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EXAMINING A WHOLE GENOME ASSOCIATIONS OF HOST CONTROL THAT ARE MAJOR DETERMINANTS AGAINST HIV DISEASE PROGRESSION

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Abstract:

A recent study examining whole genome associations of host control found that the six most significant protective determinants against HIV disease progression were found in the MHC region of the genome. Several HLA alleles are associated with long-term non-progression and control of viremia. Furthermore, not only were certain HLA-types better than others, but individuals homozygous for HLA I alleles appear to progress more rapidly to AIDS compared to heterozygous individuals. This finding may relate to the ability of individuals with a heterozygous genotype to present a more diverse set of epitopes, resulting in a broader HIV-specific immune response, making it more difficult for the virus to effectively escape from host immune responses. These results suggest that HAART will reduce sexual transmission of HIV at a population level. However, as some HIV-infected individuals on therapy continue to shed infectious HIV RNA in their semen, the individual risk of transmitting HIV is unlikely to be zero.

Keywords: *HAART, HLA, T cell immune response, genital mucosa*

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INTRODUCTION:

The immune system is the body defence system and is essential for the clearance of invading pathogens from the body. The host immune system recognizes a plethora of antigens through several mechanisms. Innate immune responses are rapidly generated following antigen exposure but do not result in the generation of immunologic memory. The innate immune system is the bodys first line of inducible defence against infectious disease and is one of the earliest forms of immunity to have developed evolutionarily [98], as several human innate immune genes share substantial homology with immune mechanisms observed in insects and certain fungal species.

The innate immune system utilizes several immune mechanisms including pathogen specific receptors, immune proteins (complement and coagulation cascades), natural killer cells (NK), macrophages, mast cells, neutrophils and eosinophils. Innate immune responses shape the adaptive immune response through the release of cytokines, chemokines and cell surface co-stimulatory molecules that direct and facilitate homing of adaptive immune lymphocytes to sites of infection and inflammation. The interplay between the adaptive and innate immune systems still remain to be elucidated. Furthermore, recent findings have demonstrated the capacity of NK cells, a component of the innate immune system, to generate immunologic memory, blurring the lines between the adaptive and innate immunity.

One mechanism for pathogen recognition utilized by the innate immune system relies on several families of pattern recognition receptors (PRR) that are expressed on the surface of cells, within intracellular compartments and/or secreted into peripheral blood. Toll-like receptors (TLRs) represent the best known family of surface and intracellular receptors (twelve receptors of this family have been identified so far), and are expressed on the majority of immune cells including B-cells, macrophages, and NK cells.

Some TLRs recognize several ligands, such as TLR2, which recognizes peptidoglycans and bacterial lipopolysaccaharides, while others are more specific. Therefore, the twelve identified mammalian TLRs recognize a plethora of ligands originating from viruses, bacteria, fungi, and parasites[279]. TLRs contain specialize motifs that recognize pathogen-associated molecular patterns (PAMPs) that are unique to pathogens [280]. Recognition of its corresponding ligand, activates TLRs inducing the expression of host defence genes via two adaptor proteins (MyD88 or TRIF) that ultimately lead to the increased expression of anti-viral cytokines, chemokines, antimicrobial peptides, co-stimulatory molecules, MHC molecules and other factors central to the generation of effective host innate and adaptive immune responses.

Secreted PRRs include mannan-binding lectins (MBL), C-reactive proteins (CRP) and serum amyloid proteins (SAP). All these secreted PRRs are produced in the liver during acute and early phase infection. MBLs recognize carbohydrate patterns commonly found on the surface bacteria, viruses, protozoa and fungi. Binding of MBL to cell surface carbohydrtes results in the activation of the lectin pathway and the complement system [75, 28], both of which represent important mechanisms for host-pathogen control they are beyond the scope of this thesis. CRP and SAP molecules bind to phosphocholine on the surface of pathogens, thus facilitating the recognition and ultimately pathogen phagocytosis.

Natural Killer (NK) cells are characterized by the absence of conventional antigen receptors such as immunoglobulin molecules or T-cell receptors (TCR) on the cell surface. NK cells are able to distinguish normal vs. abnormal expression of MHC class I molecules on somatic cells. As somatic cells express MHC class I molecules on their cell surface, NK cells are able to detect perturbations in the expression of MHC molecules on somatic cells, which are often a sign of viral infection or tumor transformation. NK cells, unlike other adaptive immune responses, recognize the absence of “self peptides” and not the presence of “non-self peptides”. Activation of NK cells is governed by a delicate balance between several activation and inhibitory killer immunoglobulin-like receptors (KIR) expressed on NK cells. Virus infection of a cell may result in decreased expression of HLA class I molecules on the cell surface, a feature that is readily recognized by NK cells and results in NK-mediated lysis of the viral infected cell.

NK cells have long been thought of as a bridge

between the innate and adaptive immune systems[282]. Activated NK cells are capable of killing immature dendritic cells that have encountered antigen, despite normal surface expression levels of HLA class I molecules. However, upon encountering antigen(s), immature dendritic cells usually mature and upregulate HLA-class I expression, thereby becoming resistant to killing by activated NK cells. This process is thought to be important in determining the quality of the adaptive immune response generated[282], since dendritic cells that fail to mature following antigen exposure provide inadequate stimulation to T cells and lead to suboptimal generation of adaptive immune responses. Furthermore, recent reports have described properties of NK cells that are more typical of the adaptive immune system, including the ability to maintain immunologic memory. Recent adoptive transfer experiments of NK cells from CMV infected mice into naive animals resulted in a robust secondary expansion of NK cells up challenge by CMV and conferred protective immunity against murine CMV in these previously naive mice.

NK cells play a central role in controlling HIV replication, both through direct killing of infected cells and indirect (secretion of cytokines that suppress HIV replication) mechanisms. NK cells represent a major source of potent suppressive cytokines, including RANTES (regulated upon expression normal T cell expressed and secreted), MIP1 and MIP1 (macrophage inflammatory proteins) that indirectly suppress replication of CCR5-trophic HIV strains. Ex vivo experiments have demonstrated an inverse correlation between NK cell function (direct and indirect) and viremia in HIV infected individuals.

Most (> 99.5%) sexual encounters do not result in HIV transmission. This suggests that natural innate immune defenses play an important role in HIV protection. Several innate mucosal factors, including secretory leukocyte protease inhibitor (SLPI), lactoferrin, Elafin and RANTES are present in genital tract sections at concentrations that have been shown to prevent infection of activated CD4+ T cells *in vitro*. Although the impact of these innate factors on HIV transmission or acquisition *in vivo* is not clear, women who are highly exposed to HIV but remain persistently HIV sero-negative (HEPS) had 10-fold higher concentrations of RANTES in the genital mucosa compared to HIV-uninfected women at risk of becoming HIV infected[90]. In addition, concentrations of SLPI in the vaginal mucosa have also been associated with a reduced probability of perinatal HIV transmission [91, 22] and elevated SLPI levels in the saliva of breastfed infants have been correlated with reduced HIV acquisition through breast milk[92, 93]. Concentrations of innate factors have also been directly implicated in reduce HIV acquisition, as elafin levels in the CVL of HIV-negative women were associated with reduced HIV acquisition in women in a prospective case-controlled trial.

Although these and other mucosal innate factors have been associated with protection against HIV in vitro and in vivo studies, the impact of these innate factors on shedding of HIV RNA in the genital tract of infected individuals is unclear.

Adaptive Immune Responses

The hallmark of the adaptive immune system is the generation of long-term immunologic memory following exposure to an antigen. The adaptive immune system developed much later on the evolutionary scale than innate immunity, and is thought to have originally developed in jawed vertebrates. The adaptive immune system can be divided into two major arms; the humoral arm (B-cells) and the cell-mediated arm (T- cells). The two branches of the adaptive immune system work in concert and result in the generation of neutralizing antibodies and cell mediate immunity against almost an infinite number of pathogens. Unlike the innate immune system, the adaptive immune system recognizes a specific antigenic sequence and generates antibodies and/or a T cell immune response targeting that epitope. Following activation, naive B and T cells mature into memory B and T cells, which will elicit a more robust and faster immune response upon antigen re-exposure. Although several facets of the adaptive immune system are elicited and involved in the control of HIV replication, this thesis focuses on the role and impact of HIV-specific CD8+ T cell responses on RNA levels in blood and semen plasma.

TCRs are member of the immunoglobulin super family of proteins that are expressed on the surface of T cells and are responsible for recognizing foreign antigens bound to MHC molecules. Germ line DNA

contains four TCR multi-gene families, each of which encodes one of the four T cell receptor chains. Two T cell receptors have been isolated to date, each a heterodimer made up of one and one chain (T cell receptor) and the other made up of one and one chain (T cell receptor). Each of the chains that make up the heterodimer through the combination of four polypeptides chains; the variable (V), diversity (D), joining (J) and constant (C) regions. All the regions are encoded on separate gene segments that recombine, which results in increased potential diversity of each of the chains. The receptor is expressed on over 95% of peripheral blood T lymphocytes and is composed of highly variable domains with almost 10 different possible combinations.

The ability of T cells to recognize and mount immune responses against antigenic peptides relates to the specificity of the TCR that is expressed on the surface of T cells. The TCR interacts with MHC class I or II molecules that are bound to non-self peptides (TCRs that recognize self-peptides are eliminated during T cell haematopoiesis in the thymus)[97]. The TCR works in conjunction with two co-receptors, CD4 and CD8, that are constitutively expressed on T cells and determine if the TCR interacts with cells expressing MHC class I (CD8+) or MHC class II molecules (CD4+)[296]. However, prior to the generation of a TCR-MHC complex, MHC molecules have to be loaded with antigenic peptides, a multistep process that ultimately determines the specificity of the host T cell immune response.

The TCR of a CD8-expressing T cell interacts with the MHC I–peptide complex and is essential in mediating the anti-viral immune response. MHC class I molecules, present on all somatic cells in the body, are loaded with viral peptides that are generated within the cell during viral replication. The majority of peptides loaded on to an MHC class I molecule are between 8 – 11 amino acids long, and are generated by the proteosome. Proteosomes are ubiquitous proteases responsible for the degradation of proteins into peptides fragments of variable lengths (ranging from between 4 and 20 amino acids long) and are responsible for the majority of peptides that are loaded onto MHC class I molecules. Usually proteosomes diffuse freely in the cytoplasmic environment of the cell and find protein substrates randomly[29]. While the exact length of generated peptides can vary, peptides that are too small to be loaded on MHC molecules are degraded, and peptides that are too long are often further trimmed by other proteases in the ER (ER-aminopeptidases; ERAP) and then loaded on MHC molecules. Following proteosomal degradation, viral peptides are transported from the cytoplasm to the site of MHC class I biogenesis – the endoplasmic reticulum (ER).

Transport of the majority of viral proteins from the cytoplasm into the ER is facilitated by an ER resident peptide transporter called transporter for antigen processing (TAP) protein. TAP readily binds cytoplasmic proteins and transfers them to the ER lumen. The large protein fragments are trmmed further by ERAP and then loaded on to the MHC class I molecules in the ER. ERAPs are designed to trim the amino terminal of the protein while proteosomes are particularly efficient at cleaving amino acids at the carboxy-terminus. Although this process appears to be quite efficient, the majority of peptides generated will not bind to MHC complexes, due to length or sequence incompatibility. In addition, the majority of proteosome processed peptides do not efficiently bind to TAP, again an issue of length. Of the peptides that are the right size, sequence and bind efficiently to TAP and the MHC as initially processed, the peptides may end up in the wrong MHC loading complex. The ER has several MHC loading complexes and as a single cell can express up to six MHC I alleles most MHC loading complexes express only four of them (at the most)[26]. Therefore getting the peptide processed and loaded right is quite an undertaking and the majority of processed peptides never get presented on an MHC molecule. The peptides that are just right and are between 8 and 11 amino acids long are then loaded on to MHC molecules (at the right MHC loading complex) and the MHC-peptide complex is transported to the plasma membrane and expressed on the cell surface awaiting recognition by a CD8+ T cell[98, 299]. A passing naive or effector (previously antigen experienced) CD8+ T cell, specific for the antigen expressed on the MHC I molecule of a somatic or antigen presenting cells, becomes activated.

RESULTS:

Insights from the SIV model of infection.

The importance of CD8+ T cell immune responses were first demonstrated in depletion studies carried out in the SIV infected non-human primates. Monoclonal antibody mediated depletion of CD8-expressing cells was associated with a rapid rise in SIV viremia and the return of CD8+ cells coincided with a rapid reduction in SIV viremia302-304. Antibody mediated depletion of CD8+ T cell in acute infection resulted in persistent high viremia and a delay in the establishment of a viral set-point until the re-emergence of CD8+ T cells 303. CD8+ T cells were also found to be important in chronically infected RM‟s as again antibody-mediated depletion of CD8+ T cell after an established viral set-point resulted in a 1 – 4 log increase in viremia, and the re-emergence of CD8+ T cells was associated with a reduction to the previously established viral set-point. Although the use of CD8-depleting monoclonal antibodies also resulted in depletion of NK cells, which have been shown to be important in virus control, these early studies in the SIV model of infection provided strong evidence for the role of CD8+ T cell responses in early and chronic SIV infection.

T cells and HIV viremi

HIV-specific CD8+ T cell immune responses represent some of the earliest adaptive immune responses detected following HIV or SIV infection 306. The first, and perhaps most obvious, evidence suggesting that CD8+ T cells play an important role in controlling HIV viremia was when a temporal association was observed between the emergence of T cells and a fold reduction in viremia during acute HIV infection. Additional studies further demonstrated that HIV-specific immune responses appeared to be associated with reduced viremia in acute, but not chronic HIV infection.

More recent studies suggest that CD8+ immune response against certain HIV proteins, notably Gag, is associated with lower viremia compared to immune responses against other HIV proteins. In fact, the detection of immune responses against HIV Env and several of the accessory proteins in these studies was associated with increased viremia . Despite the lack of direct evidence linking HIV-specific immune responses to reduced HIV viral replication in vivo, several lines of evidence now suggest that HIV- specific T cell immune responses place considerable immunologic pressure on the HIV virus in vivo (discussed below) even during chronic HIV infection.

HLA and T cell immune response

Recent study examining whole genome associations of host control found that the six most significant protective determinants against HIV disease progression were found in the MHC region of the genome. Several HLA alleles HLA-B57, HLA- B27, and HLA-B51 are associated with long-term non-progression and control of viremia. Furthermore, not only were certain HLA-types better than others, but individuals homozygous for HLA I alleles appear to progress more rapidly to AIDS compared to heterozygous individuals. This finding may relate to the ability of individuals with a heterozygous genotype to present a more diverse set of epitopes, resulting in a broader HIV-specific immune response, making it more difficult for the virus to effectively escape from host immune responses.

Characterizing T cell in the genital mucosa

As the majority of HIV is transmitted sexually, the male and female genital tracts represent the initial sites of the majority of exposures to HIV and SIV. Perhaps the presence of HIV-specific CD8+ immune responses in the genital tract of uninfected individuals may be able to protect against infection following exposure. HIV-specific CD8+ T cell immune responses have been previously characterized in the genital tracts.

T cell has been detected in the vaginal mucosa of SIV-infected rhesus macaques, HIV-infected women339 and in the semen of HIV-infected men. In addition, the majority of T cell immune responses expressed the TCR, similar to T cell populations in peripheral blood. Similar to evidence found in peripheral blood, T cell population isolated from the vaginal and cervical mucosa of SIV-infected macaques and HIV- infected women were able to directly lyse SIV and HIV infected cells; respectively . However, it is not clear if HIV-specific immune cells in the semen of HIV infected men are able to control HIV RNA shedding in semen.

Table 1 Study participant characteristics of HIV-infected individuals on long-term suppressive HAART.

No	ID	Age (yrs)	Abs. CD4 count (/mm^3)	Blood viral load (copies/mL)	HIV Time on HAART (months)	Nadir CD4 Count (/mm^3)	Duration of complete viral suppression* (months)
1.	003	47	540	<50	132	56	120
2.	004	50	440	<50	86	136	75
3.	006	51	2260	<50	92	110	91
4.	007	44	970	<50	192	91	103
5.	008	51	730	<50	168	360	102
6.	009	54	560	<50	180	n/a	112
7.	010	59	730	<50	110	n/a	107
8.	011	55	970	<50	120	82	115
9.	012	50	830	<50	92	156	73
10.	013	47	710	<50	54	330	116
11.	014	58	580	<50	198	90	53
12.	017	65	590	<50	216	300	82
13.	018	43	580	<50	102	n/a	70
14.	020	61	590	<50	85	n/a	69
15.	021	62	420	<50	105	n/a	115
16.	022	27	780	<50	51	n/a	49
17.	037	41	810	<50	84	260	76
18.	173	38	640	<50	61	240	49
19.	211	43	530	<50	175	170	56
20.	222	38	950	<50	59	308	57
21.	249	50	1030	<50	141	n/a	130
22.	271	47	480	<50	206	120	96
23.	285	44	320	<50	61	n/a	55

*The sensitivity of viral load assays varied over time, from a threshold of >1000 RNA copies/ml (1995) to >50 RNA copies/ml (currently).

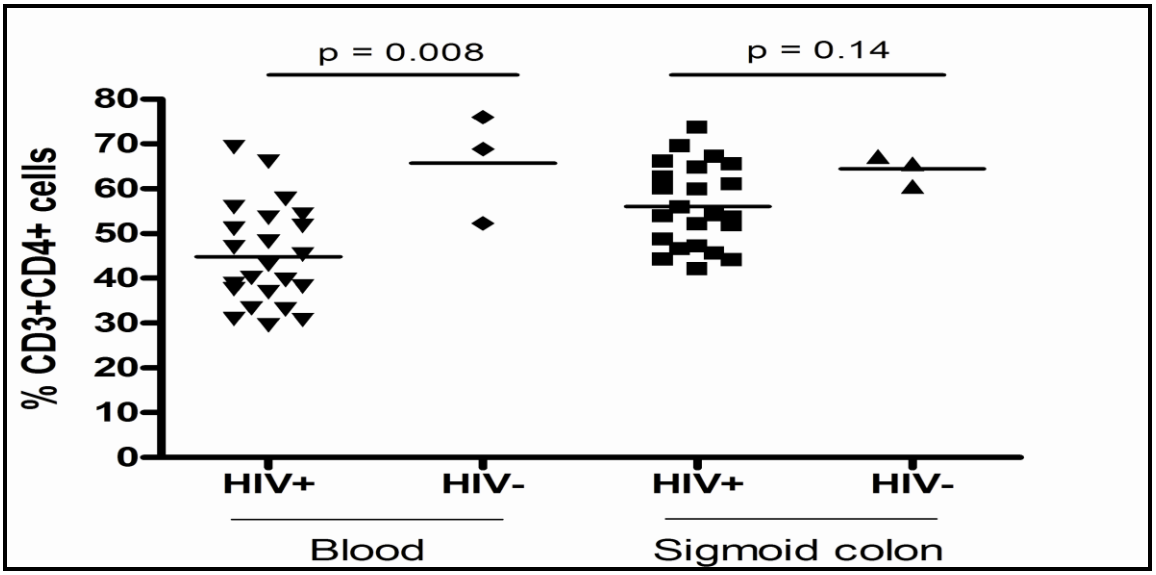

Figure 1 – Percentage of T-cells expression CD4 in blood and the sigmoid colon

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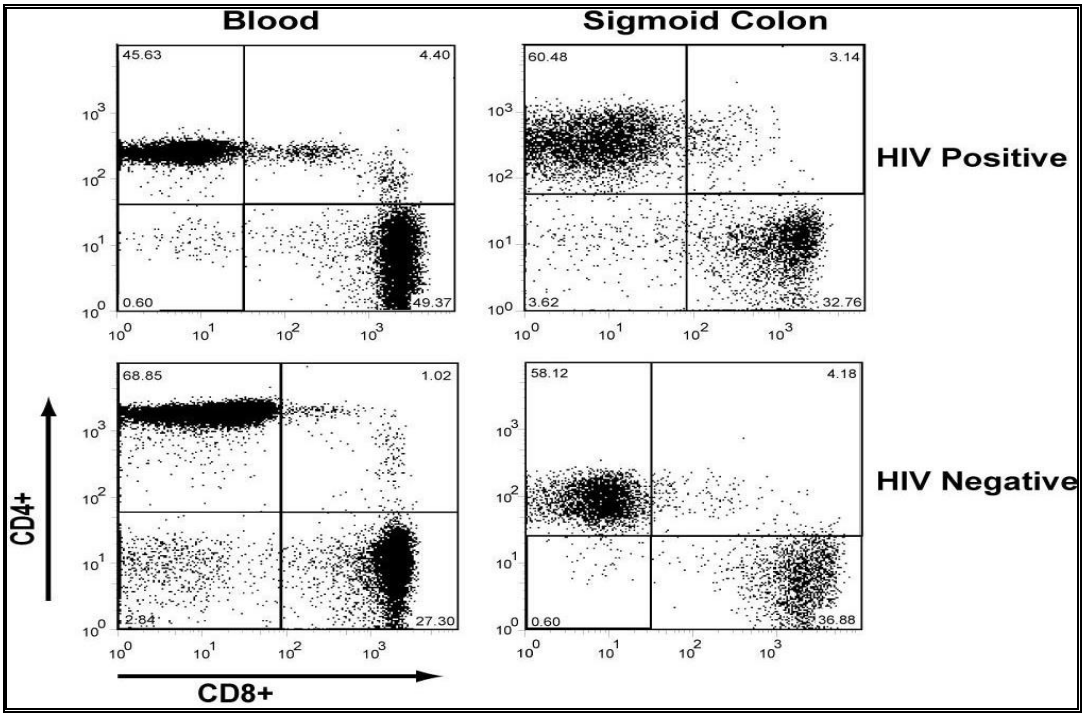


Figure 2 – CD4 and CD8 T cell expression in blood and the sigmoid colon.

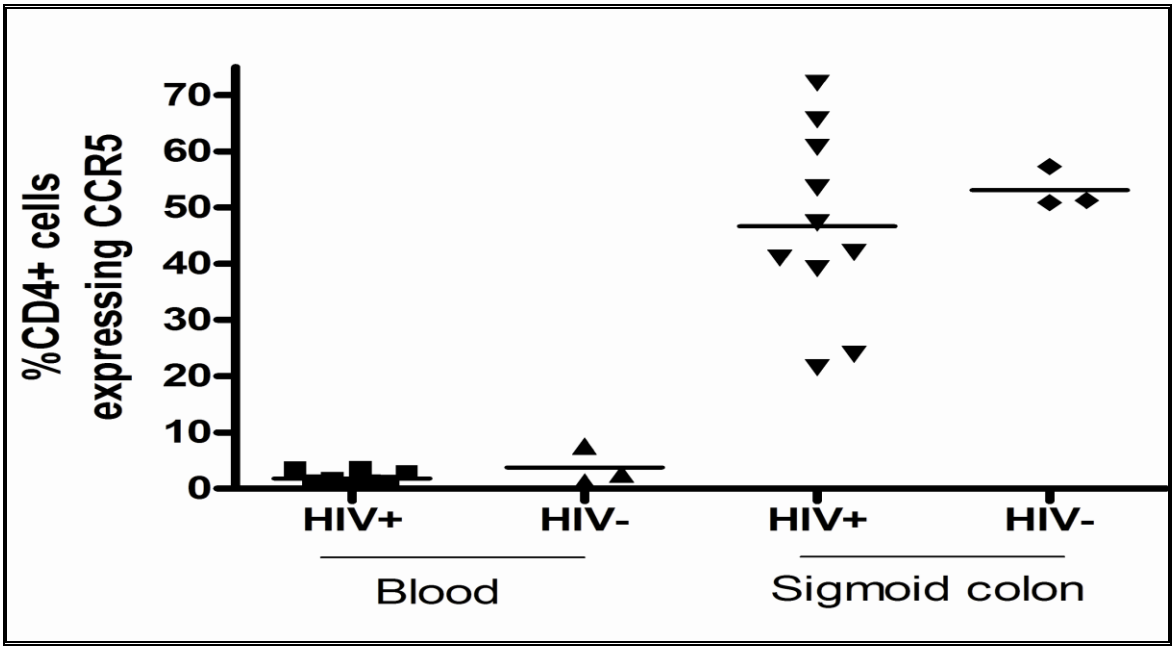


Figure 3 – Comparing CCR5 expression on CD4+ T cells in blood and the sigmoid colon

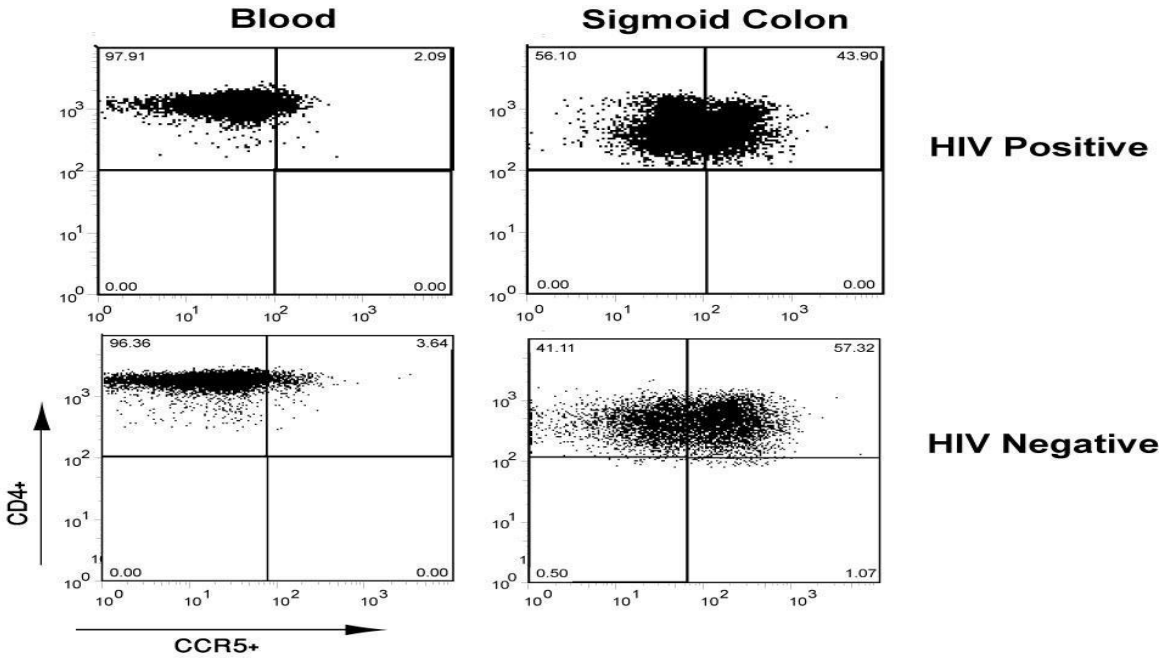
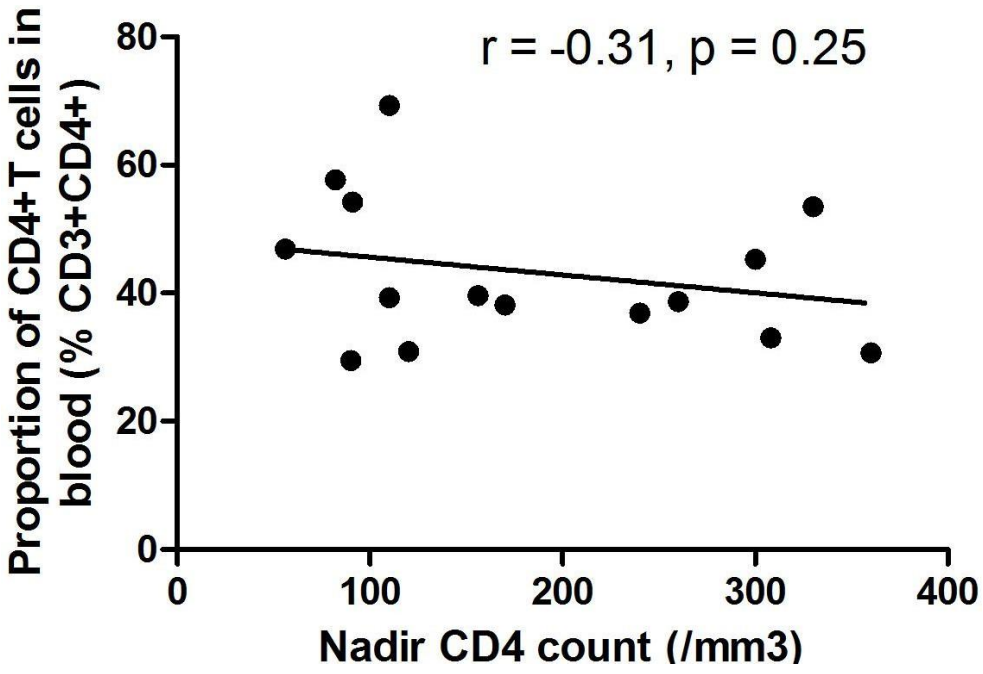


Figure 4 – CCR5 expression on CD4+ T cells from blood and the sigmoid colon.

A.



B.

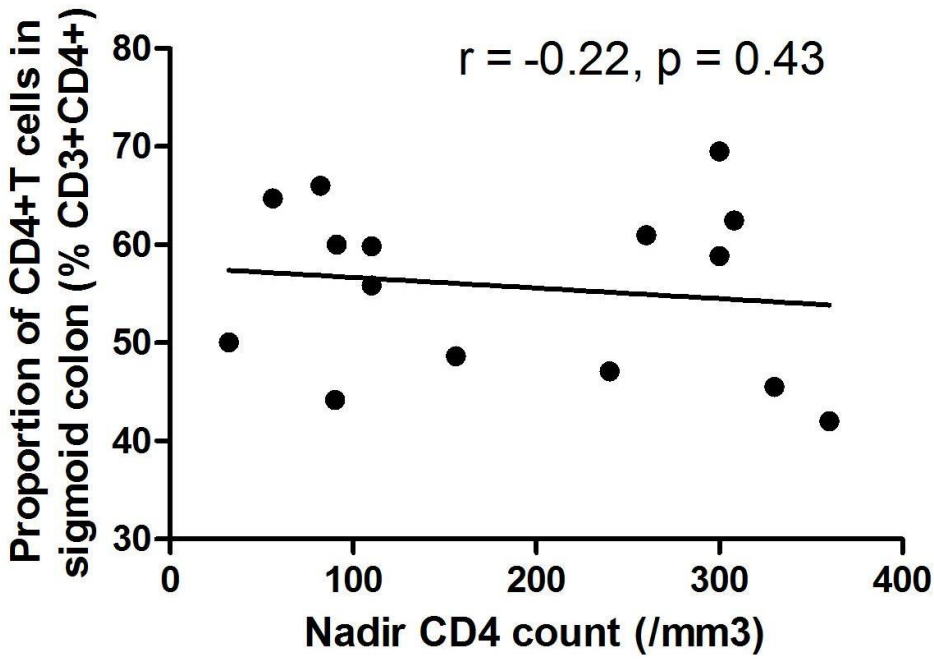


Figure 5 – Blood nadir CD4 count and immune reconstitution of CD4+ T cells in blood and the sigmoid colon.

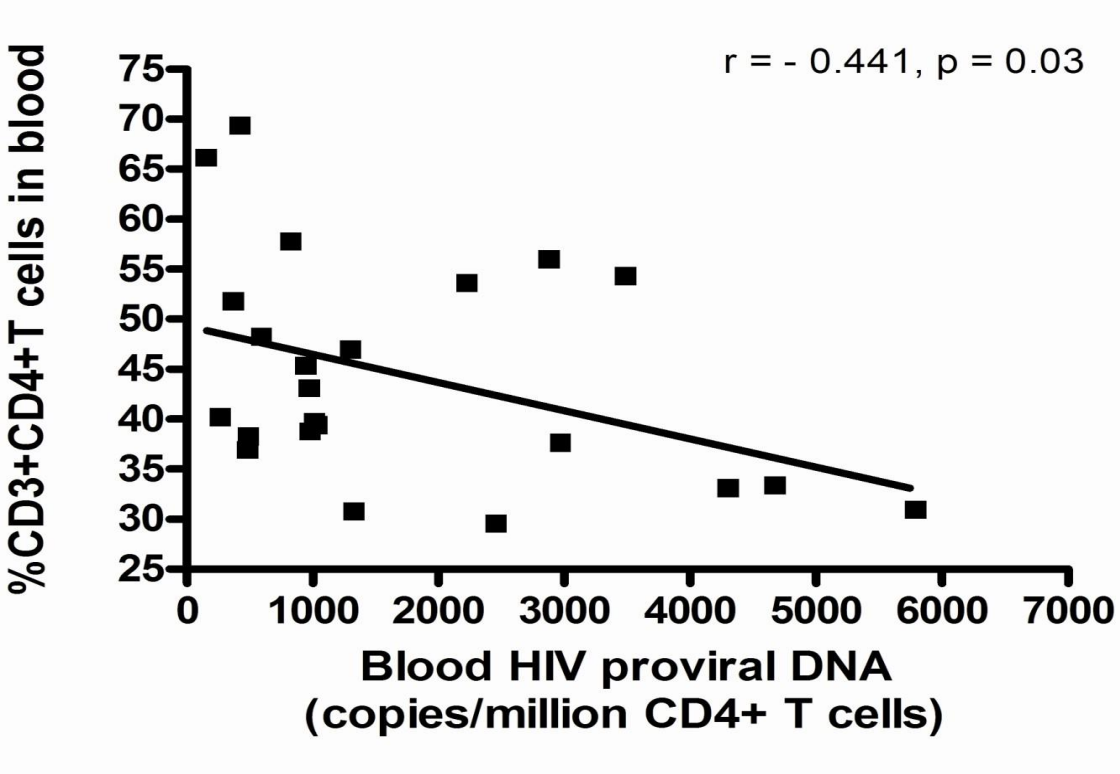


Figure 6 – Correlation between %CD4 and HIV proviral DNA in blood.

DISCUSSION:

The majority of HIV is transmitted sexually, and so understanding the correlates of HIV RNA levels in semen is important from a public health perspective. The focus of my doctoral work was to identify immunologic and clinical correlates of HIV RNA levels in semen with the overall aim to inform novel immunotherapeutic and clinical interventions to reduce sexual transmission of HIV.

I next evaluated the correlation between common viral co-pathogens and semen HIV RNA levels. We found a strong positive correlation between levels of HIV RNA and CMV DNA in semen, and disproportionately high HIV RNA shedding in semen was associated with CMV reactivation. Although, the cross-sectional nature of our studies made it impossible to determine the direction of causality, suggesting that asymptomatic reactivation of this near ubiquitous pathogen may alter the infectiousness of an HIV infected individual. A logical next step could be treat CMV and evaluate the impact that has on HIV RNA levels in semen. In fact these studies have been recently undertaken within our research group and preliminary results suggest that treating CMV has an impact of semen HIV RNA levels, suggesting that CMV reactivation was promoting HIV RNA replication in semen.

Perhaps the most intuitive way to reduce HIV levels in semen and subsequently reduce sexual transmission is HAART, which is associated with complete suppression of HIV RNA in blood. However, a better understanding of the impact of HAART on HIV RNA levels in semen is needed.

These results suggest that HAART will reduce sexual transmission of HIV at a population level. However, as some HIV-infected individuals on therapy continue to shed infectious HIV RNA in their semen, the individual risk of transmitting HIV is unlikely to be zero. Despite our data suggesting the impact of HAART on HIV transmission, data from clinical trials examining the impact of therapy on HIV transmission in the real world are urgently needed. In the meantime it is imperative that studies continue to identify immune and viral correlates of HIV shedding in semen in hopes that better understanding of correlates can lead to the development of novel immunotherapeutic interventions as well as better inform public health policy.

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