control temperature fluctuations. Nevertheless, the lowest reading in our study which was 0.2 ng/mL was above the minimum detectable dose for human HLA-B7 which is 0.156 ng/mL (User Manual, www.cusabio.com). Plasma concentration of HLA-B7 as an indicator of vaccine immunogenicity, after administration of HIV-1 DNA MVA boost vaccine did not show any difference in immunogenicity by sex and age [31]. However the distribution of HLA-B7 which belongs to HLA class I showed a statistically significant difference between baseline and vaccines ($p<0.001$) indicating that HLA-B7contributes to provide a protective role against HIV. This could be due to consistent immune-dominance patterns of HIV-1-specific CD8 (+) T cell responses during primary infection that is said to provide a protective effect on HIV-1 disease progression [2]. The consistent rise in plasma HLA-B7 could also be related to the MVA adjuvant that result into increased expression of HLA class I alleles. This proposition however, needs to be further researched. Nevertheless, our findings provide an insight on the use of MVA boosted HIV-1 DNA to confer future protection against the troublesome HIV virus. This can be taken into consideration in future vaccine development.

Moreover, HLA-B7 didnt show any difference by mode of delivery that was intramuscularly and intradermally the same applies to vaccine and placebo groups (Figure 2, panel IV). HLA-B7 is always in a linkage disequilibrium with other HLA subtypes the common one being HLA-Cw0702 [31]. This could be due to probaly other HLA subtypes masking the effect of HLA-B7 as the method we used quantify HLA subtypes only identify specifically HLA-B7 necessitating the need to study other subtypes as well.

Research by Rajapaksa [32] documented that HLA-B cytoplasmic domains are more resistant to negative factor (Nef) mediated down-regulation than HLA-A cytoplasmic domains and demonstrated different effects CTL recognition of virus infected cell in vitro, The study concluded that resistance to nef mediated down-regulation by the cytoplasmic domains of HLA-B contributes to better control of HIV-1 infection associated with HLA-B restricted CTLs. Our study dealt with HIV-1 DNA MVA boosted vaccine specifically DNA codes for pKCMVgp160A, KCMVgp160B, pKCMVgp160C, pKCMVrev, pKCMVp37A(ba), pKCMVp37B, and pKCMVpRTB and MVA boost only codes gp160 (Subtype E, CM235), and gag and pol (integrase-deleted and reverse transcriptase non-functional, Subtype A, CM240) [4]. HIV-1-DNA MVA boost vaccine does not code for nef. II as an immunogen thus could be the reason that HLA B7 levels are expressed the same as in both vaccines placebo groups.

HLA B27 effect didn't show any significant statistical difference between placebo and vaccines, too, between age and sex but showed a highly significant difference between baseline and after vaccine explaining the role of vaccine boost in activating immune response to HIV infection. Prevailing evidence [33] reports some individuals to be naturally HLA-B27 negative while others having HLA-B27 positive at baseline, implying possible ethnic difference in plasma levels of HLA-B27 [17, 28]and possibly ethnic adaptive divergence [43]. This can also explain for the different reactions observed in subjects edged <20 yrs after a boost at visit 21. However, due to the peculiar changes being restricted to that specific age of <20 yrs old participants, there might be an age-related component that is masking these observations. Varying expression in HLA-types has been reported [17, 36] with distinct HLA allele and haplotype distributions [38, 37]. Research by Mudd [9] showed that HLA-B27 elite control induce high frequencies of CD8(+) T cells acts against Viral infectivity factor (Vif) and Negative factor (Nef) epitopes in blood, lymph nodes and colon, and were associated with viral control. The HIV-1, MVA boost vaccine used in our study was not coding Vif and Nef as described in chapter three under vaccine immunogens. This could be the reason why we couldn't get a highly significant difference between placebo and vaccines.

Moreover research by Uyl [39], explains the protective role of HLA-27 in HIV patients in which HLA-B27-positive patients have a specific and strong CTL responses against p24 epitope a conservative HIV protein that does not easily mutate. The HIV-1, MVA boost vaccine p24 protein was not one of the immune-gens as we could not observe significant effect between the groups after administration of either a placebo and or vaccines. The same study Uyl [44] explains individuals' difference in HLA-B27 between groups thus the positive and the negative results.

Interestingly, it was found in our study that even in the placebo group (which consisted of only saline alone) the response was such that the levels of HLA was even higher than that in MVA boosted HIV-1 DNA vaccine (Figure 1, Panel IV and Panel VIII). This can be explained by the previous reported findings that placebo may have positive effect; sometimes significantly influencing subjective symptoms [24]. This is critical because of the suggested impact it may also have on patients' subjective experience who knows that the given treatment is without any drug.

Our findings provide clues on the use of MVA as an HIV-1 DNA vaccine. We have seen increase in levels of HLA-B7 and HLA-B27 that could be compared between characteristic groups and at different levels of application of this vaccine boost. The resultant comparison indicates MHC molecules to be best markers of HIV immunogenicity and thus more HIV vaccine trials should opt to use these molecules to improve the efficacy of HIV vaccine.However, more research still needs to be done to ensure and confirm its sole role as a vaccine boost under different settings with HIV pandemic.

CONCLUSION

The findings from this study suggest that further HLA B7 and HLA B27 levels increase after MVA boost a vector virus which contains gp160 (Subtype E, CM235), gag and pol (integrase-deleted and reverse transcriptase non-functional, Subtype A, CM240),MVA should be deployedas future HIV-1 DNA vaccine boost for increased efficacy. However, more research still needs to be done to ensure and confirm its sole role as a vaccine boost under different settings with HIV pandemic.

There is no data on which HLA subtypes are encoded by HIV in sub saharan Africa, we suggest that molecular techniques preferably sequencing of the MHC region should be done to get a clear picture of the MHC subtypes found in sub-saharan Africa setting. By so doing we can have a list of known HLA subtypes and onecan be comparethem among the placebo and after vaccines.

Also MHC classes are highly polymorphic, by doing only ELISA test to target a single HLA subtype could be difficult, for instance a researcher can target HLA B57, but HLA B 58:02 is the one that has been coded, as the ELISA test is highly specific and can target the only single HLA subtype, therefore we suggest that a multiplex cellular ELISA method should be employed so that multiple number of HLA subtypes can be detected.

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