THE ROLE OF HUMAN HERPESVIRUS AND GBV-C COINFECTIONS IN THE DEVELOPMENT OF NON-HODGKIN'S LYMPHOMA IN HIV-1 POSITIVE INDIVIDUALS IN THE MULTICENTER AIDS COHORT STUDY

by

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ABSTRACT

Introduction: Infection with the human immunodeficiency virus (HIV) can result in severe immune suppression, often allowing infections that would normally be controlled by the immune system to persist in HIV-positive individuals. Many of these infections can in turn affect the dynamics of HIV disease progression. Specifically, we hypothesize that herpesvirus infections are associated with more rapid progression of HIV-1 disease, and chronic immune activation that can lead to the development of HIV/AIDS-associated cancers. Alternatively, we postulate that GB Virus C coinfection has been shown to have a protective effect against HIV-1 disease progression, and may reduce chronic immune activation in HIV-1 positive individuals.

Methods: This study investigated the associations of cytomegalovirus, Epstein-Barr virus, human herpesvirus 6, human herpesvirus 8, and GB Virus C viral coinfections on the development of non-Hodgkin's lymphoma in MACS HIV-1 seroconverters pre-combination antiretroviral therapy (cART). 536 seroconverters pre-cART were included in the investigation, including 22 who developed non-Hodgkin's lymphoma.

Results: HIV-1 seroconverters who developed non-Hodgkin's lymphoma pre-cART experienced CMV and EBV viremia significantly more frequently than those who did not develop non-Hodgkin's lymphoma. HIV-1 seroconverters who did not develop non-Hodgkin's lymphoma pre-cART were more frequently viremic for GBV-C. Levels of EBV viremia were significantly higher

in HIV-1 seroconverters who developed non-Hodgkin's lymphoma, and levels of GBV-C viremia were significantly higher in the HIV-1 seroconverters who did not develop non-Hodgkin's lymphoma. Additionally, HIV-1 seroconverters who had experienced EBV viremia progressed from HIV-1 seroconversion to lymphoma diagnosis more rapidly than those from whom EBV viremia was never detected. Conversely, those who experienced GBV-C viremia, but never experienced EBV viremia progressed more slowly from HIV-1 seroconversion to non-Hodgkin's lymphoma.

Discussion: This investigation supports the hypothesis that EBV and CMV coinfections drive HIV-1 disease progression and the development of HIV-associated non-Hodgkin's lymphoma. The results also suggest that GBV-C plays a protective role against the development of non-Hodgkin's lymphoma in HIV-1 positive individuals. These findings are of public health significance, because knowledge gained regarding the risks associated with non-Hodgkin's lymphoma development can contribute to the prevention and early detection of non-Hodgkin's lymphoma.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would like to thank my committee members, Dr. Clareann Bunker, and Dr. Yue Chen, for all of their assistance and support throughout the completion of my thesis project. I also want to extend my appreciation for all of the hard work of Arlene Bullotta that made this project possible. Lastly, I want to express my deepest gratitude to my advisor and committee chair, Dr. Charles Rinaldo, for affording me the opportunity to work on this project, and for his guidance over the last two years.

ABBREVIATIONS

AIDS: Acquired Immunodeficiency Syndrome

cART: Combination Antiretroviral Therapy

CDC: Centers for Disease Control and Prevention

CMV: Cytomegalovirus

EBV: Epstein-Barr virus

GBV-C: GB Virus C

HIV: Human Immunodeficiency Virus

HIV-1: Human Immunodeficiency Virus Strain 1

HHV-6: Human herpesvirus 6

HHV-8: Human herpesvirus 8

KS: Kaposi's sarcoma

KSVH: Kaposi's sarcoma-associated herpesvirus (HHV-8)

MCD: Multicentric Castleman's disease

MSM: Men who have sex with men

NHL: Non-Hodgkin's Lymphoma

PEL: Primary effusion lymphomas

SES: Socioeconomic Status

1.0 INTRODUCTION

Viral coinfections have important implications for human immunodeficiency virus type 1 (HIV-1) disease progression. For example, human herpesvirus coinfections are associated with chronic immune activation and inflammation in individuals with HIV-1 (1). Chronic immune activation and inflammation not only contribute to HIV-1 disease progression, but also are liked to the development of negative health outcomes associated with HIV/AIDS such as cardiovascular disease and cancer. Previous Multicenter AIDS Cohort Study (MACS) investigations have documented associations between elevated levels of cytokines and chemokines as biomarkers of non-Hodgkin's B-cell lymphoma in MACS HIV-1 seropositives (2, 3). Similarly, herpesvirus coinfections have been associated with the development of various types of non-Hodgkin's lymphomas (NHL) in HIV-1 positive individuals; although, other studies have shown no significant association between herpesvirus infection and NHL (4, 5).

GB Virus C (GBV-C) coinfection results in slower HIV-1 disease progression, and better health outcomes in HIV-1 positive individuals (6). Recently, GBV-C has been associated with reduced monocyte and natural killer cell activation, and a trend toward reduced B-cell activation in HIV-1 positive individuals (7). Chronic B-cell activation is associated with the development of HIV-associated non-Hodgkin's lymphomas (8).

Thus, while herpesvirus coinfections drive HIV-1 disease progression and chronic immune activation in HIV-1 positive individuals, GBV-C has a protective effect on HIV-1 disease

progression, and is associated with reduced immune activation in HIV-1 positive individuals (1, 9). Therefore, it is possible that GBV-C also has a protective effect against the development of HIV-1-associated NHL in HIV-1 positive individuals.

Specific Objective: The specific aim of this investigation is to examine the role of activated human herpesvirus and GBV-C coinfections defined by the presence of nucleic acid viremia in the development of NHL in HIV-1 seroconverters in the MACS. It is hypothesized that in HIV-1 seroconverters, the risk of NHL development is enhanced by herpesvirus coinfections and reduced by GBV-C coinfection.

1.1 HUMAN IMMUNODEFICIENY VIRUS

HIV is a single stranded RNA *lentivirus* that is responsible for causing acquired immunodeficiency syndrome (AIDS) (10). AIDS is characterized by the deterioration of one's immune system leading to life-threatening opportunistic infections, or cancers.

1.1.1 Epidemiology and Transmission of HIV

Since the Centers for Disease Control and Prevention (CDC) first reported the occurrence of severe immunodeficiency in five California men in 1981, it is estimated that over 75 million people have become infected with HIV and 36 million have died due to their infection (11). According to the World Health Organization (WHO), an estimated 35.3 million people were living with HIV in 2012 (11). Although the prevalence of HIV varies greatly from country to country, the burden of HIV is greatest in sub-Saharan Africa where an estimated 70% of all infections occur (11). The

majority of HIV infections are due to the HIV-1 subtype of the virus, while the less common HIV-2 strain is not widely seen outside of Africa (12).

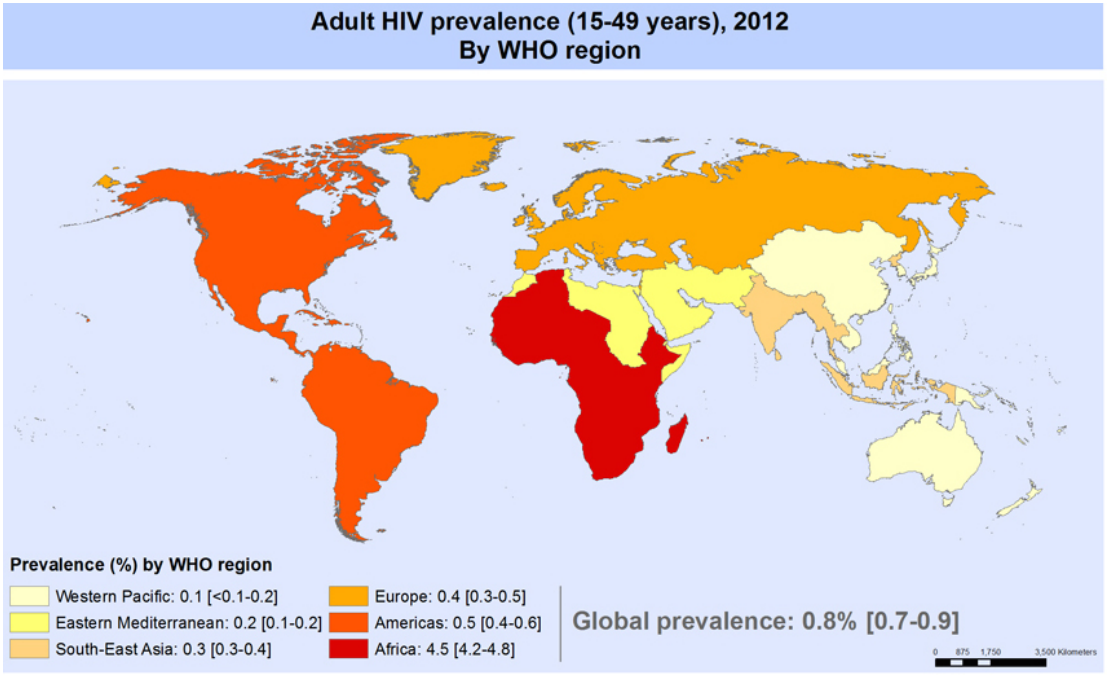

Figure 1. Global adult HIV prevalence

Source: The World Health Organization, http://www.who.int/gho/hiv/hiv_013.jpg?ua=1

In the United States, an estimated 1.1 million individuals are currently living with HIV, and an estimated 50,000 new infections occur each year (13). The epidemiology of HIV infection in the U.S. has changed greatly since the onset of the epidemic in the early 1980s. In the 1980s HIV predominately affected white men who have sex with men (MSM) living in U.S. cities. By 2006, an estimated 46% of all people living with HIV in the U.S. were African American although African Americans constitute only 12% of the U.S. population (14). Although men still account for three-quarters of all persons living with HIV in the U.S., the percentage of new HIV infections

in women has increased steadily since the mid-1980s (13). High-risk heterosexual contact is now the second most common transmission route in the U.S. following male-to-male sexual contact (14). Additionally, the age of people living with HIV has continued to increase in the United States due to the introduction of combination antiretroviral therapy (cART). 24% of all people currently living with HIV are now over the age of 50, compared to only 17% in 2001 (14).

HIV is transmitted through the sharing of bodily fluids including blood, semen, anal and vaginal fluids, and breast milk (15). Transmission routes of infection are unprotected sex, sharing of contaminated needles, blood transfusions or transplants, and mother-to-child transmission during pregnancy, birth, or breastfeeding (15). Certain high-risk sexual practices, and infection with other sexually transmitted infections (STIs) can place an individual at higher risk of contracting HIV infection (14).

1.1.2 HIV-1 Replication Cycle

HIV predominately infects CD4 T cells, but is also capable of infecting dendritic cells and macrophages (10). HIV-1 enters cells through interactions of gp120 envelope glycoproteins with CD4 molecules, and CCR5 or CXCR4 co-receptors on the cell surface (12). The glycoprotein gp41 on the viral envelope then facilitates fusion with the plasma membrane so that the viral capsid may enter the cell. Upon entry, the viral reverse transcriptase transcribes the viral RNA into a complementary DNA copy that is integrated into the host genome by the viral integrase (12). The HIV-1 RNA genome may then be transcribed in activated T cells, or the infection can remain latent, which particularly occurs in memory T cells or dormant macrophages (12). First, transcripts of viral RNA are translocated from the nucleus and extensively spliced for the production of Tat and Rev proteins (12). When levels of these proteins increase, viral transcripts are left unspliced

or singly spliced in order to produce all necessary structural components of the mature virion (10). These structural components assemble along with unspliced viral RNA in order to produce mature HIV-1 virus particles that bud from the cell (12).

1.1.3 HIV-1 Pathogenesis and AIDS

Acute HIV infection occurs at two-six weeks post infection, and is characterized by the onset of flu-like symptoms, and HIV viremia (16). During this time CD8 T cells are activated to kill HIV-1 infected cells, CD4 T cell count decreases due to large quantities of cell death within the gut-associated lymphoid tissue, and antibody production marks seroconversion (10). HIV infection then disseminates and establishes infection in lymphoid tissues throughout the body. Following the acute phase of HIV-1 infection, the level of virus that is maintained in blood plasma, known as the viral set point, has been shown to be indicative of future HIV disease progression (12). The asymptomatic latent phase of HIV infection can last between six months and twenty years, but on average lasts about ten years (12). Although infection is asymptomatic during this time, HIV-1 infection persists and numbers of CD4 T cells decline. The persistence of HIV-1 infection during this time has been associated with the upregulation of markers of CD4 and CD8 T cell activation (17). Moreover, chronic immune activation is associated with the persistence of HIV infection and helps drive CD4 T cell depletion (18, 19). Once CD4 T cells decline, typically from normal levels of 800-1200 to levels of $\leq$500 cells per milliliter of blood, the symptomatic phase begins and opportunistic infections or cancer begin to occur (12). Once CD4 T cells reach levels of 200 cells per milliliter an individual is typically clinically diagnosed with AIDS (12). Several negative health outcomes are associated with HIV progression and the onset of AIDS. Common opportunistic infections associated with AIDS include viral diseases due to herpesvirus infections

that will be described later in this study, and bacterial diseases such as tuberculosis or pneumonia. Additionally, HIV and AIDS-associated malignancies and neurodegeneration can develop.

1.1.3.1 HIV-Associated Non-Hodgkin's Lymphoma

Approximately 90% of all lymphomas, the malignancies of the lymph system, are classified as NHLs (20). NHLs can occur in lymph nodes, the digestive tract, central nervous system, or proximal to the tonsils, and they are classified either as indolent or aggressive (21). The majority of NHLs (85%) are of B cell origin, but a small percentage are of T cell origin (20). Unlike Hodgkin's lymphoma, which originates only in abnormal Reed-Sternberg cells and commonly occurs in young adults, NHL exhibits over 30 unique subtypes and typically occurs in those over the age of 65 (22). Risk factors for NHL include age, male gender, Caucasian race, certain infections (e.g. EBV), chronic B cell stimulation, and immune deficiency disorders (20, 23).

NHL is the second most common malignancy seen in HIV-1-positive individuals, and is considered an AIDS-defining illness (21). Research suggests that between 5-20% of all HIV-positive individuals will develop NHL compared to less than 1% of the general population (24, 25). NHL in HIV-1-positive individuals is typically intermediate or high-grade, meaning that it will progress rapidly if left untreated (20). HIV-1-related lymphomas are categorized into three main groups; systemic NHLs, primacy central nervous system lymphomas, and primary effusion lymphomas (26).

Systemic NHLs are the most common type of HIV-associated NHL and they include diffuse large B cell lymphomas and Burkitt lymphomas (26). While diffuse large B cell lymphomas typically occur late in HIV disease progression when CD4 T cell counts are low (<50

per ml), Burkitt lymphoma may develop among sustained levels of CD4 T cells (26). Chronic active B cell receptor signaling has been implicated as a pathogenic mechanism of development in diffuse, large B cell lymphomas, and Burkitt lymphoma has been shown to be associated with both EBV and HIV-1 infection (27, 28). The introduction of cART has reduced the incidence of NHL in those with HIV-1, and has helped improve the prognosis in those who develop NHL. However, unlike other subtypes of HIV-1-associated NHL, Burkitt's lymphoma prognosis does not improve following the use of cART, for which the mechanism is not yet understood (29).

Primary central nervous system lymphomas are B cell lymphomas that most frequently occur in the brain, but may also develop on the spinal cord (30). EBV is identified in nearly all primary central nervous system B cell tumors (20, 30). Previous to the introduction of cART the prognosis for HIV-positive individuals with primary central nervous system lymphomas was poor. This particular subtype is usually treated with radiation and chemotherapy, and the use of cART has increased mean survival time to 55 months (31).

Primary effusion lymphoma (PEL) is a rare HIV-1 or AIDS-associated NHL that was previously known as body-cavity lymphoma. PEL is of B cell origin, and accounts for an estimated 4% of all HIV-related NHLs, and they typically occur in the pleural space or abdomen (24). Human herpesvirus 8 (HHV-8; aka Kaposi's sarcoma associated herpesvirus) is associated with the development of PEL, and most individuals who develop PEL already have either Kaposi's sarcoma or multicentric Castleman's Disease (24). The mechanism of oncogenesis promoted by HHV-8 in the development of PEL is not yet understood. In addition to HHV-8 infection, associations between EBV and PEL development have been suggested (24). cART initiation and combination chemotherapy are the recommended treatment for PELs; however, the prognosis remains poor with a mean survival time of five-seven months (24).

1.2 HUMAN HERPESVIRUSES

Herpesviruses, of the *Herpesviridae* family, are widespread in nature with over 130 viruses utilizing a variety of host species as reservoirs. Highly prevalent in both the U.S. and global populations, there are eight types of herpesviruses that infect humans: herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7), and (HHV-8) (10). Estimates suggest that 60-95% of the global population has been infected with at least one HSV, and in the U.S. 89% of children test positive for the EBV antibody by age 18 (32, 33).

Herpesviruses are composed of a linear, double-stranded DNA genome encased in an icosahedral capsid (10). During symptomatic infection, viral DNA synthesis and capsid assembly occur in the nucleus of the host cell, which usually results in the destruction of the host cell (10). Following primary infection, herpesviruses are capable of remaining latent in host cells indefinitely while expressing only a small number of viral genes. Reactivation, and subsequent symptomatic infection can occur due to a variety of factors, particularly, immune suppression. Because of the high prevalence of herpesviruses, and the shared transmission routes of many herpesviruses and HIV-1, human herpesviruses are common coinfections with HIV-1 (34). Immune suppression and CD4 T cell depletion due to HIV-1 infection allows for the reactivation of persistent herpesvirus infections that contribute to the chronic immune activation and inflammation that drive the progression of HIV-1 disease and result in other negative health outcomes such as cardiovascular disease, cancer, and neurodegeneration (34).

1.2.1 Cytomegalovirus

CMV is a betaherpesvirus that is capable of infecting a wide variety of tissues and cell types within a host, and causing systemic infection (10). Viral entry occurs following the adherence and fusion of the virion envelope protein and cellular membrane, and then capsid proteins deliver the viral genome to the nucleus for replication (35). CMV replication typically occurs in epithelial cells, endothelial cells, fibroblasts, or smooth muscle cells (36). The prevalence of CMV in the United States is estimated to be over 58% in people over the age of six (37). The infection is more prevalent in minority groups, particularly Hispanic Americans (81.7% prevalence) versus the prevalence in non-Hispanic white Americans (51.2%) (37). CMV is transmitted sexually, perinatally, by close contact, or through contaminated blood transfusions or organ transplants. The virus is shed in bodily fluids including urine, saliva, tears, breast milk, vaginal secretions, and semen (38). CMV is typically asymptomatic in immune-competent hosts, but may cause mononucleosis-like symptoms such as fatigue, fever, and enlarged lymph nodes in healthy individuals (38). In immune-competent individuals, CMV infection is usually self-limiting and does not require treatment. Like all herpesviruses, CMV infection persists in a host and can reactivate later in life causing illness if one becomes immune suppressed. This infection can be very serious in immune-compromised individuals such as transplant recipients, infants, and HIV-1-positive individuals.

1.2.1.1 CMV Infection in HIV-1 Positive Individuals

CMV is one of the leading opportunistic infections affecting those living with HIV/AIDS. CMV disease commonly manifests as retinitis, colitis, esophagitis, or meningoencephalitis (16).

T-cell immunity is a key host defense to CMV infections, and for this reason disease normally occurs in severely immune compromised individuals with CD4 T cell counts below 100 per ml (16).

CMV is considered a cofactor in HIV-1 disease progression. Upon cell entry of the virus, interferon-stimulated genes are upregulated, with increased levels of interferon-β and other inflammatory cytokines (35). The immune activation resulting from persistent CMV infection can result in further T cell depletion, and diseases associated with chronic inflammation. Specifically, increased CMV-specific T cells have been associated with atherosclerosis, and CMV-infected tumor cells are associated with increased malignancy and chemoresistance (35, 39). In immune-compromised individuals, CMV disease is usually treated with ganiciclovir or valganciclovir antiviral medications depending on clinical presentation of the infection (16).

1.2.2 Epstein-Barr Virus

EBV is a gammaherpesvirus that primarily infects B-cells and epithelial cells, but may infect T cells, NK-cells, and smooth muscle cell (35). Separate methods of entry are required for different cell types. In order to enter B cells, EBV proteins interact with the CD21 protein, and in order to enter epithelial cells EBV proteins interact with β integrins prior to viral fusion with the cell membrane (40). EBV is transmitted through saliva, blood transfusions, and organ transplants (16). As previously mentioned, EBV is highly prevalent in the U.S. with 89% of children infected by age 18 (32). Infection rates are higher in minority groups. (41).

Primary infection typically occurs in children and is asymptomatic (90%). However, if primary infection occurs at an older age, infectious mononucleosis becomes more likely. Mononucleosis is characterized by elevated lymphocyte counts in the blood consisting of “atypical mononuclear” cells in the blood, usually CD8 T cells (10). Symptoms of infectious mononucleosis

set in approximately five-seven weeks post-infection and include fever, pharyngitis, lymphadenopathy, headache, abdominal pain, and nausea (16, 40). Symptoms typically last for three weeks, are self-limiting, and are treated with supportive care. In addition to a few serious complications that may occur due to infection, EBV has been implicated in numerous lymphoproliferative disorders that are most often seen in immune-compromised individuals (42, 43).

1.2.2.1 EBV Infection in HIV-1 Positive Individuals

HIV-1 positive individuals are at an increased risk of developing EBV-associated malignancies (43). Reactivation of EBV infection is not necessary for the development of these proliferative disorders; instead, the viral genes expressed during latent EBV infection of lymphocytes are sufficient for the development of various lymphomas (43). Burkitt lymphoma can develop in individuals regardless of HIV-1 status. However, EBV DNA is detected in 30-40% of HIV-associated Burkitt lymphoma cases (43). Additionally, EBV is implicated in over 95% of primary central nervous system lymphomas in HIV-1-positive individuals (42). Similar to HHV-8, EBV DNA is also associated with PEL (35). EBV is most commonly associated with B-cell lymphomas, but can also play a role in the development of other proliferative disorders such as T cell lymphomas and nasopharyngeal carcinoma (40). Treatment of EBV-associated malignancies in individuals with HIV/AIDS depends on the classification, and advancement of the disease. The utilization of cART resulted in a dramatic decrease in the prevalence of AIDS-associated malignancies in the 1990's (40). However, as HIV-1 positive individuals continue to live longer, it is likely that the burden of EBV and HIV-associated lymphomas will rise.

1.2.3 Human herpesvirus 6

HHV-6 is the collective name of the betaherpesviruses HHV-6A and HHV-6B. HHV-6 utilizes the CD46 receptor, and enters cells through fusion of the viral envelope protein and the cellular membrane (44). The wide array of cells that HHV-6 is capable of infecting includes B cells, T cells, DCs, NK Cells, and γ/δ T Lymphocytes (44). The virus often replicates in the salivary glands following infection, and is shed through saliva. This is believed to be the most common route of HHV-6 transmission, however, evidence suggests that it may also be transmitted vertically and sexually (45). Like most herpesviruses, HHV-6 is highly prevalent both globally, and in the U.S. with an estimated 95% of all people over the age of two being seropositive for HHV-6. Primary HHV-6 infection commonly results in roseola infantum, a combination of high fever and rash that is not typically severe in the immune-competent children in which this disease most frequently occurs (45).

1.2.3.1 HHV-6 Infection in HIV Positive Individuals

Reactivation of HHV-6 infection can be dangerous in people living with HIV/AIDS, and can result in HHV-6 viremia, lymphadenitis, pneumonitis, retinitis, meningoencephalitis, and disseminated infection of organs (45). The frequency of HHV-6 reactivation in HIV-1 positive individuals has been reduced since the introduction of cART. HHV-6 infection contributes to HIV-1 disease progression through the upregulation of cytokines, transactivation, and interference with dendritic cells' ability to capture antigen (45, 46). Although HHV-6 accelerates HIV-1 disease progression, HHV-6 detection in peripheral blood mononuclear cells decreases during AIDS likely due to reduced levels of CD4 and CD8 T cells. The possible association between HHV-6 infection and AIDS-associated malignancies is not currently well understood. Recently, HHV-6 DNA was

detected in 86% nodular sclerosis Hodgkin's Lymphoma (NSHL) cases, a finding that has been demonstrated in previous studies (47, 48). While investigations have found increased HHV-6 viral load in individuals with various types of lymphoma and leukemia, the mechanism or reason for increased HHV-6 load has not been defined, and may be due to increased dysregulation and dysfunction of the immune system due to HIV-1 disease progression and AIDS.

1.2.4 Human herpesvirus 8

HHV-8 is a gammaherpesvirus that has been associated with the development of several proliferative disorders most notably, Kaposi's sarcoma (KS) (10). HHV-8 primarily infects B cells, but may also infect endothelial cells, prostate epithelial cells, nerve cells, and macrophages (35). Spindle cells are the primary target of HHV-8 in KS tumors (49). HHV-8 may be transmitted through infected sexually, vertically, through organ transplantation, and contaminated saliva (16). Prevalence of HHV-8 infection varies greatly by geography and among groups at various risks for contracting the virus. The overall prevalence of HHV-8 in the U.S. is estimated between 1-6%, but may be as high as 9.4% in heterosexual males, and 22% in MSM (35, 50). The prevalence is also much higher in some Mediterranean locations (Sicily 23.1%), and in Africa where the prevalence is estimated to be greater than 50% (10, 51). In those experiencing primary infection, HHV-8 rarely causes symptoms, but may result in a rash and fever. However, HHV-8 is associated with proliferative disorders including KS, PEL, and multicentric Castleman's disease (35).

1.2.4.1 HHV-8 Infection in HIV-1 Positive Individuals

HIV-1 positive MSMs are a high-risk group for HHV-8 infection, for example 60% of MACS seroconverters have been viremic for HHV-8 at least once. Like all herpesviruses, latent

HHV-8 infection may be reactivated and cause disease in immune-compromised individuals. The reactivation and lytic replication of HHV-8 is critical to the development of HHV-8 -associated proliferative disorders (10). KS is a vascular tumor of proliferating spindle cells that typically affects the face, mouth, upper body, or even the respiratory and gastrointestinal tract (52) . There are four types of KS: classic KS that occurs typically in Mediterranean men, African endemic KS, immunosuppression-associated KS, and AIDS-associated KS (10). Inflammatory cytokines and vasoproliferative cellular cytokines induce lytic replication of HHV-8, and vasoproliferative cytokines and the HIV Tat protein contribute to tumor maintenance and KS pathogenesis (35). Antivirals aimed at reducing herpesvirus replication such as ganiciclovir have been shown to reduce the occurrence of KS, and cART can result in remission of KS in individuals with HIV/AIDS (52).

PEL is a rare, non-Hodgkin's B cell lymphoma that typically occurs in visceral cavities (24). PEL is treated with a combination of chemotherapy and cART, which can result in regression of the disease (24). Unfortunately, the prognosis remains poor for PEL with a mean survival from diagnosis of 5-7 months (10). MCD is a systemic lymphoproliferative disorder that is characterized by expanded germinal centers, and hyperproliferation of B cells (10). Dysregulation and hypersecretion of IL-6, which promotes B cell growth and inhibits B cell apoptosis, is a common cause of MCD (35). In HHV-8-associated MCDs (approximately 50% of all MCDs) HHV-8 viral-encoded cytokine, vIL-6, is hypersecreted (53).

1.3 GB VIRUS C

GVB-C of the *Flaviviridae* family is a positive-polarity single stranded RNA virus (9). GBV-C, also previously known as the hepatitis G virus (HGV), is phylogenetically related to hepatitis C virus (HCV); however, unlike hepatitis viruses, GBV-C is lymphotrophic and non-pathogenic (9). A relatively common infection, estimates suggest that 1-2% of healthy blood donors in the United States are viremic for GBV-C, but the estimated prevalence is much higher in MSMs (54). A previous MACS investigation demonstrated that 85% of all MACS subjects have been infected with GBV-C (7). GBV-C transmission occurs via parenteral, sexual, and vertical routes, and because of shared transmission modes with HIV-1, HIV-1-positive individuals are often coinfected with GBV-C. Although immune-competent hosts typically clear GBV-C viremia, infection can persist for years in immune-compromised individuals. Over the last 15 years, several studies have demonstrated an association between GBV-C infection, and increased survival, and better health outcomes including increased CD4+ count in HIV-positive individuals (6, 55, 56).

1.3.1 GBV-C in HIV-1 Positive Individuals

Questions remain regarding the mechanism of the protective effect that GBV-C seems to have against HIV-1 infection, and the potential interactions between GBV-C and HIV-1. Recent studies have suggested that the GBV-C envelope E2 protein inhibits TCR activation-induced IL-2 release, and is associated with high levels of double-negative T cells that limit immune activation (57, 58). Additionally, GBV-C has been associated with reduced NK cell and monocyte activation, and may be associated with reduced B cell activation (7). This reduction in immune activation can have an

impact on the clinical complications associated with the chronic immune activation and inflammation that accompany HIV-1 disease progression.

2.0 METHODS

The MACS is a prospective cohort study designed specifically to examine HIV-1 infection in MSMs. The MACS is the largest study of its kind, and has recruited nearly 7,000 participants since its creation in 1984. Men have been recruited at four sites: Johns Hopkins University in Baltimore, Northwestern University in Chicago, the University of California Los Angeles, and the University of Pittsburgh. Thus far, the MACS has resulted in over 1,300 publications, and has contributed greatly to current knowledge of the epidemiology and the natural history of HIV and AIDS.

This study specifically examines the role of various viral coinfections in the development of NHL in MACS seroconverters pre-cART. The University of Pittsburgh MACS site conducted testing for CMV, EBV, HHV-6, and HHV-8 DNA and GBV-C RNA in the blood plasma of 536 MACS seroconverters pre-cART collected from over 5,000 person-visits. Within this group of 536 seroconverters, 26 cases of pre-cART NHL within the group were identified to be included within this analysis.

2.1 LABORATORY METHODS

Nucleic acid was extracted from human plasma specimens utilizing a NucliSENS easyMAG automated extractor (bioMerieux Inc., Durham, NC). Known amounts of phocine herpesvirus-1 (PhHV) an animal herpesvirus and equine arteritis virus (EAV) a single stranded RNA virus were added to all samples prior to nucleic acid extraction in order to confirm extraction efficiency, and to detect the presence of PCR inhibitors. Five microliter aliquots of the extracts were tested for the

presence of CMV, EBV, HHV-6, and HHV-8 viral DNA by real-time PCR conducted in Dr. Charles Rinaldo's University of Pittsburgh Clinical Virology Research Laboratory by Arlene Bullotta. The presence of GBV-C RNA was detected utilizing real-time reverse transcriptase PCR assay at the University of Pittsburgh Graduate School of Public Health under the direction of Dr. Yue Chen. Negative controls consisting of nuclease-free water, and positive controls consisting of known amounts of viral nucleic acid were included in each real-time PCR assay. The limits of detection and quantitation of viral DNA and RNA were extrapolated using known quantities of viral DNA and RNA from Advanced Biotechnologies (ABI, Columbia, MD). The upper and lower limits of detection for each of the viral assays are described below in the number of quantities per reaction in Table 1. Contamination of the negative control, or failure to detect internal control (PhHV or EAV) within a specified Ct value range resulted in the re-extraction of samples and repetition of the PCR assay. Primers and probes utilized in the real-time PCR assays are listed below in Table 2.

Table 1. Upper and lower limits of detection in number of quantities per reaction

	CMV	**EBV**	**HHV-6**	**HHV-8**	**GBV-C**
Upper LOD (qtx/rx)	500,000	650,000	500,000	400,000	190,000,000
Lower LOD (qtx/rx)	1	2.5	2	1.5	1,900

Table 2. Primers and probes utilized for real-time PCR assay

Target	Forward Primer	Reverse Primer	Probe	Reference
CMV	CGATCAAGAACGCGATAACG	ACCGTCGATGGCAGGTCAT	6FAM-CGATCACAAACAGCG-MGBNFQ	Snghavi, et al., 2008
EBV	AAACCTCAGGACCTACGCTGC	AGACACCGTCCTCACCAC	6FAM-TAGAGGTTTTGCTAGGGAGGAGACGTGTG-TAMRA	Jebbink et al., 2003
GBV-C	CCAACCCTGTCATCACCCATCCACC	GRTGACCGGGATTTACGACCT	Cy5-GCGACCGGCCAAAAGG-BHQ	Li et al. 2006
HHV6	CGCTAGGTTGAGGATGATCG	CAAAGCCAAATTATCAGACG	6FAM-CACCAGACGTCACACCCGAAGGAAT-TAMRA	Locatelli et al., 2000
HHV8	GTCTCTTGGACAAGCTCGCTG	AGTGAGCATGGCAGATGTTCGT	6FAM-CGGTCTGTGAAACGGTCATTGACCTTAC	Jenkens, personal communication
PHHV	GGGCGAATCACAGCTTGAAT	GCGGTTCCAAACGTACCAA	VIC-TTTTATGTGTCCGCCACCAT-MGB-NFQ	Neisters et al., 2002
EAV	GGCGACAGCCTACAAGCTACA	CGGCATCTGCAGTGAGTGA	6FAM-TTGCGGACCCGCATCTGACCAA-MGBNFQ	

2.2 STATISTICAL ANALYSIS

A total of 26 HIV-1 MACS seroconverters with NHL pre-cART were identified by the Center for Analysis and Management of MACS Data (CAMACS) to have specimens included in viral testing conducted at the University of Pittsburgh by Dr. Rinaldo's and Dr. Chen's laboratories. Four of the seroconverters had only completed one visit, and DNA testing had not been fully completed for these individuals so they were excluded from the sample. Thus, 22 HIV-1 MACS seroconverters with NHL were included in the sample. Additionally, 514 MACS seroconverters who did not develop NHL pre-cART were included in the analysis. Data were described and analyzed using STATA/SE 13.0 software (StataCorp College Station, TX).

Fisher's exact tests were used to measure non-random association of categorical variables between the seroconverters who developed NHL and those who did not. The categorical variables included race, tobacco use, presence of viremia at least once, and the proportion of person-visits from which viremia was detected. The proportion of person-visits from which CMV, EBV, HHV-6, HHV-8, or GBV-C viremia was detected was determined by the proportion of visits from which viremia for each coinfection was detected among HIV-1 seroconverters who developed NHL and those who did not develop NHL. Two-sample t-tests of unequal variance were used to compare means of continuous variable (age at HIV-1 seroconversion, and HIV RNA set point) between the seroconverters who developed NHL and those who did not develop NHL.

A Mann-Whitney U test was used to compare the quantities of CMV, EBV, HHV-8, and GBV-C viremia between the HIV-1 seroconverters who developed NHL and those who did not. HHV-6 was not included in this portion of the analysis, because none of the HIV-1 experienced HHV-6 viremia detected from the blood plasma. Finally, the 22 seroconverters who developed NHL pre-cART were divided into groups based on which viral DNA or RNA had been detected in their blood plasma at least once over the course of the study. Two-sample t-tests of unequal variance were used to compare the mean length of time from HIV-1 seroconversion to NHL diagnosis between HIV-1 seroconverters with various coinfection profiles.

3.0 RESULTS

In total, the study sample included 536 HIV-1 seroconverters pre-cART, including the 22-participant subsample that developed NHL pre-cART. Characteristics of the 22 seroconverters who developed NHL pre-cART, and the 514 seroconverters who did not develop NHL can be found in Table 3. The mean age at seroconversion was 35 for all participants, and the average years of seroconversion for seroconverters who developed NHL and those who did not develop NHL were 1988 and 1989, respectively. In both groups, the majority of participants were white non-Hispanic individuals; however, there were significantly more black and Hispanic men in the group of seroconverters who did not develop NHL ($P = 0.036$). The proportions of current and former smokers (36% and 49%) were significantly higher in the group of seroconverters who developed NHL compared to that measure within the group that did not develop NHL (30% and 40%). Table 3 also describes the proportion of individuals from each group whose plasma had detectable quantities of CMV, EBV, HHV-6, and HHV-8 DNA or GBV-C RNA at least once. The proportions of seroconverters who had detectable CMV, and EBV viremia at least once were slightly higher in those who developed NHL (CMV 59.09% vs. 50.23%, EBV 81.82% vs. 67.91%), and the proportion who had detectable HHV-8 and GBV-C viremia at least once was higher in those who did not develop NHL (HHV-8 33.18% vs. 18.18%, GBV-C 47.45% vs. 36.36%). HHV-6 DNA was not detected in the blood plasma of the seroconverters who developed NHL, and very few individuals from either group never experienced detectable viremia for any of the herpesvirus or GBV-C coinfections (4.76% and 2.09%). None of these differences between the two groups were statistically significant. In order to better compare sustained viremia between the two groups, the percentage of person-visits from which viral DNA and RNA were detected was

compared between the two groups for each virus. Additionally, the percentage of person-visits from which no herpesvirus DNA or GBV-C RNA were detected was compared between the seroconverters who developed NHL, and those who did not develop NHL. The group of seroconverters who developed NHL had a significantly higher of percentage of person-visits from which CMV, and EBV DNA were detected ($P < 0.001$ for each). Contrarily, the group who did not develop NHL had a significantly higher percentage of person-visits from which either no viremia was detected, or from which GBV-C RNA was detected ($P = 0.000$ and 0.018 respectively). Lastly, the HIV-1 RNA set point was found to be higher in the individuals who developed NHL, but this difference was not significant until the measurement was converted to $\log_{10}$ form.

Table 3. Characteristics of the study sample

		HIV-1 Seroconverters Who Developed NHL	HIV-1 Seroconverters Who Did Not Develop NHL	P-value *Fisher's exact or t-test
Total Number of Individuals		22	514	536
Avg. Year of Seroconversion		1988	1989	NA
Avg. Year of NHL Diagnosis		1996	NA	NA
Mean Age at Seroconversion		35.56	35.6	0.982
Race	**Black**	5% (1)	7% (37)	0.036
	White	95% (21)	92% (472)	
	Other	0	1% (5)	
Smoke Tobacco	**Never Smoked**	15%	30%	0.000
	Former Smoker	49%	40%	
	Current Smoker	36%	30%	
CMV viremia detected at least once		59.09%	50.23%	0.514
EBV viremia detected at least once		81.82%	67.91%	0.239
HHV-6 viremia detected at least once		0.00%	3.94%	1.000
HHV-8 viremia detected at least once		18.18%	33.18%	0.167
GBV-C viremia detected at least once		36.36%	47.55%	0.384
Viremia never detected		4.76%	2.09%	0.395
Person-visits where CMV viremia detected		25.84%	12.24%	0.000
Person-visits where EBV viremia detected		43.89%	26.24%	0.000
Person-visits where HHV-6 viremia detected		0	0.58%	1.000
Person-visits where HHV-8 viremia detected		7.87%	9.04%	0.688
Person-visits where GBVC viremia detected		28.89%	37.72%	0.018
Person-visits where no viremia detected		20.25%	37.95%	0.000
HIV RNA Set Point		90,594 copies per ml	58,481 copies per ml	0.410
HIV RNA Set Point log 10		4.66	4.24	0.026

Next, within those whose plasma contained detectable levels of herpesvirus DNA or GBV-C RNA the quantities of viral DNA and RNA were compared between the two groups using a two-sample Mann-Whitney U test. Differences in the quantities CMV and HHV-8 DNA were not found to be significant by the Mann-Whitney test. The median quantity of EBV DNA was significantly

higher in the HIV-1 seroconverters who developed NHL (207.5 vs. 121 copies per ml; $P < 0.0001$). Contrarily, the quantity of GBV-C RNA per ml of plasma was found to be significantly higher in those who did not develop NHL ($P = 0.0002$) using the Mann-Whitney U test. These results are described in Table 4.

Table 4. Comparison of viral quantities between HIV-1 seroconverters who did and did not develop NHL

		Percentiles			
Virus & Group	**Mean Viral Copies per ml**	**25th**	**50th (median)**	**75th**	**P-Value** *Mann-Whitney U
CMV					0.866
NHL	896.52	104	360	882	
No NHL	668.21	74.5	374	909.5	
EBV					0.0000
NHL	931.32	114	207.5	499	
No NHL	82.47	60	121	242	
HHV-8					0.111
NHL	554.35	51	338	1227	
No NHL	1233.51	149	1188	3273	
GBV-C					0.0002
NHL	2.33E+08	8.90E+06	7.89E+07	4.51E+08	
No NHL	1.10E+09	6.23E+07	4.04E+08	1.05+09	

The various combinations of viral coinfections within the group of seroconverters who developed NHL pre-cART were determined based on the presence of viral DNA or RNA having been detected in the participant's blood plasma at least once. The most frequently observed coinfection profile was concurrent CMV and EBV (six individuals), and the least common coinfection profiles were GBV-C coinfection alone, and concurrent CMV and GBV-C coinfections (one individual each). Only one seroconverter who developed NHL pre-cART had no detectable herpesvirus DNA or GBV-C RNA in blood plasma obtained from at least one person-visit. The average time from HIV-1 seroconversion to NHL diagnosis was calculated for each

participant who developed NHL. The overall average number of years from HIV-1 seroconversion to the development of NHL was 7.47 years.

The average number of years from HIV-1 seroconversion to NHL diagnosis was then compared based on detection of CMV, EBV, HHV-8, and GBV-C at least once in order to determine any potential effects of viremia on NHL development. The time to NHL diagnosis was also compared based on the various combinations of coinfections observed within the study population. Unfortunately, due to the small sample size and insufficient frequency of select coinfection profiles, select profiles could not be included in the comparison. Lastly, because it was hypothesized that the presence of herpesvirus DNA and the presence of GBV-C RNA have the opposite effect HIV-1 disease progression and the development of NHL, the average time to NHL diagnosis was examined for each of the herpesviruses without GBV-C and vice versa. All data regarding the differences in mean time to NHL diagnosis can be found in Table 5.

Table 5. Average number of years from HIV-1 seroconversion to NHL diagnosis by coinfection

	Average Years from Seroconversion to NHL Diagnosis (n)		
Any Presence Of:	**Viremia Detected**	**Viremia Not Detected**	**p-value** *Fisher's exact
CMV	7.38 (13)	7.63 (9)	0.782
EBV	7.05 (18)	10 (4)	0.007
HHV-8	6 (4)	7.82 (18)	0.077
GBVC	8.13 (8)	7.08 (14)	0.218
Viruses with Competing Effects:	**Combination Present**	**Combination Absent**	
CMV + GBV-C -	7.53 (8)	7.53 (14)	0.824
EBV + GBV-C -	6.92 (12)	8.22 (10)	0.113
HHV-8 + GBV-C -	6.33 (4)	7.67 (18)	0.267
GBV-C + CMV -	7 (3)	7.53 (19)	0.694
GBV-C + EBV -	10.5 (3)	7.16 (19)	0.0219
GBV-C + HHV-8 -	8.29 (8)	7.07 (14)	0.171
Unique Combinations:	**Combination Present**	**Combination Absent**	
EBV + GBV-C +	7.5 (2)	7.47 (20)	0.98
CMV + EBV +	6.82 (6)	8.2 (16)	0.089
EBV + HHV-8 +	6 (2)	7.82 (20)	0.038
CMV + EBV+ GBV-C +	7.5 (4)	7.47 (18)	0.967
CMV + EBV + HHV-8 +	5.5 (2)	7.68 (20)	0.048

4.0 DISCUSSION

There were several differences observed between the MACS HIV-1 seroconverters who developed NHL pre-cART and the MACS HIV-1 seroconverters who did not develop NHL. First, there was a significantly higher percentage of white males in the group of seroconverters who developed NHL compared to those who did not. This observation is in agreement with body of evidence suggesting that in the United States white individuals are at an increased risk of NHL. Additionally, smoking (former or current) was significantly associated with the development of NHL in MACS HIV-1 seroconverters. This observation is interesting, because whether smoking is a risk factor associated with the development of NHL has been frequently debated in literature. HIV RNA viral set point was also significantly higher in those who developed NHL. This association is in agreement with existing evidence that demonstrates the effects that HIV-1 RNA set point may have on HIV-1 disease progression over time.

Although the percentage of seroconverters with detectable herpesvirus or GBV-C viremia at least once differed slightly between those who developed NHL and those who did not, none of these differences were significant. However, the HIV-1 seroconverters who developed NHL exhibited CMV, and EBV viremia significantly more frequently than those who did not develop NHL. Contrarily, those who did not develop NHL experienced GBV-C viremia significantly more frequently than the seroconverters who did develop NHL. Furthermore, the seroconverters who did not develop NHL were significantly more likely to have no detectable herpesvirus or GBV-C viremia during annual person-visits. These observations are in agreement with the hypothesis that CMV, and EBV coinfections may contribute to NHL development in HIV-1 positive individuals,

and that GBV-C coinfection has a protective effect against the development of NHL in HIV-1 positive individuals.

Only 3.94% of the study sample ever experienced HHV-6 viremia that was detectable by DNA in the blood plasma, and none of these HHV6-positive individuals developed NHL. The association of HHV-6 with the development of various HIV/AIDS-associated cancers including NHL remains unclear in literature. This investigation did not find any association between HHV-6 DNA and NHL development. Additionally, the observations that the prevalence of HHV-8 viremia, and the frequency of HHV-8 viremia did not differ significantly between the two groups are interesting. Research has suggested that HHV-8 infection is associated with the development of certain types of NHL in HIV-1 positive individuals, and HHV-8 viral DNA has been detected NHL tumors by numerous investigators. However, there was not a significant association between HHV8 viremia and the development of NHL observed in this study. Instead, there were slightly fewer seroconverters who had ever experienced HHV-8 viremia detectable from blood plasma among those who developed NHL. This finding is in agreement with that of another MACS investigation that found no associated between serum levels of HHV-8 DNA and NHL development in HIV-1 positive individuals. In order to better understand the role of HHV-8 infection in NHL development it may be helpful, and necessary, to expand this investigation to other body compartments that may be infected by HHV-8. It was found that seroconverters with detectable EBV and HHV-8 coinfection, and seroconverters with detectable CMV, EBV, and HHV-8 coinfection progressed to NHL significantly more quickly than the seroconverters with other detected coinfection profiles.

When comparing the average number of viral copies of each of the herpesviruses and GBV-C detected in the blood plasma of the seroconverters who developed NHL and those who did not,

only the quantities of EBV and GBV-C differed significantly between the two groups (p-value: 0.00). Higher quantities of EBV DNA were detected in those who developed NHL and higher quantities of GBV-C RNA were detected in those who did not develop NHL. Again, this finding is in agreement with the hypothesis that EBV contributes to the development of NHL in HIV-1 seroconverters, and that GBV-C helps reduce immune activation associated with HIV-1 infection, and has a protective effect against the development of negative health outcomes associated with HIV-1 disease progression. Higher mean quantities of CMV DNA were detected in the seroconverters who developed NHL; however these differences were not significant. Because none of the seroconverters in the NHL group had detectable HHV-6 viremia, the average quantities of HHV-6 DNA could not be compared between the two groups.

The seroconverters who developed NHL pre-cART were categorized based on the various coinfections that had been detected in each of their blood plasma. Next the average number of years from HIV-1 seroconversion to NHL diagnoses was determined for each coinfection profile. Seroconverters who progressed to NHL most slowly were those with no detectable coinfections (9 years), only detectable GBV-C coinfection (10 years), or CMV and GBV-C coinfection (11 years). Since these scenarios each only occurred in one individual, the average time to NHL diagnosis could not be determined, and therefore, could not be compared to the other groups utilizing a mean comparison test. The average time to NHL diagnosis was instead only compared between coinfection profiles with a sufficient number of seroconverters to determine an average. Two coinfection combinations were associated with significantly less time between HIV-1 seroconversion and NHL diagnosis: EBV and HHV-8 coinfection (6 years average, $P = 0.038$), and CMV, EBV and HHV-8 coinfection (5.5 years average, $P = 0.048$). This finding suggests

these combinations of herpesvirus coinfections are associated with more rapid development of NHL in HIV-1 positive individuals.

Additionally, the mean time from seroconversion to NHL diagnosis was compared between individuals who had ever experienced detectable viremia, and those who had never experienced detectable viremia for each of the viral coinfections. Seroconverters who had ever experienced detectable CMV, or HHV-8 viremia developed NHL more rapidly than those who had never experienced detectable viremia for those viruses; however this difference was not significant. Similarly, those who had been viremic for GBV-C at least once had a greater average length of time between seroconversion and NHL diagnosis, but the difference was not significant. However, GBV-C positive individuals who did not have detectable EBV viremia had the greatest average time length from HIV-1 seroconversion to NHL diagnosis (10.5 years $P = 0.022$), suggesting that GBV-C coinfection is associated with slower development of NHL in HIV-1 positive individuals. Contrarily, seroconverters who had ever experienced detectable EBV viremia were diagnosed with NHL an average of three years sooner than those who never had detectable EBV viremia (7.05 years vs. 10 years, $P = .007$), suggesting that the presence of EBV DNA in blood plasma is associated with more rapid development of NHL in HIV-1 positive individuals. Both of these findings support the hypothesis that the chronic immune activation and inflammation associated with herpesvirus infection may contribute to the development of NHL in HIV-1 positive individuals, and that GBV-C coinfection may have a protective effect against the development of NHL in HIV-1 positive individuals.

4.1 LIMITATIONS & FUTURE DIRECTIONS

The greatest limitation of this investigation was the small sample size of the HIV-1 seroconverters who developed NHL pre-cART. Because some occurrences, such as lack of detectable coinfections, were so rare within the sample the options for statistical analysis were quite limited. In the future, it would be valuable to expand this investigation to a larger study sample. Including HIV-1 seroprevalent individuals, and individuals who were diagnosed with NHL post-cART would allow for a more robust statistical analysis.

Additionally, in order to fully determine the effects of CMV, EBV, HHV-6, HHV-8 and GBV-C coinfection on chronic immune activation and inflammation in HIV-1 positive individuals, biomarkers of immune activation and inflammations should be included in the analysis. Comparing levels of biomarkers of immune activation and inflammation with the presence of viral coinfections in HIV-1 positive individuals would illuminate many unanswered questions regarding the dynamics of viral coinfections in HIV-1 disease progression and their role in the development in inflammatory diseases. In addition to including immune biomarkers in future analysis, it would be valuable to expand viral DNA and RNA detection to other body compartments in addition to blood plasma. This study suggests that although detectable HHV-8 DNA in blood plasma is not associated with the development of NHL, among those who develop NHL, detectable HHV-8 DNA in the blood plasma is associated with more rapid development of NHL following seroconversion. Testing for HHV-8 DNA in blood cells that it targets, e.g. B cells, monocytes, dendritic cells, and additional body compartments may better clarify the true role of HHV-8 infection in the development of NHL.

5.0 PUBLIC HEALTH SIGNIFICANCE

NHL is the seventh most common cancer in the U.S. and is estimated to be the tenth most common cancer in the world, resulting in over 350,000 new cases each year (59). It affects individuals of all ethnicities and socioeconomic status, and the global incidence of NHL has continued to increase in recent years (59). Better understanding the risks associated with the development of NHL can contribute to the early detection and prevention of cases. Although this study focused on NHL in HIV-1 positive individuals, knowledge gained regarding the association of chronic immune activation due to infection on the development of NHL can be applied to the general population. This is particularly true for immune activation due to herpesvirus coinfections, because they are so widespread among the general population.

HIV-1 infection is the most important factor contributing to one's likelihood of developing NHL, and it is estimated that 5-25% of all HIV-1-positive individuals will develop NHL. Although the introduction of cART has reduced the incidence of NHL tremendously, other risk factors for NHL in HIV-1-positive individuals include age, and length of time from seroconversion. It is likely that as a greater number of HIV-1-positive individuals receive treatment, and live longer lives, the burden global burden of NHL will continue to increase. In the U.S. NHL is typically treated with chemotherapy, radiation, or immunotherapy, and the average five-year survival rate is over 70% (59). However, the mean survival times differ greatly among the various subtypes of NHL, and the means survival times are much lower in those with HIV. In some areas of the world, particularly those where the HIV-1 burden is highest, these treatment options are not always accessible. Moreover, estimates suggests that over 70% of cancer patients are diagnosed at late stage of illness in the developing world (21). For these reasons, it is essential that a greater

understanding of the risks associated with NHL development in HIV-1-positive individuals are better understood in order to detect and prevent cases in populations with high HIV prevalence. Additionally, programs aimed at increasing the accessibility of HIV-1 treatment globally should consider incorporating efforts to help build the capacity of health care systems so that they not only address the burden of HIV-1, but also the increasing burden of HIV-1-associated malignancies such as NHL.

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