The Human Leukocyte Antigen associations of survival in an acquired immunodeficiency syndrome

Masters of Science by Research



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ABSTRACT

An understanding of the molecular basis of immune recognition in human disease is essential for the development of antigen specific immunotherapy. Here, a new method for unambiguous human leukocyte antigen typing (HLA) is assessed and followed by an immunogenetic study exploring the association of HLA with disease outcomes in a group of adolescents who have survived with untreated, perinatal human immunodeficiency virus type-1 (HIV-1) infection.

The new method for high-resolution, unambiguous, mono-allelic genotyping of HLA-B and C alleles was tested on a subset of samples from a cohort of Zimbabwean Shona adolesents. Three novel HLA-C alleles were described and named by the World Health Organisation Nomenclature Committee for Factors of the HLA System.

Next, the association of HLA in HIV-1 disease progression was assessed in the Shona adolescents perinatally infected with the virus. Previous reports have shown that African infants with vertically-acquired HIV-1 infection progress rapidly, with only 50% surviving beyond two years in the absence of treatment. Recent reports, however, have described a substantial burden of older children living with untreated vertically-acquired HIV-1 infection in Southern Africa. The immunological and genetic factors associated with long-term survival following vertical infection are poorly understood. Medium-to-high resolution HLA typing was performed on DNA samples obtained from a cohort of presumed vertically HIV-1 infected children and age-matched uninfected controls in Harare, Zimbabwe. Overall, 93 HLA class I alleles were detected in the study population with a significant enrichment of HLA-C*08:02 and -C*08:04 in the HIV-1 infected long-term survivor group. Conversely, HLA-A*02:01, -A*34:02 and -B*58:02 were over-represented in the uninfected control group.

Together, these preliminary studies serve as a common starting point to identify pathways of immune recognition inherent in disease susceptibility and survival.

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This work has given me the great privilege of working with outstanding individuals in the United Kingdom and Zimbabwe. In Zimbabwe, I am indebted to many people whom I have yet to meet but hope to have the good fortune of crossing paths with. Our international exchanges have shown a spotlight on this country and with great expectation I seek to find any commonality that I can for friendship and scientific exchange. I owe an immense amount of gratitude to the adolescents plagued by HIV who willingly shared their blood so that we may find insights that could help others around the world. May their lives always be blessed.

Following this, I would like to thank the members of the Sarah Rowland-Jones lab for their spirit, friendship, and community. This attitude will take everyone far in the field of science and beyond.

In addition to the lab, my senior tutor and academic advisor at Balliol College demonstrated great dedication in guiding me through this process and for that I am grateful.

I would also like to thank my friends, both old and new, for accepting nothing less than completion from me.

My family, I would like to thank you for believing in me during the highs and the lows. Your compassion and love has given me the opportunity to grow and develop into a stronger woman and for that I am forever grateful.

And finally, I would like to thank God for the compassion and love that always guides me.

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CHAPTER 1

Full-length allelic sequencing and detection of novel alleles in HLA class I genes

HLA
Immune Response Genetics

Isolation of full-length *HLA-C*18:02* allele in an individual from Sub-Saharan Africa

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Key words: HLA-C; new allele; SBT; Southern Africa; Zimbabwe

The full-length sequence of *HLA-C*18:02* differs from that of *HLA-C*18:01* by a single nucleotide polymorphism.

Human leukocyte antigen (HLA) molecules are encoded within the major histocompatibility complex located on human chromosome 6 (6q21) which contains several other genes. Several associations have been showed between HLA class I genes and outcomes of autoimmune and infectious diseases (1, 2). According to the latest release of the IMGT/HLA Database version 3.23.0 of January 2016 (3), 2902 HLA-C alleles have been described from different world populations albeit with fewer full-length sequences deposited in the database. HLA-C molecules are expressed by most cells of the human body and play a central role in the immune system by presenting peptides derived from endoplasmic reticulum. Recent studies have shown that the expression levels of HLA-C molecules correlate with the outcomes of certain infectious diseases and transplantation (4, 5).

We recently isolated the full-length nucleotide sequence of *HLA-C*18:02* allele which is similar to that of *HLA-C*18:01* except for a non-synonymous single nucleotide polymorphism in exon 5 at position 2040T>C (Figure 1). The full-length sequence of *HLA-C*18:02* was isolated from genomic DNA obtained from a heterozygous donor belonging to the Shona ethnic group of Zimbabwe using a recently developed sequence-based typing technique that independently amplify and bi-directionally sequence HLA alleles from heterozygous individuals. The nucleotide sequences of *HLA-C*18:02* have been deposited into GenBank (accession number KR920752) and IPD IMGT/HLA database (submission ID HWS10025314).

The name *C*18:02* has been officially assigned by the World Health Organization (WHO) Nomenclature Committee in December 1996. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report (6), names will be assigned to new sequences as they are identified. Lists of such new names will be published in the following WHO Nomenclature Report.

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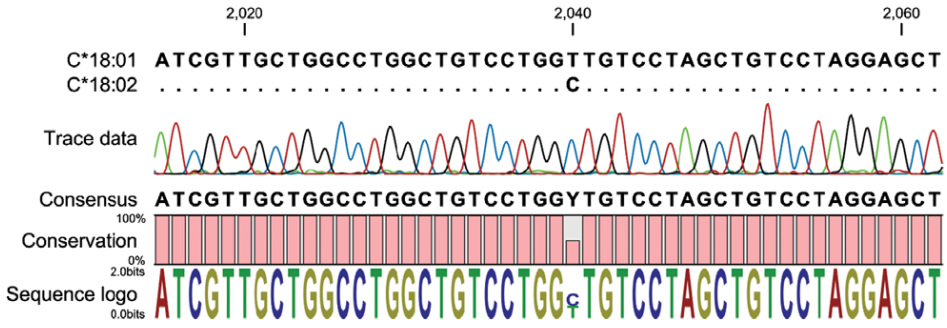


Figure 1 Partial nucleotide alignment of *HLA-C*18:01* and *HLA-C*18:02*.

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Conflict of interest

The authors have declared no conflicting interests.

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6. Marsh SG, Albert ED, Bodmer WF et al. Nomenclature for factors of the HLA system, 2010. *Tissue Antigens* 2010: **75**: 291–455.

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NEW ALLELE Alerts

*HLA-C*06:04:02* a novel HLA-C allele isolated from donors of Southern African descent

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Key words: HLA-C; new allele; sequence-based typing; full length; Southern Africa

*HLA-C*06:04:02* allele differs from *HLA-C*06:02:01:01* with two nucleotide substitutions in exon 3 and 3′ untranslated region (UTR_.

The human leukocyte antigens (HLA) are coded by a number of genes located on the short arm of human chromosome 6 – the most highly polymorphic region of the human genome. These genes encode groups of related proteins that help the human immune system distinguish between body's own proteins (also known as 'self') and those of foreign origin ('non-self'). Humans have three classical HLA class I genes, known as HLA-A, HLA-B, and HLA-C, which are present on the surface of almost all human cells (1). According to the most recent release 3.23.0 of January 2016, a total of 10574 HLA class I alleles isolated from different world populations have been deposited in the IPD-IMGT/HLA Database for HLA – a specialist repository for sequences of the human major histocompatibility complex (2). To date, 2902 HLA-C alleles have been described but fewer full length HLA-C sequences ($n = 116$) have been deposited in international databases. This is probably due to the lack of an appropriate technique to comprehensively isolate full length alleles at the population level.

We recently designed a high-resolution sequence-based technique in our laboratory at the University of Oxford to detect diversity within HLA class I loci. This technique has the ability to independently amplify any two HLA alleles present in heterozygous DNA samples and bi-directionally sequence them with no ambiguity. Herein, we describe a novel HLA class I allele (*HLA-C*06:04:02*) that was isolated from several heterozygous DNA samples obtained from people of Shona descent in Zimbabwe.

Exons 2 and 3 of *HLA-C*06:04:02* are identical to those of *HLA-C*06:04:01* except for a synonymous point mutation in exon 3 from C (*HLA-C*06:04:01*) to T (*HLA-C*06:04:02*) at nucleotide position 914 (Figure 1). The data available for *HLA-C*06:04:01* in the IPD-IGMT/HLA Database is incomplete (lacking all other exons 1, 4–8). For this reason, we were unable to compare the full coding sequences of both alleles. Moreover, the full-length sequence of *HLA-C*06:04:01* is not available in any database. Comparison of full-length sequences of *HLA-C*06:04:02* with those available in the IPD-IMGT/HLA Database revealed that this novel allele was closest to *HLA-C*06:02:01:01* but differed from it at two nucleotide positions 915G>T (exon 3) and 3005T>C [(3′untranslated regions (UTR)], respectively (Figure 1). The full length sequence of *HLA-C*06:04:02* has been deposited into GenBank with the accession number KR920751 and in IPD-IMGT/HLA database (submission ID: HWS10025312).

The name *C*06:04:02* has been officially assigned by the WHO Nomenclature Committee in August 2015. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report (3), names will be assigned to new sequences as they are identified. Lists of such new names will be published in the following WHO Nomenclature Report.

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Conflict of interest

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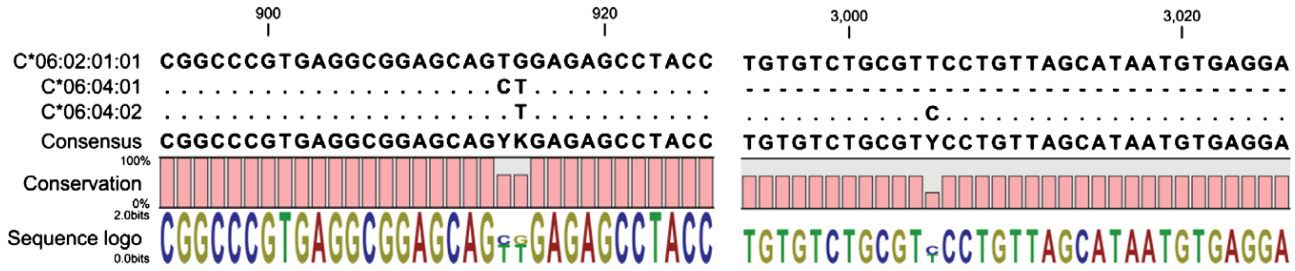


Figure 1 Partial nucleotide alignment comparing the novel allele *HLA-C*06:04:02* to *HLA-C*06:02:01:01* and *HLA-C*06:04:01*.

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3. Marsh SG, Albert ED, Bodmer WF et al. Nomenclature for factors of the HLA system, 2010. *Tissue Antigens* 2010: **75**: 291–455.

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NEW ALLELE Alerts

*HLA-C*16:01:01:02*, a recently isolated HLA class I allele from Southern Africa

B. L. Shepherd[1], T. Bandason[2], R. Ferrand[2,3], S. Rowland-Jones[1] & L.-M. Yindom[1]

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3 Infectious Disease Department, London School of Hygiene and Tropical Medicine, London, UK

Key words: human leukocyte antigen-C; new allele; sequence-based technique; Southern Africa; Zimbabwe

The full-length nucleotide sequence of *HLA-C*16:01:01:02* differs from that of *HLA-C*16:01:01:01* with a SNP at 2996C>T.

Human leukocyte antigen (HLA) represents the human's major histocompatibility complex (MHC) and is one of the most polymorphic systems within the human genome. It is located on the short arm of chromosome 6 at 6p21.3 (1). HLA-C is one of three classical HLA class I loci. The genes that map to this locus code for heterodimers consisting of a heavy chain glycoprotein anchored into the membrane and a water-soluble light chain. HLA-C molecules are expressed by most cells of the human body and play a central role in the immune system by presenting peptides derived from endoplasmic reticulum. The number of HLA class I gene variants differs between individuals at the population level, and efforts are being made to discover and report full-length HLA variants present in different parts of the world. Given the high degree of polymorphism observed in the HLA system, it is expected that changes in the nucleotide or amino acid composition of HLA allotypes may influence the specificity of antigen presentation through their levels of expression.

We report here a novel HLA-C allele (*C*16:01:01:02*) that was recently isolated in our laboratory using an in-house sequence-based technique designed to independently amplify and sequence in both directions single HLA alleles from heterozygous individuals. This newly described HLA-C allele was observed in two genomic DNA samples extracted from individuals of South African descent belonging to the Shona ethnic group of Zimbabwe. The sequence traces of *C*16:01:01:02* were similar to that of *HLA-C*16:01:01:01* except for the presence of a single nucleotide polymorphism at position 2996 in the 3′ unstranslated region of the locus (Figure 1). The full-length sequence of *HLA-C*16:01:01:02* has been deposited into GenBank with the accession number KR920753 and in IPD-IMGT/HLA database (submission ID: HWS10025316).

The name *C*16:01:01:02* has been officially assigned by the World Health Organization (WHO) Nomenclature Committee in August 2015. This follows the agreed policy that, subject to the conditions stated in the most recent nomenclature report (2), names will be assigned to new sequences as they are identified. Lists of such new names will be published in the following WHO Nomenclature Report.

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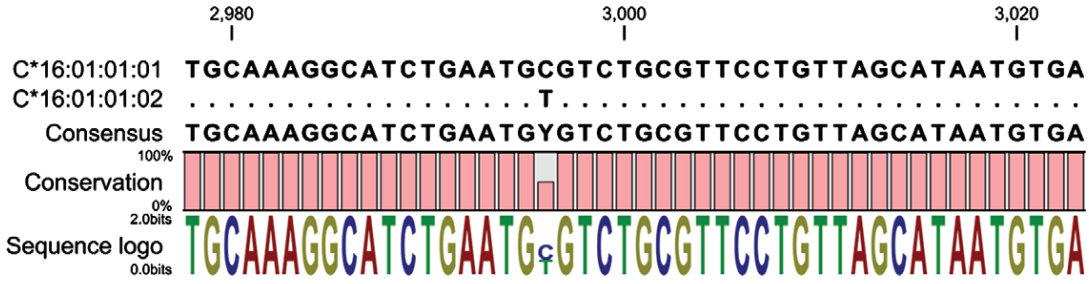


Figure 1 Partial nucleotide alignment comparing the novel allele *HLA-C*16:01:01:02* to *HLA-C*16:01:01:01*.

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1. Marsh SGE, Parham P, Barber LD. *The HLA Factsbook*. *San Diego*: *Academic Press*, *2000*.
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Key words: human leukocyte antigen-C; new allele; sequence-based technique; Southern Africa; Zimbabwe

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Human leukocyte antigen (HLA) represents the human's major histocompatibility complex (MHC) and is one of the most polymorphic systems within the human genome. It is located on the short arm of chromosome 6 at 6p21.3 (1). HLA-C is one of three classical HLA class I loci. The genes that map to this locus code for heterodimers consisting of a heavy chain glycoprotein anchored into the membrane and a water-soluble light chain. HLA-C molecules are expressed by most cells of the human body and play a central role in the immune system by presenting peptides derived from endoplasmic reticulum. The number of HLA class I gene variants differs between individuals at the population level, and efforts are being made to discover and report full-length HLA variants present in different parts of the world. Given the high degree of polymorphism observed in the HLA system, it is expected that changes in the nucleotide or amino acid composition of HLA allotypes may influence the specificity of antigen presentation through their levels of expression.

We report here a novel HLA-C allele (*C*16:01:01:02*) that was recently isolated in our laboratory using an in-house sequence-based technique designed to independently amplify and sequence in both directions single HLA alleles from heterozygous individuals. This newly described HLA-C allele was observed in two genomic DNA samples extracted from individuals of South African descent belonging to the Shona ethnic group of Zimbabwe. The sequence traces of *C*16:01:01:02* were similar to that of *HLA-C*16:01:01:01* except for the presence of a single nucleotide polymorphism at position 2996 in the 3′ unstranslated region of the locus (Figure 1). The full-length sequence of *HLA-C*16:01:01:02* has been deposited into GenBank with the accession number KR920753 and in IPD-IMGT/HLA database (submission ID: HWS10025316).

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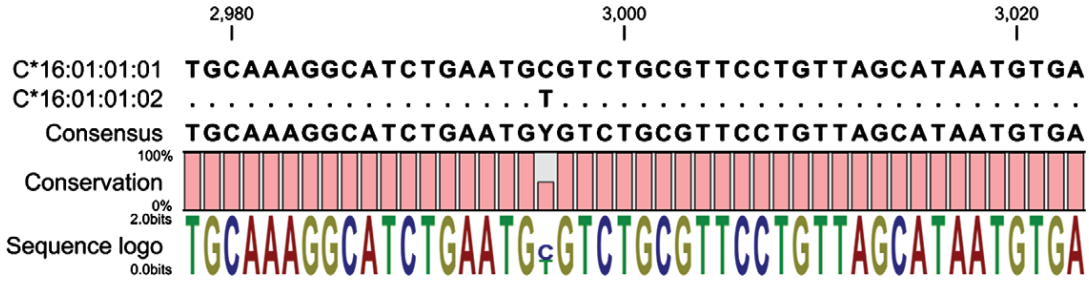


Figure 1 Partial nucleotide alignment comparing the novel allele *HLA-C*16:01:01:02* to *HLA-C*16:01:01:01*.

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CHAPTER 2

HLA associations of long-term survival in a group of vertically infected, treatment naïve HIV-1 positive adolescents

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HLA Correlates of Long-Term Survival in Vertically Infected HIV-1-Positive Adolescents in Harare, Zimbabwe

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Abstract

African infants with vertically acquired HIV infection progress rapidly, with only 50% surviving beyond 2 years in the absence of treatment. Despite this high initial mortality, recent reports describe a substantial burden of older children living with untreated vertically acquired HIV infection in Southern Africa. The immunological and genetic factors associated with long-term survival following vertical infection are poorly understood. We performed medium-to-high resolution HLA typing on DNA samples obtained from a cohort of presumed vertically HIV-1-infected children and age-matched uninfected controls in Harare, Zimbabwe. Overall, 93 HLA class I alleles were detected in the study population with a significant enrichment of HLA-C*08:02 and -C*08:04 in the HIV-1-infected long-term survivor group. Conversely, HLA-A*02:01, A*34:02, and -B*58:02 were overrepresented in the uninfected control group. Our data indicate that HLA alleles may have differential effects against HIV acquisition and disease progression in vertical HIV-1 infection.

GLOBALLY, 35.3 MILLION PEOPLE were estimated to be living with HIV infection in 2012.[1] Southern Africa remains the worst affected region with adult HIV prevalence rates exceeding 20% in some countries[2]: this rate is even higher among pregnant women.[1] Without intervention the risk of mother-to-child transmission (MTCT) of HIV is 25–35%, which has resulted in a regional epidemic of vertically acquired HIV, in parallel to the adult epidemic, that has had a major impact on child mortality.[1,3]

Unlike adults, in which the median time from HIV infection to development of AIDS is about 10 years, the natural history of HIV in infants is dominated by high rates of rapid disease progression with a 50% probability of survival by the age of 2 years in African cohorts.[4–6] As a result, survival into adolescence without antiretroviral treatment was considered rare in Africa. However, recent reports have revealed that up to one-third of HIV-infected infants have more slowly progressive disease with mean survival rates ranging from 8 to 16 years of age.[7–10]

Population-based surveys of older children in Southern Africa report high HIV prevalence rates of 2–6% among older children and young adults.[11] Although many untreated HIV^+ adolescents present with severe disease manifestations, predominantly respiratory and cardiac, initial studies suggest that a small proportion (5–10%) of newly diagnosed presumed vertically infected HIV^+ adolescents in Harare, Zimbabwe have no symptoms and a normal $CD4^+$ count,[12] and could be termed long-term slow-progressors (LTSP). Further studies in the now sizable group of adolescents who have survived long term with vertical HIV-1 infection may provide important information about immune correlates of delayed disease progression following acquisition of HIV infection in infancy.

To date, there have been few studies to identify the genetic determinants of long-term survival following vertical acquisition of HIV-1 infection in African adolescents. The highly polymorphic human leukocyte antigen (HLA) gene network on chromosome 6 is strongly predictive of adult HIV-1 infection outcomes,[13] with striking associations between HLA alleles and viral control.[14] HIV-1-infected adults with ''protective'' HLA class I alleles who go on to exhibit spontaneous viral control show rapid viral clearance in acute HIV-1 infection.[15] In contrast vertically infected infants have very high peak viral loads (VL) in the first few months of life

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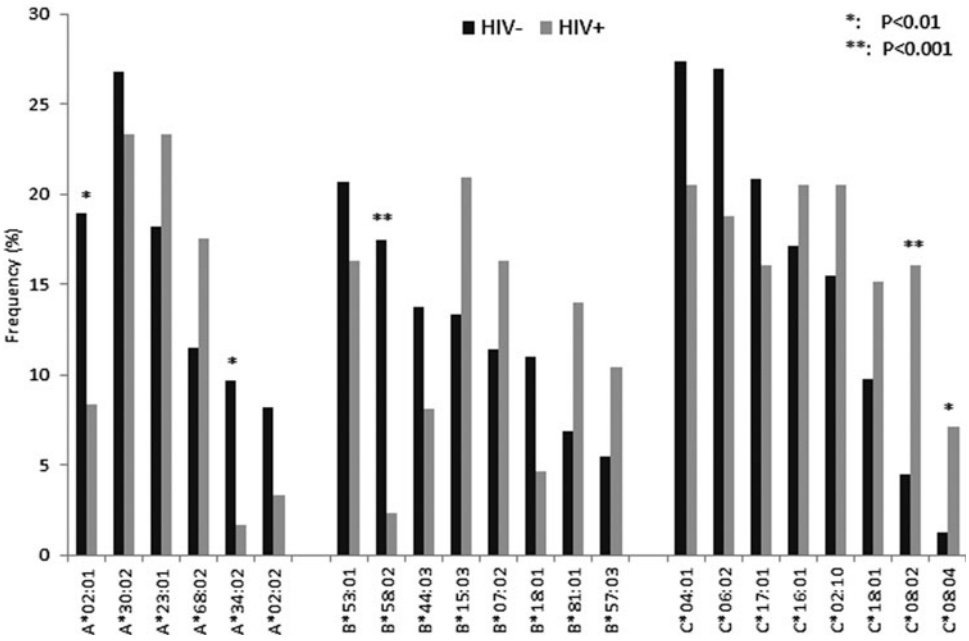


FIG. 1. Distribution of HLA class I alleles between infected and uninfected groups. Only alleles with an overall frequency >3% of the study population are represented. *Differences remained significant after Bonferroni correction for multiple comparisons. *p*-values estimated using Chi-squared or Fisher's exact test as appropriate.

that decline only slowly[16]: there is no obvious ''controller'' group in infant HIV-1 infection, raising the question of whether the HLA alleles linked with viral control in adults mediate a similar effect in children.

One previous study in a relatively small population of 61 HIV-infected children in Durban, South Africa showed that slow progression was associated with possession of at least one of three predefined ''protective'' class I HLA alleles (B*57:03, B*58:01, B*81:01), particularly when these were not shared with the mother[17]: however, these children were not followed into adolescence. A better understanding of the role played by host immunogenetic factors in the long-term control of vertical HIV-1 infection could lead to the design of new therapeutic and preventive strategies for pediatric HIV-1 infection.

We sought to identify the HLA correlates of delayed pediatric HIV-1 disease progression using samples previously collected from a cohort of 123 children and adolescents aged 10–18 years old with (presumed) vertically acquired HIV-1 infection and 276 age-matched uninfected controls recruited from public hospitals and primary health care clinics in Harare, Zimbabwe, as previously described.[12,18] Vertical HIV infection was assumed to be the most likely source of infection on the basis of criteria developed in Zimbabwe: these include self-report of no sexual debut or blood transfusions, a history of maternal or natural sibling HIV disease/death, and characteristic clinical features of longstanding HIV [≥1 of stunting; history of recurrent minor infections (skin, upper respiratory tract)].

The clinical samples were collected as part of a study focusing on the diagnosis and care of older children with previously undiagnosed HIV-1 infection,[12,18] hence at recruitment the majority of HIV^+ participants were not taking antiretroviral therapy (ART). The ideal comparison group would have been untreated vertically infected rapid progressors infected with HIV-1 contemporaneously with the slow progressors: however, these children inevitably had died well before the start of this study. We reasoned that the enrichment of specific HLA alleles in the HIV^+ group compared to healthy controls would be the result of either increased susceptibility to HIV-1 infection or (more likely) a survival advantage. The study was approved by the Medical Research Council of Zimbabwe (MRCZ), the Biomedical Research and Training Institute (BRTI) IRB, the Harare Hospital Ethics Committee, and the Oxford Tropical Research Ethics Committee (OxTREC). Informed consent/assent was obtained from each participant/guardian, and participants largely belonged to the Shona ethnic group.

HLA class I genotyping was performed by sequencing using locus-specific primers and the BigDye Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems, Foster city, CA) as previously described.[19,20]

Overall, we identified 30 HLA-A, 37 HLA-B, and 26 HLA-C subtypes, with the most frequent being A*30:02 (25.7%), B*53:01 (19.4%), and C*04:01 (25.2%), respectively. Figure 1 depicts the assignment of HLA class I alleles (present at >3% frequency in this population) between the long-term survivors of vertical HIV-1 infection and the uninfected controls. Alleles present at significantly increased frequency in the control population include HLA-A*02:01, -A*34:02, and -B*58:02: of note, HLA-B*58:02 has previously been linked to rapid disease progression in African populations (Zambians and Rwandans).[21] The only alleles showing significant enrichment in the slow progressors were HLA-C alleles, C*08:02 and C*08:04. There was a trend to enrichment for B*57:03, which is strongly linked with delayed disease progression in African adults,[21,22] but this did not reach significance in our study (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/aid).

These data show that HLA-C*08:02 and C*08:04 are significantly enriched in the HIV-1 long-term survivor group, and may therefore confer a survival advantage following vertical infection (Fig. 1). These alleles have not previously been linked with either progression or disease susceptibility

in other cohorts. The level of cell surface expression of some HLA-C alleles is strongly linked with delayed disease progression,[23] suggesting that HIV-specific T cells restricted by HLA-C molecules may contribute to viral control: however, in this study HLA-C*08:02 was in the middle of the range for HLA-C expression. Alternatively, as ligands for natural killer (NK) cell recognition, HLA-C molecules may contribute to HIV control through NK cell activity: HLA-C*08 alleles belong to the C group 2 allele family, and would therefore be expected to bind to the two domains inhibitory killer immunoglobulin-like receptors (KIRs) 2DL2 and 2DL3. KIR typing and further investigations into potential mechanisms by which HLA-C*08:02 and C*08:04 may contribute to HIV control will be addressed in future studies.

In a large population study, it was shown that HLA-influenced viral polymorphisms correlated with higher viral loads. In the same study, escape mutants were associated with the most prevalent class I allele in most populations, HLA-A*02:01.[24] This indicates that HLA immune pressure may lead to the evolution of circulating HIV variants that are able to avoid detection by the most common HLA alleles, which, in turn, contributes to the failure of the immune system to control viral replication. In a mixed American population, a strong relationship was found between rapid disease progression in children and the presence of the HLA-A*23:01 allele, but a discordant effect of HLA-A*02:01 alleles in the black and white ethnic groups was noted.[17,25] In our study, HLA-A*02:01, A*34:02, and HLA-B*58:02 were significantly overrepresented in the uninfected group: this could indicate that these alleles have a beneficial effect against HIV acquisition, but most immunogenetic studies on HIV infection have reported much stronger links between HLA alleles and disease progression than either resistance or susceptibility to HIV acquisition. Alternatively, the relatively low frequencies of these alleles in the long-term survivor group could reflect associations with rapid progression to AIDS and early death following vertical HIV-1 transmission. Further investigation in well-established pediatric cohorts, in which both rapid and slow progressors can be reliably characterized, will be required to resolve this issue.

Host genetic factors have been shown to have a profound impact on antigen presentation and immune responses, greatly influencing HIV pathogenesis and progression to AIDS. The HLA class I region, the most polymorphic region of the human genome, has a stronger association with HIV disease outcome than any other group of genes. Here, we provide the HLA class I gene profiles of a population of adolescent long-term survivors of HIV-1 infection that serve as an essential first step for further study design to elucidate the immunological and genetic factors mediating delayed disease progression in a minority of African children with vertically acquired HIV-1 infection.

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Author Disclosure Statement

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CHAPTER 3: GENERAL DISCUSSION AND FUTURE DIRECTIONS

This thesis aimed to characterise the association of class I HLA alleles in the pathology of an acquired immune deficiencies, as well as to introduce a new method of long-range, high resolution HLA typing for the identification of novel alleles that may provide insight into further investigations of disease pathology.

Chapter 1: Long-range HLA class I sequencing

Chapter 3 introduced a new method for high-resolution, unambiguous, mono-allelic genotyping of HLA-B and C alleles. While the traditional method of sequence based techniques for HLA typing focuses on the highly polymorphic binding sites in exon 2 and 3 of class I molecules[1,2], this new in-house technique produces a sequence that involves all 8 exons of the coding region and noncoding region of class I alleles. This provides an opportunity to identify polymorphisms in other regions—such as the leader peptide (exon 1), α3 of the extracellular domains (exon 4), the transmembrane anchor (exon 5), the cytoplasmic tail (exons 6 and 7), and the 3' untranslated region (exon 8)—that may have functional consequences previously overlooked. Additionally, novel allele combinations will contribute to the ever-growing IMGT/HLA sequence database to provide reliable sequences for future experiments, reagent design, and transplant matching.

As a pilot study, 13 samples available from the Zimbabwean Shona adolescent cohort were used to demonstrate the fidelity of this technique for the typing of HLA-B and HLA-C alleles. By generating full-length DNA sequences of each allele, this method allowed for a detailed analysis of ambiguities from the consensus sequence of the known allelic variant; where, at the nucleotide

level, SNPs were identified in 3 samples that were submitted to GenBank® to be reviewed for quality control measures and to confirm the presence of novel mutations within these sequences. Ideally, future studies will use a larger number of samples to demonstrate the effectiveness of this technique. For the current study, only a small number of samples had a high enough concentration of DNA to perform the experiment. Currently, our lab's continued collaboration with Dr. Rashida Ferrand from the London School of Hygiene and Tropical Medicine has enabled the prospective collection of new DNA samples from 400 Zimbabwean Shona adolescents with vertically acquired HIV infection. With this in mind, further typing of class I alleles with this new method will become achievable for future researchers in the lab of Sarah Rowland-Jones who work on this population.

Additionally, forthcoming efforts should be made to validate this method in different populations across different continents. Validation of the technique is essential to both confirm its fidelity and detect weaknesses in the technique that may need further improvements to ensure that single alleles can be obtained from any part of the world. Finally, the method can be further optimised to define both known and unique HLA class II heterozygous sequences.

Chapter 2: HLA class I associations of survival in long-term nonprogressors of mother to child transmission

In this chapter, investigations were performed to identify the HLA class I associations with slow progression of HIV infection in a group of Zimbabwean adolescents with either direct or indirect epidemiological evidence of having acquired the virus through mother-to-child transmission. Many studies have confirmed the importance of class I associations in the pathogenesis of HIV infection[3,4]. Nevertheless, it is still essential to perform HLA association studies on new

populations to characterise the distribution of alleles in different regions worldwide. Identifying the baseline distribution of alleles allows for investigators to monitor the impact of disease processes on the selection of alleles that persist or decrease in frequency within a population over time. Changes in population allele frequencies observed give insight into the selective constraints that contribute to survival or death as a result of different pathogens and disease[3,5-9]. This is especially true in African populations, which exhibit some of the greatest differences in HLA genetic variability worldwide[10-15]. Second, aside from ethnic differences in HLA type, different HLA antigens may be associated with differential disease outcomes in HIV based on a number of different factors related to the host or the virus itself[3,16-22]. Finally, much less is known about the HLA associations in paediatric HIV as compared to adults. More studies need to be performed to identify new HLA associations or confirm those already known in adults to better study the implications of disease progression in the maturing immune system. With this in mind, as a first step toward delineating the pathway of CD8+ T-cell immune recognition, the HLA class I associations were determined in a cohort of long-term survivors of vertically acquired HIV infection.[23]

This study identified several HLA-C alleles that may be associated with slower progression of HIV infection in this cohort[24]. Furthermore, this study demonstrated that the only alleles significantly enriched in the slow progressors were HLA-C alleles, C*08:02 and C*08:04. The results of this analysis may indicate a survival advantage after vertical infection with HIV-1 for those positive for HLA-C*08:02 and/or HLA-C*08:04.

As presented in this thesis, HLA class I alleles, specifically HLA-B, have consistently been the

strongest independent, genetic association with differential rates of HIV disease outcome[3,4]. Studies to date continue to provide evidence for HLA-dependent influences on viral control and evolution, which, subsequently, will have important implications for the design of an effective HIV vaccine.

As previously discussed, HLA-C is interesting and controversial in the context of HIV Infection [25-27]. HIV-Nef has been shown to selectively down regulate HLA-A and HLA-B while maintaining HLA-C and HLA-E expression at the cell surface[28,29]. To this end, it is speculated that highly expressed HLA-C alleles could trigger HIV-specific responses through increased antigen presentation to CTLs, through the binding of KIRs on NK cells, or by a combination of these mechanisms[30]. In the population of Zimbabwean adolescents with vertically infected HIV-1 disease, HLA-C*08 was significantly associated (<0.001) with slow progressing infection. Previously, Blais *et al*. have demonstrated in a Chinese Han infected with a single source virus that HLA-C*08 was in LD with the -35 CC SNP variant previously shown by several groups to be independently associated with HIV control and HLA-C expression[31].

That the -35 CC SNP associates with better clinical outcome than that of -35TT in the vertically infected population of Shona adolescents has yet to be determined. To explore the mechanism of immune protection during HIV infection in this population enriched by the moderately-expressed HLA-C*08, further steps should be taken to assess the potential for HLA-C restricted immune responses that may be associated with viral control. Additionally, the relative expression of different HLA-C subtypes it not known and should be explored. These studies can be used as a springboard to assess whether HLA- C*08 restricted T-cell responses associate with viral

control. If so, further studies can utilise HLA-C*08 restricted CTL clones to determine whether they have unusually high avidity or suppress viral replication in *in vitro* assays. To consider whether HLA-C restricted immune responses contribute to HIV evolution, the frequency of C-associated mutations in plasma viral RNA should correlate with the magnitude of immune pressure exerted on the virus. C-mutations should be exclusively associated with HLA-C alleles and used with C-mutations as markers for HLA-C restricted immune pressure.

Additionally, as ligands for NK cell recognition, HLA-C molecules may contribute to HIV control through NK cell activity: HLA-C*08 alleles belong to the C group 2 allele family, and would therefore be expected to bind to the two domains inhibitory KIRs 2DL2 and 2DL3. KIR typing and further investigations into potential mechanisms by which HLA-C*08:02 and C*08:04 may contribute to HIV control will be addressed in future studies.

Final Remarks

Each of the studies presented within this thesis have opened up opportunities for new investigations into the role of class I antigens in developing an immune response against human acquired immune deficiency syndrome. Additionally, the further refinement of the long-range sequencing technique developed for high resolution HLA class I typing, will provide an opportunity for more detailed investigations of polymorphisms within the coding and noncoding regions of HLA alleles class alleles and their relationship to the pathogenesis of these diseases.

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